UNIWERSYTET MIKOŁAJA KOPERNIKA W TORUNIU

Collegium Medicum
im. Ludwika Rydygiera w Bydgoszczy

Bydgoszcz 2024 r.

UNIWERSYTET
MIKOŁAJA KOPERNIKA
W TORUNIU
Wydział Lekarski
Collegium Medicum w Bydgoszczy

Damian Jaworski

Wpływ zaburzeń ekspresji wybranych białek mechanizmów naprawy DNA na progresję raka gruczołu krokowego

Rozprawa na stopień doktora nauk medycznych

Promotor:

Prof. dr hab. n. med. Łukasz Szylberg

Bydgoszcz 2024r.

Składam serdeczne podziękowania mojemu promotorowi
Panu prof. dr hab. Łukaszowi Szylbergowi
za cenne wskazówki, poświęcony czas i opiekę naukową podczas realizacji
niniejszej pracy doktorskiej.

Spis treści

Wykaz stosowanych skrótów 5

Rozdział 1. Nota informacyjna i wykaz publikacji stanowiących podstawę rozprawy doktorskiej 6

Rozdział 2. Wprowadzenie 7

2.1 Mechanizmy naprawy DNA 8

2.2 Mechanizm naprawy DNA błędnie sparowanych zasad azotowych (MMR) 9

2.3 Mechanizmy naprawy podwójnych pęknięć nici DNA (DSB) 12

2.4 Wpływ zaburzeń mechanizmów naprawy DNA na raka gruczołu krokowego 16

Rozdział 3. Cel pracy 17

Rozdział 4. Publikacje będące przedmiotem rozprawy doktorskiej 18

4.1 Artykuł 1 18

4.2 Artykuł 2 29

4.3 Artykuł 3 38

Rozdział 5. Podsumowanie 59

Rozdział 6. Wnioski 62

Rozdział 7. Bibliografia 63

Rozdział 8. Oświadczenia autorów publikacji włączonych do cyklu 69

Rozdział 9. Zgoda Komisji Bioetycznej 91

Rozdział 10. Streszczenie w języku polskim 93

Rozdział 11. Streszczenie w języku angielskim 95

Wykaz stosowanych skrótów

Skrót	Pełna nazwa w języku angielskim	Pełna nazwa w języku polskim
DSBR	Double-strand break repair	Mechanizm naprawy podwójnego pęknięcia nici DNA
MMR	Mismatch repair	Mechanizm naprawy DNA błędnie sparowanych zasad
PARP	Poly (ADP-ribose) polymerase	Polimeraza poli(ADP-rybozy)
HRR	Homologous recombination repair	Proces rekombinacji homologicznej
HR	Homologous recombination	Rekombinacja homologiczna
NHEJ	Non-homologous end joining	Scalanie niehomologicznych końców DNA
SSB	Single-strand break	Pojedyncze pęknięcie nici DNA
BER	Base excision repair	Naprawa przez wycinanie zasady
NER	Nucleotide excision repair	Naprawa przez wycięcie nukleotydu
DSB	Double-strand break	Podwójne pęknięcie nici DNA
MSI	Microsatellite instability	Niestabilność mikrosatelitarna
IDLs	Insertion-deletion loops	Pętle insercja-delecja
ssDNA	Single –stranded DNA	Pojedyncze nici DNA
mCRPC	Metastatic castration-resistant prostate cancer	Rak gruczołu krokowego z przerzutami oporny na kastrację
FDA	Food and Drug Administration	Amerykańska Agencja Leków i Żywności
GP	Gleason Pattern	Architektonika Gleasona
GS	Gleason Score	Skala Gleasona
PCa	Prostate Cancer	Rak gruczołu krokowego

Rozdział 1. Nota informacyjna i wykaz publikacji stanowiących podstawę rozprawy doktorskiej

Przedmiotem niniejszej dysertacji doktorskiej jest spójny tematycznie zbiór artykułów, które zostały opublikowane w recenzowanych czasopismach naukowych umieszczonych w ministerialnym wykazie czasopism naukowych i recenzowanych materiałów z konferencji międzynarodowych. Sumaryczna wartość współczynnika oddziaływania (Impact Factor, IF) publikacji stanowiących niniejszą rozprawę doktorską wynosi 13,398 oraz 340 punktów Ministerstwa Nauki i Szkolnictwa Wyższego (MNiSW). Publikacje zostały uporządkowane według chronologicznej kolejności ich wydania.

1. Gzil Arkadiusz, **Jaworski Damian**, Antosik Paulina, Zarębska Izabela, Durślewicz Justyna, Dominiak Joanna, Kasperska Anna, Neska-Długosz Izabela, Grzanka Dariusz, Szylberg Łukasz, The impact of TP53BP1 and MLH1 on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice, Urol. Oncol.-Semin. Orig. Investig.; 2020 : Vol. 38, nr 6. s. 600.e17-600.e26. DOI: 10.1016/j.urolonc.2020.02.012
   IF: 3,498 MNiSW: 100,000
2. **Jaworski Damian**, Gzil Arkadiusz, Antosik Paulina, Zarębska Izabela, Dominiak Joanna, Neska-Długosz Izabela, Kasperska Anna, Grzanka Dariusz, Szylberg Łukasz, Expression differences between proteins responsible for DNA damage repair according to the Gleason grade as a new heterogeneity marker in prostate cancer, Arch. Med. Sci.; 2023 :Vol. 19, nr 2. s. 499-506. DOI: 10.5114/aoms.2019.89773
   IF: 3,900 MNiSW: 100,000
3. **Jaworski Damian**, Brzoszczyk Bartosz, Szylberg Łukasz, Recent research advances in double-strand break and mismatch repair defects in prostate cancer and potential clinical applications, Cells; 2023 :Vol. 12, nr 10, s. 1-20; 1375, DOI: 10.3390/cells12101375
   IF: 6,000 MNiSW: 140,000

Rozdział 2. Wprowadzenie

Rak gruczołu krokowego (z ang. prostate cancer, PCa) jest najczęstszym złośliwym nowotworem wśród mężczyzn i stanowi 20,6% nowotworów złośliwych w Polsce. Roczna umieralność z powodu PCa wynosi 10,3%, co czyni go trzecim najbardziej śmiertelnym nowotworem w Polsce[1]. Dotychczasowe badania dowodzą, że od 10% do 20% pacjentów z przerzutami PCa w obserwacji pięcioletniej rozwinie PCa opornego na kastracje, ze średnią przeżywalnością około 14 miesięcy[2]. Z uwagi na powyższe dane, nowotwór ten nadal stanowi duże wyzwanie w kontekście optymalizacji diagnostyki i strategii terapeutycznych. W obszarze biologii molekularnej znaczenie mechanizmów naprawy DNA błędnie sparowanych zasad znany jako MMR (ang. Mismatch repair) i naprawy podwójnego (dwuniciowego) pęknięcia nici DNA znany jako DSBR (ang. double-strand break repair) jest coraz bardziej dostrzegane w kontekście wielu typów nowotworów, w tym PCa[3,4]. Podczas gdy bezpośrednia przyczyna powstawania PCa nadal pozostaje przedmiotem badań, dotychczasowe doniesienia sugerują, że zdolność do inicjacji nowotworzenia tego guza posiadają zarówno komórki zróżnicowane, jak i komórki macierzyste lub progenitorowe[5–7]. Niemniej jednak, powszechnie uznaje się, że przewlekły stan zapalny jest jedną z głównych przyczyn nowotworzenia. Długotrwała ekspozycja na stres oksydacyjny oraz reaktywne formy tlenu mogą prowadzić do uszkodzeń DNA, co skutkuje selekcją komórek z mutacjami i progresją neoplazji śródnabłonkowej do raka[8].

Zaburzenia mechanizmów naprawy uszkodzeń DNA stanowią przyczynę wielu nowotworów. Klasycznym przykładem są mutacje w genach BRCA1/2 w raku piersi i jajnika, zaburzenia w obrębie szlaku MMR w zespole Lyncha predysponującego do raka jelita grubego, a także zespołu Xeroderma Pigmentosum stanowiącego podłoże dla nowotworów skóry[9–12]. W PCa przyjmuje się, że od 5% do 15% przypadków powstaje na podłożu czynników dziedzicznych, w tym zaburzeń MMR i DSBR. Nowotwory w tej grupie charakteryzują się agresywnym przebiegiem i gorszym

rokowaniem[13,14]. Identyfikacja zaburzeń w obrębie szlaków naprawy DNA takich jak MMR i DSBR, w procesach nowotworzeni oraz ocena ich wpływu na przebieg choroby otwierają nowe możliwości dotyczące planowania terapii, szczególnie w przypadkach, kiedy konwencjonalne metody leczenia nie są skuteczne. Dotychczasowe badania w tematyce zaburzeń wyżej wymienionych szlaków naprawy DNA umożliwiły wprowadzenie inhibitorów polimerazy poli(ADP-rybozy) (inhibitory PARP), między innymi w leczeniu raka piersi u pacjentów z mutacją genu BRCA1/2, w nowotworach z mutacją w obrębie genu PTEN, w raku jelita grubego u pacjentów z zespołem Lyncha (zaburzenia MMR), a także PCa opornego na kastrację z zaburzeniami w obrębie genów naprawy DNA zaangażowanych w proces rekombinacji homologicznej (z ang. homologous recombination rep air, HRR)[13,15–17].

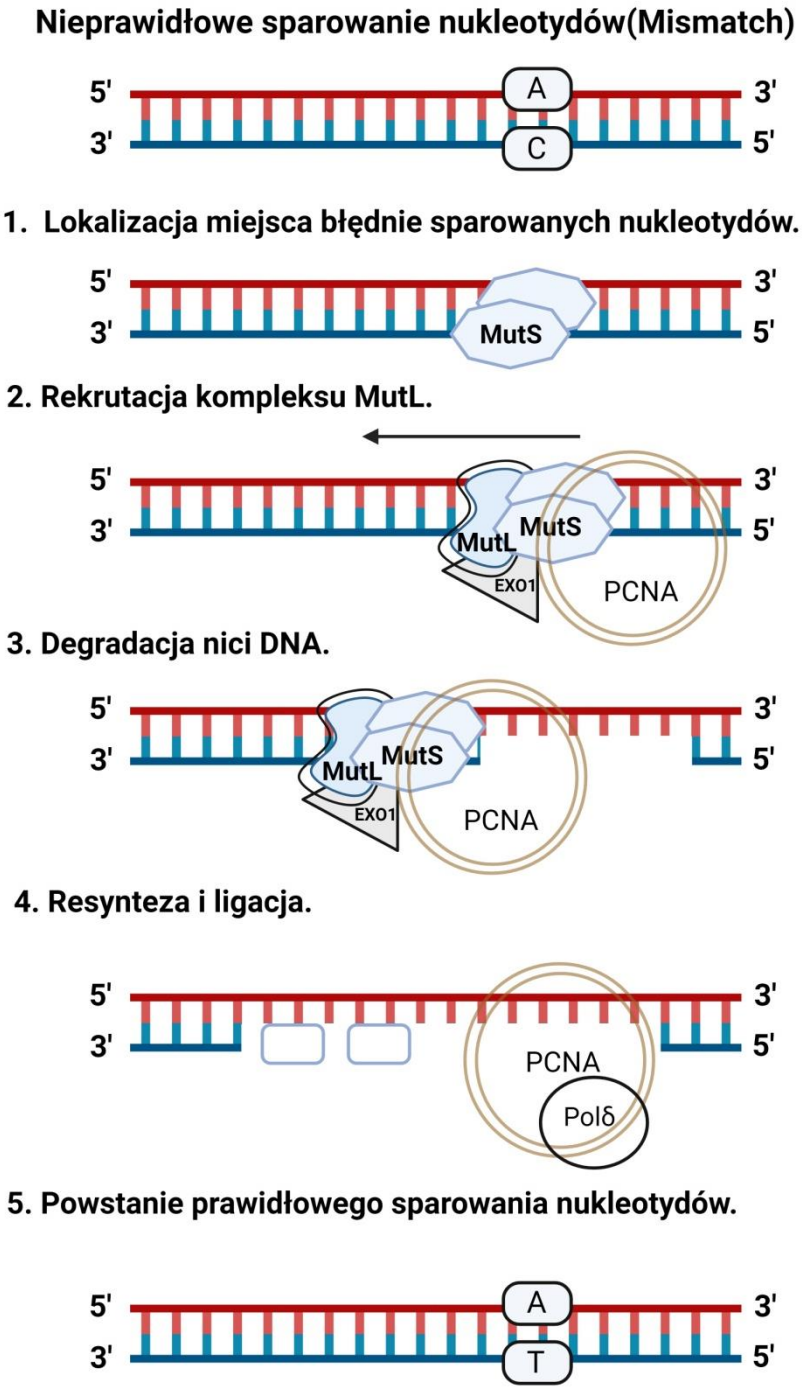
2.1 Mechanizmy naprawy DNA

Mechanizmy naprawy DNA aktywowane w przypadku różnorodnych uszkodzeń materiału genetycznego są kluczowe dla zachowania integralności genetycznej oraz prawidłowej funkcjonalności komórkowej organizmów. Przykładami takich uszkodzeń są pojedyncze pęknięcia nici DNA (ang. single-strand break; SSB), które są naprawiane przez szlak MMR. Dodatkowo, inne mechanizmy, takie jak naprawa przez wycinanie zasad (ang. base excision repair; BER) oraz naprawa przez wycięcie nukleotydów (ang. nucleotide excision repair, NER), również przyczyniają się do utrzymania stabilności genomu. W przypadku podwójnych pęknięć nici DNA (ang. double-strand breaks, DSB), uruchamiane są mechanizmy takie jak rekombinacja homologiczna (ang. homologous recombination, HR) oraz scalanie niehomologicznych końców DNA (ang. non-homologous end joining, NHEJ). Dzięki inicjowaniu prawidłowych form naprawy uszkodzeń DNA, mechanizmy te zapewniają efektywną naprawę, zapobiegając powstawaniu błędów podczas replikacji[18]. W mechanizmach tych uczestniczą geny odpowiedzialne za naprawę uszkodzonego DNA nazywane genami mutatorowymi (naprawczymi). Nieprawidłowe działanie mechanizmów naprawy błędów po replikacji, takich jak niewłaściwe parowanie zasad, może

prowadzić do niestabilności genomu, co z kolei sprzyja indukcji i postępowi procesów kancerogennych.

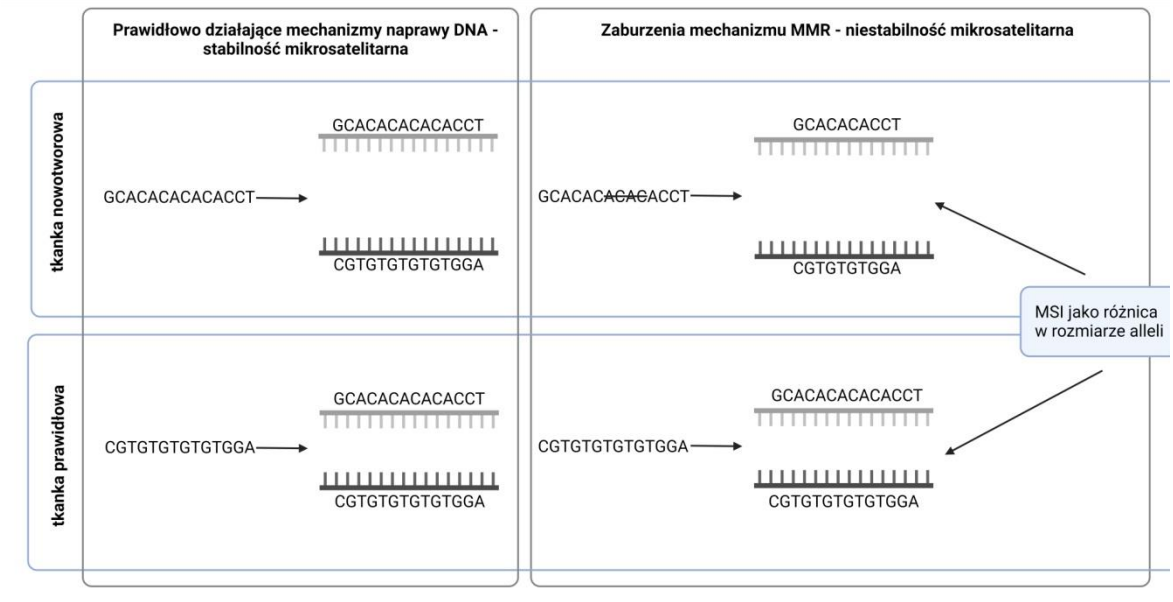
2.2 Mechanizm naprawy DNA błędnie sparowanych zasad azotowych (MMR)

Szlak MMR naprawia błędy powstałe podczas replikacji, rekombinacji oraz naprawy DNA, utrzymując stabilność genomu poprzez rozpoznawanie i korygowanie niesparowanych bądź błędnie sparowanych zasad azotowych, pętli insercja-delecja (z ang. insertion-deletion loops, IDLs) oraz uszkodzeń DNA typu SSB[19,20]. W szlaku MMR bierze udział wiele białek, w tym homologi MutS (MSH2, MSH3, MSH6), homologi MutL (MLH1, PMS1, PMS2), a także exonukleaza 1 (EXO1)[19,20]. Białka te umożliwiają rozpoznawanie, wiązanie się z miejscem uszkodzenia oraz naprawę DNA. Szlak MMR może być podzielony na dwie główne fazy, rozpoznania i naprawy uszkodzenia. W fazie pierwszej, homologi MutS tworzą heterodimery i rozpoznają miejsce błędnego sparowania zasad lub IDLs. Następnie homologi MutS rekrutują homologi MutL, co w konsekwencji aktywuje drugą fazę, naprawczą. W tej fazie biorą udział białka naprawcze, takie jak PCNA oraz EXO1. Po natrafieniu na przerwę w nici DNA, na przykład pomiędzy fragmentami Okazaki, na nici opóźnionej lub nacięcie nici wiodącej przez PMS2, rozszczepiają one przylegającą do miejsca działania nić DNA i rozpoczynają degradację. W dalszym etapie następuje stabilizacja fragmentów pojedynczej nici przez białko replikacyjne A (z ang. replication protein A, RPA), uzupełnienie przerw nici DNA przez polimerazę δ oraz wiązanie pozostałych przerw za pomocą ligazy DNA[21]. Działanie mechanizmu MMR zostało schematycznie przedstawione na Rycinie 1.



Rycina 1. Schemat przedstawiający sposób działania mechanizmu MMR. Do okolicy nieprawidłowo sparowanej pary nukleotydów przyłącza się hetero dimer MSH2/MSH6 (MutS), do którego po zmianie konformacyjnej dołącza się drugi heterodimer MLH1/PMS2 (MutL). Podczas przesuwania się wzdłuż nici w momencie natrafienia na nieprawidłowość w postaci zaburzenia ciągłości nici, razem z PCNA oraz EXO1 rozpoczynają degradację nici DNA. Następnie RPA stabilizuje pojedyncze fragmenty nici DNA. Nić DNA uzupełniana jest przez polimerazę, a fragmenty nici łączone są przez ligazę DNA[20,21].

Mutacje w genach MMR prowadzą do niestabilności mikrosatelitarnej (z ang. microsatellite instability, MSI), co objawia się zwiększoną częstością mutacji typu insercji i delecji w sekwencjach DNA o charakterze powtarzalnym. W odniesieniu do MSI, mikrosatelity to fragmenty DNA z powtarzalnymi sekwencjami nukleotydów, które są szczególnie podatne na pojawienie się błędu w przypadku zaburzeń MMR. MSI jest zdefiniowane jako różnica w liczbie powtarzających się sekwencji nukleotydów między tkanką zdrową, a nowotworową (Rycina 2.). Nowotwory mogą być klasyfikowane na podstawie poziomu MSI na nowotwory z wysokim poziomem markerów niestabilności (MSI-high) oraz nowotwory z niskim poziomem markerów niestabilności (MSI-low). Obecność MSI jest jedną z charakterystycznych cech zaburzeń w MMR. Zaburzenie funkcji szlaku MMR prowadzi do akumulacji zaburzeń w DNA, co z kolei skutkuje niestabilnością genomu. Taka niestabilność może prowadzić do powstania mutacji, które są kluczowymi czynnikami w procesie nowotworzenia. Niemniej, MSI będąc efektem tych zaburzeń, jest powszechnie obserwowana w wielu rodzajach nowotworów. Szczególnie często związana jest z rakiem jelita grubego, żołądka, jajnika i trzustki, gdzie akumulacja mutacji w genomie może przyspieszać progresję nowotworową i wpływać na agresywność tych chorób[22].

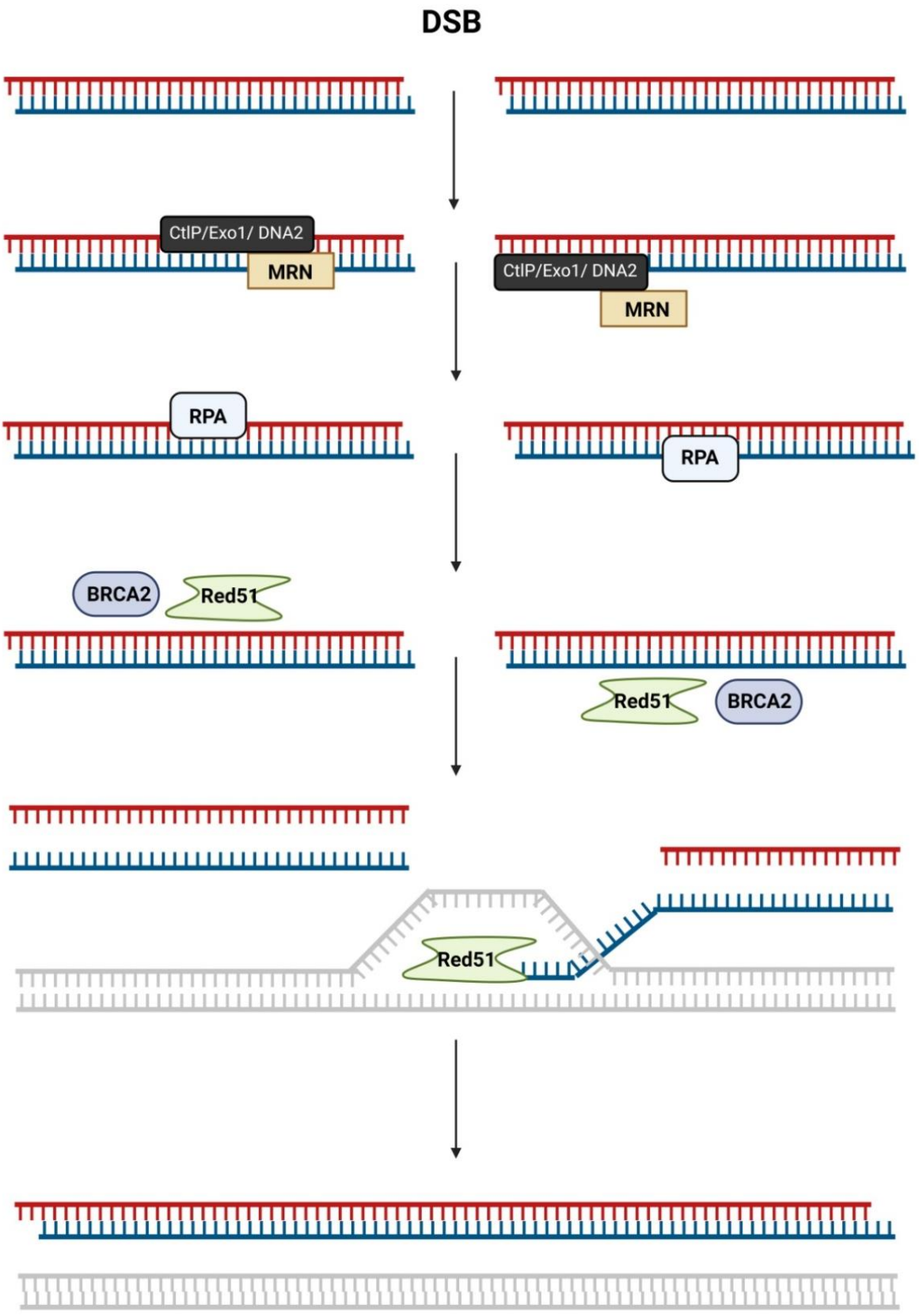


Rycina 2. Schemat przedstawiający niestabilność mikrosatelitarną jako różnicę ilości powtarzających się par nukleotydów w allelach pomiędzy tkanką prawidłową oraz nowotworową[21].

Zaburzenia szlaku MMR mogą powstawać na podłożu terminalnym, somatycznym oraz epigenetycznym[23]. Utrata bądź osłabienie aktywności MMR prowadzi do akumulacji mutacji, w tym mutacji onkogenów i genów supresorowych nowotworów, co w konsekwencji skutkuje powstaniem i proliferacją komórek nowotworowych[19,20]. Komórki z tak wysokim obciążeniem mutacyjnym przyczyniają się do powstawania wysokiej ilości nowych antygenów nowotworowych, co z kolei uwrażliwia je na inhibitory punktów kontrolnych[24]. Nowotwory z zaburzeniami w obrębie szlaku MMR są również bardziej podatne na terapie z udziałem inhibitorów PARP oraz preparatów uszkadzających DNA[25,26].

2.3 Mechanizmy naprawy podwójnych pęknięć nici DNA (DSB)

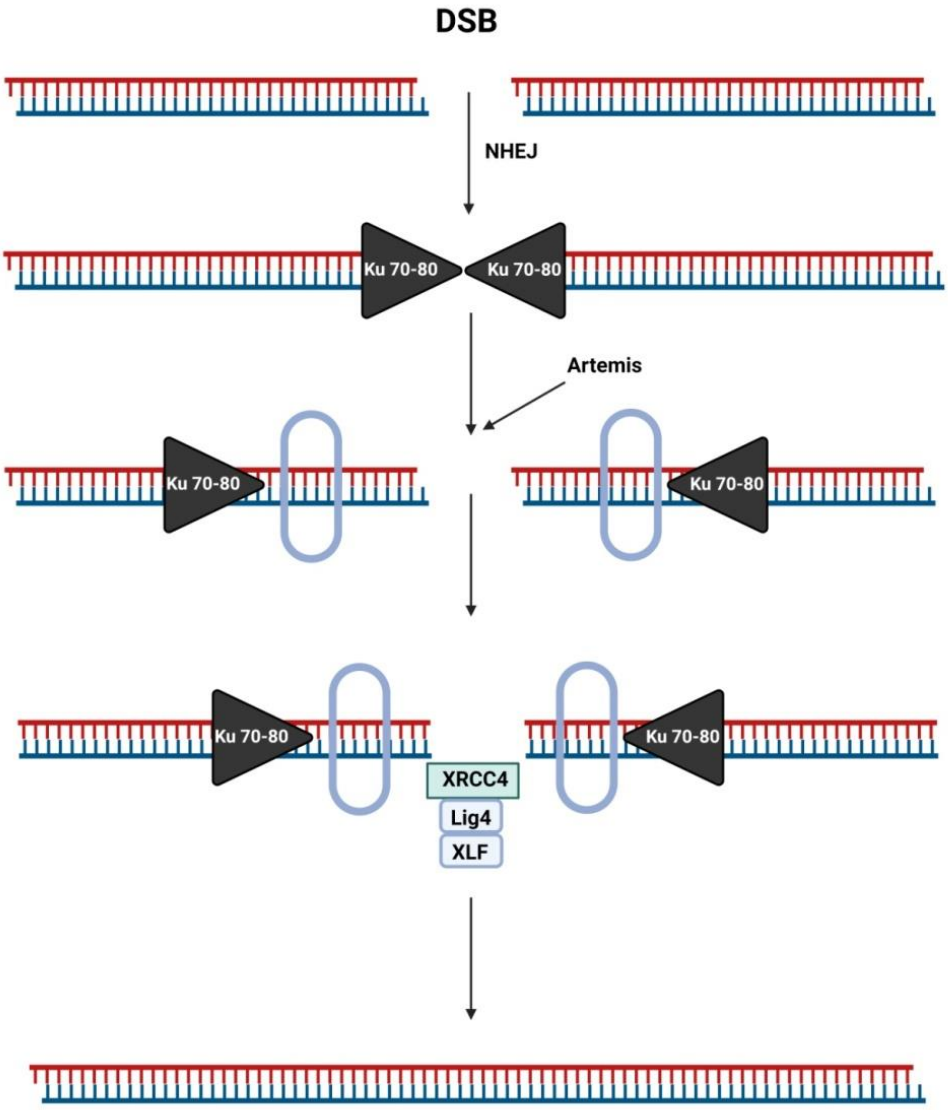
Uszkodzenia podwójnej nici DNA mogą powstawać na podłożu endogennych czynników jak wolne rodniki tlenowe powstające podczas procesów metabolicznych zachodzących w komórce lub czynników egzogennych jak promieniowanie jonizujące czy chemioterapeutyki. Komórki eukariontów dysponują dwoma głównymi szlakami naprawy tego typu uszkodzeń. Pierwszym, głównym szlakiem jest HR, natomiast drugim NHEJ[22]. Pierwszym punktem naprawy DSB jest rozpoznanie uszkodzenia. W HR kompleks MRN (MRE11-RAD50-NBS1), po utworzeniu platformy przez MDC1 związany z H2AX, rozpoznaje DSB i inicjuje resekcje końców DNA przy pomocy nukleaz, takich jak CtlP, Exo1 i DNA2. Powstałe pojedyncze nici DNA (z ang. single–stranded DNA; ssDNA) zostają zabezpieczone przez białko RPA, następnie zastępowane przez rekombinazę RAD51 przy udziale BRCA2. Powstały filament nukleoproteinowy RAD51-ssDNA wnika w homologiczny szablon DNA i inicjuje naprawę z następczą syntezą DNA i ligacją[27–29]. Przebieg procesu HR został schematycznie przedstawiony na Rycinie 3.


DSB
CtIP/Exo1/ DNA2
MRN
CtIP/Exo1/ DNA2
MRN
RPA
RPA
BRCA2
Red51
Red51
BRCA2
Red51

Rycina 3. Schemat przebiegu procesu HR.

Kompleks MRN rozpoznaje uszkodzenie o typie podwójnej nici i angażuje nukleazy takie jak CtlP, Exo1 i DNA2 do resekcji końców DNA. Powstałe pojedyncze nici DNA zostają zabezpieczone przez białko RPA które zostaje następnie zastąpione przez rekombinazę RAD51 przy udziale BRCA2. Powstały filament nukleoproteinowy RAD51-ssDNA wnika w homologiczny szablon DNA i inicjuje naprawę z następczą syntezą DNA i ligacją[27–29].

Drugi mechanizm naprawy DSB, NHEJ, będący podatny na powstawanie błędów w trakcie naprawy, przeprowadza bezpośrednią ligazę końców przerwanego łańcucha DNA, bez korzystania z homologicznego szablonu DNA. W tym procesie DSB rozpoznawane są przez heterodimery Ku70-80, które wiążą się z końcami przerwanej nici DNA i rekrutują DNA-zależne kinazy białek (z ang. DNA-dependent protein kinase; DNA-PKcs). DNA-PKcs fosforyluje substraty, w tym Artemis i XRCC4, które razem z kompleksem MRN prowadzą do przetworzenia końców. Wypełnienie przerw zostaje dokonane przez polimerazy μ oraz λ, a ligacja za pośrednictwem ligazy IV[30]. Alternatywnie, w przypadku braku Ku lub DNA-PKcs, kompleks MRN inicjuje resekcję końca, co może promować NHEJ przez ścieżkę zwaną alt-NHEJ[29,31,32]. Przebieg procesu NHEJ przedstawiono schematycznie na Rycinie 4.



Rycina 4. Schemat przebiegu procesu NHEJ.

Dwuniciowe przerwanie nici DNA zostaje rozpoznane przez heterodimetry Ku70-80, które następnie rekrutują DNA-PKcs. DNA-PKcs fosforyluje substraty, w tym Artemis i XRCC4, które razem z kompleksem MRN prowadzą do przetworzenia końców. Wypełnienie przerw zostaje dokonane przez polimerazy μ oraz λ, a ligacja za pośrednictwem ligazy IV, czego efektem jest scalenie przerwania nici DNA[30].

Wybór pomiędzy tymi dwiema ścieżkami naprawy DNA zależy od wielu czynników, w tym od etapu cyklu komórkowego. W fazach S i G2, gdy dostępne są chromatydy siostrzane służące jako matryce naprawy, preferowany jest HR. NHEJ jest natomiast aktywny przez cały cykl komórkowy, zwłaszcza w fazach G1 i G0, gdy matryce nie są dostępne. Ponadto proces resekcji końców DNA również determinuje wybór pomiędzy HR a NHEJ. HR wymaga, aby końce DNA zostały przetworzone do jednoniciowych odcinków, do czego potrzebne są białka takie jak RAD51, NBS1 i MRE11. NHEJ jest natomiast preferowany w przypadkach, gdy występuje podwójne pęknięcie nici z powstaniem kompatybilnych końców DNA, podczas gdy HR jest angażowany w przypadkach skomplikowanych uszkodzeń ze zmienionymi końcami, wymagającymi bardziej precyzyjnej naprawy. Dodatkowo, w proces regulacji wyboru metody DSBR zaangażowane są również białka regulatorowe takie jak 53BP1, promujący NHEJ i BRCA1, wspierający HR.

2.4 Wpływ zaburzeń mechanizmów naprawy DNA na raka gruczołu krokowego

Obecność zaburzeń mechanizmów naprawy DNA pod postacią mutacji w obrębie genu BRCA2 i ATM u pacjentów z PCa wskazuje na konieczność ściślejszej kontroli w związku z większym prawdopodobieństwem progresji nowotworu i istotnie gorszym rokowaniem[33]. Ponadto dotychczasowe badania wskazują na trzykrotnie częstsze występowanie zaburzeń w obrębie genów mechanizmu HR w przypadku PCa z przerzutami (11% vs. 33%)[34]. Podobne wnioski wysunięto z badania klinicznego Profound, gdzie wykazano, iż wśród pacjentów z opornym na kastrację rakiem gruczołu krokowego (z ang. metastatic castration-resistant prostate cancer, mCRPC) tkanka guza pierwotnego (27%) ma mniej mutacji w genach mechanizmów naprawy DNA w porównaniu z tkanką przerzutową (32%)[35]. Dotychczasowe badania umożliwiły zaakceptowanie przez Amerykańską Agencję Leków i Żywności (z ang. Food and Drug Administration, FDA) dwóch leków z grupy inhibitorów PARP takich jak Olaparib i Rucaparib. Leki te wykazały skuteczność w leczeniu mCRPC z obecnością mutacji w obrębie genów mechanizmów HR[36,37].

Ponadto 5 leków z grupy inhibitorów punktów kontrolnych Nivolumab, Atezolizumab, Durvalumab, Ipilimumab, Pembrolizumab oraz 3 kolejne leki z grupy inhibitorów PARP Niraparib, Talazoparib oraz Veliparib znajdują się w trakcie badań klinicznych w leczeniu mCRPC[38–40].

Rozdział 3. Cel pracy

Niniejsza praca doktorska dąży do rozwiązania kluczowych zagadnień poprzez zdefiniowanie następujących celów badawczych:

1. Porównanie ekspresji białek (MLH1, MSH2, MSH6, PMS2, MDC1 i TP53BP1) szlaków MMR i DSBR w tkance raka gruczołu krokowego bez przerzutów (pN0), w tkance raka gruczołu krokowego z przerzutami do węzłów chłonnych (pN+) oraz w tkance węzła chłonnego z przerzutem nowotworowym.
2. Analiza korelacji między poziomem ekspresji białek MLH1, MSH2, MSH6, PMS2, MDC1 i TP53BP1, a stopniem zróżnicowania nowotworu, ocenianym na podstawie skali Gleasona (z ang. Gleason Score, GS) oraz architektoniką Gleasona (z ang. Gleason Pattern, GP) w raku gruczołu krokowego.
3. Określenie wpływu zaburzeń białek szlaków MMR i DSBR na skuteczność terapii inhibitorami PARP oraz inhibitorami punktów kontrolnych cyklu komórkowego w raku gruczołu krokowego.

Rozdział 4. Publikacje będące przedmiotem rozprawy doktorskiej

4.1 Artykuł 1

Gzil Arkadiusz, Jaworski Damian, Antosik Paulina, Zarębska Izabela, Durślewicz Justyna, Dominiak Joanna, Kasperska Anna, Neska-Długosz Izabela, Grzanka Dariusz, Szylberg Łukasz, The impact of TP53BP1 and MLH1 on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice, Urol. Oncol.-Semin. Orig. Investig.; 2020 : Vol. 38, nr 6. s. 600.e17-600.e26. DOI: 10.1016/j.urolonc.2020.02.012

ELSEVIER

Urologic Oncology: Seminars and Original Investigations 38 (2020) 600.e17–600.e26

UROLOGIC ONCOLOGY

Laboratory-Prostate cancer

The impact of TP53BP1 and MLH1 on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice

Arkadiusz Gzil, M.D.[a,1,*], Damian Jaworski, M.D.[a], Paulina Antosik, M.S.[a], Izabela Zarębska, M.D.[a], Justyna Durślewicz, M.S.[a], Joanna Dominiak, M.D.[a], Anna Kasperska, M.D.[a], Izabela Neska-Długosz, M.D.[a], Dariusz Grzanka, M.D.[a], Łukasz Szylberg, M.D.[a,b,c,1]

[a] *Department of Clinical Pathomorphology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, Poland*
[b] *Department of Pathomorphology, Military Clinical Hospital, Bydgoszcz, Poland*
[c] *Department of Tumor Pathology and Pathomorphology, Oncology Center, Prof., Franciszek Łukaszczyk Memorial Hospital, Bydgoszcz, Poland*

Received 29 June 2019; received in revised form 30 January 2020; accepted 9 February 2020

Abstract

Background: Lymph node (LN) metastases increase the risk of death from prostate cancer (CaP). The dysfunction of factors responsible for DNA injury detection may promote the evolution of localized primary tumors into the metastatic form.

Methods: In this study, 52 cases of CaP were analyzed. The cases were divided into groups of CaP without metastases (N0), with metastases to the LNs (N+), and metastatic LN tissue. Immunohistochemical examinations were performed with antibodies against MDC1, TP53BP1, MLH1, MSH2, MSH6, and PMS2.

Results: Statistical analysis showed lower nuclear expression of TP53BP1 in N+ cases than in N0 cases ($P = 0.026$). Nuclear TP53BP1 expression was lower in LN cases than in N+ cases ($P = 0.019$). Statistical analysis showed lower nuclear expression of MLH1 in N+ cases than in to N0 cases ($P = 0.003$).

Conclusion: Decreased expression of both MLH1 and TP53B1 were demonstrated in N+ cases of CaP. This observation could help to determine the risk of nodal metastasis, and to select appropriate treatment modalities for patients with locally advanced CaP.

Keywords: TP53BP1; MDC1; MLH1; MSH2; MSH6; PMS2; Prostate cancer

1. Background

Deaths from prostate cancer (CaP) amount to about 10% of cancer related mortality in men, the third highest rate in this group [1]. Lymph node (LN) metastases increase the risk of death from this disease [2]. The risk of cancer-related death increases with the number of affected LNs [3]. The acquisition of phenotypic features, through the accumulation of undetected or unrepaired DNA lesions, results in the development of metastatic capability in CaP [4,5].

There are two major types of error in DNA. Damage results in physical abnormalities in the DNA (single or double-strand breaks) and changes in the base sequence of the DNA (i.e. mutation). Each DNA lesion triggers complex and sophisticated cellular signaling networks, collectively termed DNA damage response (DDR). The system relies on various mechanisms which facilitate the assembly of repair and checkpoint factors at the sites of DNA damage (Fig. 1). One of the most important DDR-related proteins appears to be MDC1 (mediator of DNA damage checkpoint protein 1; also known as nuclear factor with BRCT domains protein 1, NFBD1). This protein acts as a regulator of the

Funding: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

[1] These authors have contributed equally.

*Corresponding author. Tel.: +48 52 5854200; fax: +48 52 5854049.
E-mail address: arkadiuszgzilresearch@gmail.com (A. Gzil).

https://doi.org/10.1016/j.urolonc.2020.02.012
1078-1439/

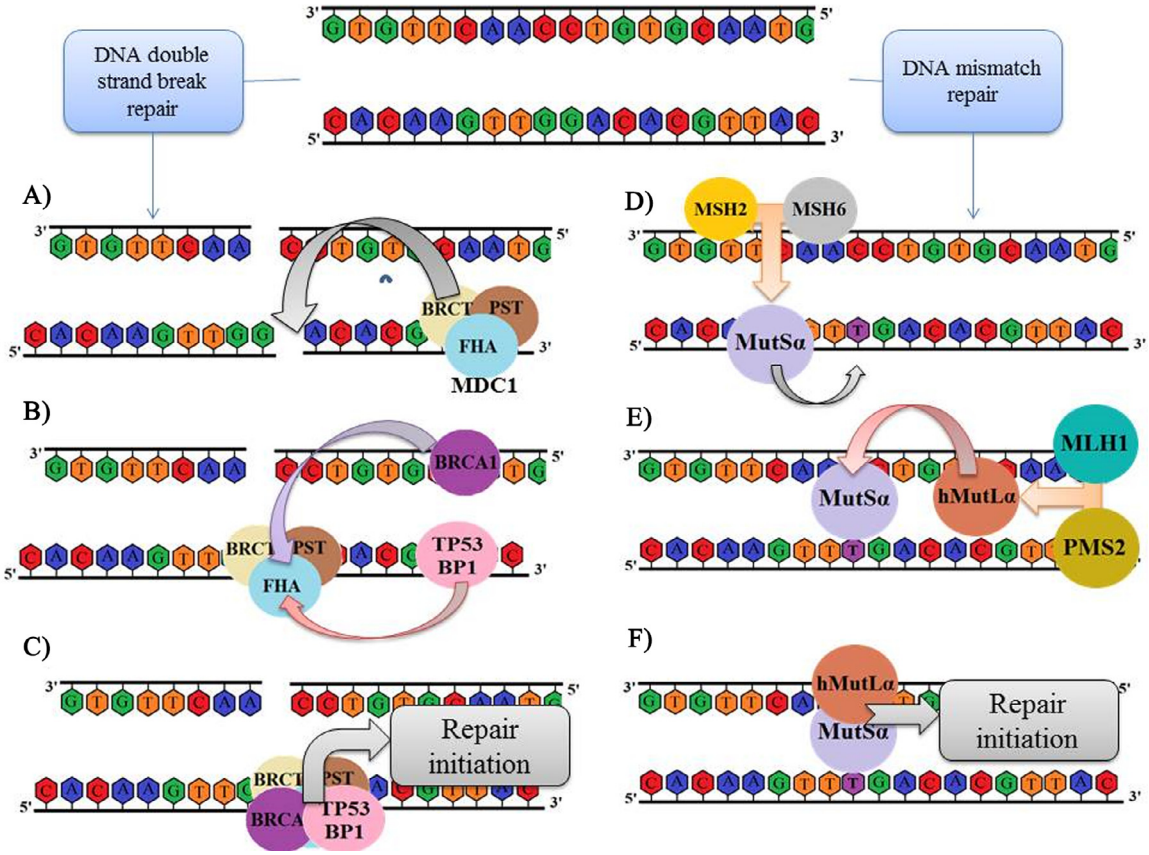


Fig. 1. Mechanisms associated with the detection of DNA alternations [6–8, 46]. (A) MDC1 is activated in response to cellular double stranded DNA breakage. MDC1 contains three structural domains, an NH2-terminal forkhead-associated (FHA) domain, a tandem COOH-terminal BRCT domain, and an internal proline/serine/threonine (PST) domain. In the first step of damage detection, the BRCT domain docks MDC1 to the site of the DNA lesion. (B) The FHA domain permits the attachment of other DSB repair factors involved in this process, such as BRCA1 and TP53BP1. (C) After attachment to the damaged region, TP53BP1 activates downstream effector molecules, leading to the initiation of the repair process. (D) In the initial step of DNA mismatch repair, Msh2 and Msh6 create a heteromodimer which is responsible for detecting mistakes in DNA. (E) After MutSα connects with DNA, other factors responsible for repair initiation are formed. MLH1 and PMS2 compose the hMutLα complex, which links to MutSα. (F) The formed complex enables the initiation of repairs to the mismatch defect.

microenvironment at sites of double-stranded DNA breakage (DSB) [6]. MDC1 recruits and maintains a myriad of DDR proteins, such as BRCA1 (breast cancer type 1 susceptibility protein.), TP53BP1 (Tumor Protein P53 Binding Protein 1) as well as the MRE11-RAD50-NBS1 (MRN) complex at chromatin flanking DSBs, where they provide a molecular platform that efficiently amplifies the DNA damage signal and activates multiple downstream effector molecules involved in cell cycle checkpoints and DNA repair [6].

A variety of cellular DNA repair pathways form the critical first lines of defense against DNA injuries. The DNA mismatch repair (MMR) pathway recognizes and corrects non–Watson-Crick base pairs and insertion/deletion loops which arise as a result of misincorporation errors during DNA replication. The MMR system is also important for the removal of mispaired bases and branched DNA formed during the recombination process, as well as such mispairings that appear due to chemical modifications of DNA, for example by chemotherapeutic agents [7]. The MutSα complex detects mistakes in DNA and recruits factors responsible for repairs at the initial step of this process. The MutSα complex is a heterodimer consisting of Msh2 and Msh6 proteins [7]. After formation, MutSα recruits the hMutLα complex which consists of MLH1 and PMS2 proteins [8]. Formation of the complex enables initiation of repairs to mismatch defects [8].

Recent studies have revealed that decreased expression of the proteins responsible for DNA-repair could be involved in many types of cancer. The loss of MDC1 function has been reported in studies of breast and pancreatic carcinomas, and associated with invasion and metastasis [9,10]. The loss of TP53PB1 function has been shown to be related to similar consequences in cases of colorectal, lung, and breast cancer [11–16]. Germline mutations in DNA MMR genes such as *MSH2, MLH1*, *PMS2*, and *MSH6* have been shown to be responsible for the development of Lynch syndrome [7,17]. This syndrome predisposes to various types of cancer, especially colorectal cancer [8]. Disorders of the MMR genes have also been found to be involved in the progression of breast, endometrial, ovarian, urinary, and biliary tract cancers [18–24]. The results of many studies

have suggested that the promoters of MMR genes are hypermethylated in cases unrelated to Lynch syndrome, which may lead to a deficiency of MMR proteins in those cases [25,26].

The dysfunction of factors responsible for DNA repair results in the accumulation of mutations in the genetic material. Increasing numbers of mutations may lead to the development of more aggressive clones of cells. In the context of carcinogenesis, this may result in the evolution of a localized primary tumor into a disseminated metastatic form. According to the TNM classification, CaP which extend through the capsule, but without showing evidence of remote metastasis, are known as locally advanced cancer [27]. Cases of CaP with these features are at a crucial step in the development from localized to metastatic disease, and the development of regional LN metastases signal an introduction to the spread of the disease. In such cases the recommended treatment methods are necessarily radical, including radical radiotherapy in combination with neoadjuvant and adjuvant androgen deprivation therapy or equipotential radical prostatectomy with pelvic lymphadenectomy [28]. It is possible that a population of patients with locally advanced CaP could profit from surgery instead of radiotherapy. In this study, we aimed to explore this possibility, and to investigate the differences in the expression of DSB and MMR proteins among metastatic and nonmetastatic forms of locally advanced CaP.

2. Materials and methods

2.1. Material

All patients with locally advanced CaP defined as a pT3a or greater T-stage according to American Joint Committee on Cancer (AJCC) and absence of distal metastasis, who underwent radical prostatectomy for CaP between January 1, 2015 and December 31, 2017 in single urologic department were selected to further analysis. The total number of 263 patients was evaluated for inclusion. The patients were aged between 52 and 78 years. The inclusion criteria for material to be used in this study were: a clear-cut diagnosis of CaP fitting the Gleason classification criteria and the presence of enough material for further work. The prostate specimens, together with the LNs, were fixed in 10% buffered formalin and processed according to a standard protocol to produce paraffin blocks. Subsequently, two independent pathologists verified the specimens. The examined materials were taken from cases of CaP, with or without nodal metastasis, and were subdivided into three groups. A group of 26 cases with nodal involvement were identified and were classified as extending through the prostate capsule (T3 according to 7th edition of the AJCC/UICC Cancer Staging Handbook [29]). These tumors comprised group N+ and the LNs from the same cases comprised group LN. Another 26 cases of CaP without evidence for metastasis were randomly selected (group N0), giving 52 cases all. There was no power calculation or reason for choosing 26 patients beyond the fact that only 26 N+ patients have been found and we decided to have 26 N0 patients chosen as controls. These latter cases were classified as stage III according to the AJCC TNM system for CaP and comprised 6 cases in stage IIIa and 20 cases in stage IIIb [29]. The average age of patients in this group was 65 years. Clinical characteristic of both investigated groups is summarized in Table 1.

2.2. Methods

The expression of MDC1, TP53BP1, MLH1, MSH2, MSH6, and PMS2 proteins was detected by immunohistochemical (IHC) staining of formalin-fixed, paraffin-embedded (FFPE) prostate tissue. For IHC, unstained 4-μm sections were cut using a manual rotary microtome (AccuCutSMRTM200, Sakura, Japan). IHC staining was performed according to standard procedures, and a series of positive and negative control reactions. 4-μm tissue sections were pretreated with high-pH Epitope Retrieval Solution, using the PT-link system (Dako; Agilent Technologies, Inc., Santa Clara, CA). Tissues were incubated in 3% hydrogen peroxide for 10 minutes to block endogenous peroxidase activity and then in a 5% solution of BSA for 15 minutes to prevent nonspecific binding. Subsequently tissue sections were incubated for 30 minutes at room temperature with specific primary antibodies: rabbit polyclonal anti-MDC1 (1:200, Sigma-Aldrich; HPA006915), rabbit polyclonal

Table 1
Basic clinical information about patients included in both the N0 and N1+ groups.

	N0	N+
Number of cases		
	26	26
Age		
< 55	0%	12.5%
55–65	58%	62.5%
> 65	42%	25%
PSA value		
< 4 ng/ml	4%	0%
4–10 ng/ml	56%	8%
> 10 ng/ml	40%	92%
Primary tumor according to AJCC TNM Staging		
pT3a	37.5%	21%
pT3b	59.5%	72%
pT4	3%	7%
Gleason score		
3 + 3	6%	0%
3 + 4	39%	7%
3 + 5	10%	0%
4 + 3	10%	28%
4 + 4	16%	38%
4 + 5	13%	7%
5 + 3	0%	0%
5 + 4	6%	20%
5 + 5	0%	0%

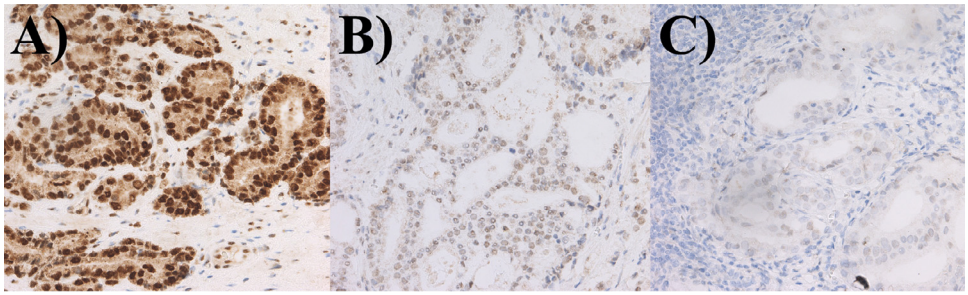


Fig. 2. Expression of TP53B1 in CaP cells (A) A representative micrograph of N0 CaP. TP53BP1 immunostaining. Primary objective magnification 20×. (B) A representative micrograph of N+ CaP. TP53BP1 immunostaining. Primary objective magnification 20 ×. (C) A representative micrograph of a CaP metastasis in a lymph node (LN). TP53BP1 immunostaining. Primary objective magnification 20× .

anti-53BP1 (1:300, Novus Biologicals; NB100-304), rabbit monoclonal anti-MLH1 (1:100, Abcam; ab92312) and mouse monoclonal anti-MSH2 (1:200, BD Pharmingen; G219-1129), mouse monoclonal anti-MSH6 (1:100, BD Biosciences; 610918), mouse monoclonal anti-PMS2 (1:100, BD Pharmingen; 556415). For negative controls, primary antibodies were replaced with phosphate-buffered saline (PBS). The antigen-antibody complex was detected using the EnVision FLEX-HRP system (Dako; Agilent Technologies, Inc., Santa Clara, CA) for 20 minutes at room temperature. After incubation, the substrate for peroxidase—3,3′-diaminobenizidine was applied and the sections were incubated for a further 10 minutes. Finally, the sections were counterstained with hematoxylin, dehydrated through graded alcohols (80%, 90%, 96%, and 99.8%), cleared in xylene, and mounted using mounting medium (Dako, Agilent Technologies, Inc. Santa Clara, CA).

2.3. Evaluation of IHC reactions

All sections were examined microscopically and scored by two independent pathologists who were blinded to the patients' clinical data. The analyses were performed using a Nikon Eclipse E800 microscope (Nikon Instruments Europe, Amsterdam, The Netherlands) at a final magnification of x400 and analyzed with the NIS-Elements 3.30 software (Nikon).

An immunoreactive score was used to evaluate the level of protein expression. The method chosen was a modified Remmele-Stegner scale, based on the intensity of expression and the number of cells/tissue areas showing positive reactions, as described elsewhere [30].

2.4. Statistical analysis

All statistical analyses were performed using Statistica version 13 (StatSoft) and Microsoft Excel 2007. Comparative statistical studies were carried out using the nonparametric *U Mann-Whitney* test. Findings were considered statistically significant where $P < 0.05$. The expression values of the analyzed proteins were presented as median and mean values.

3. Results

3.1. Expression of TP53B1 in CaP cells

Nuclear expression of TP53BP1 was demonstrated in 100% of N0, 88% of N+, and in 88% of LN cases . Statistical analysis showed significantly lower nuclear expression of TP53BP1 in the N+ group in comparison to the N0 group ($P = 0.026$, Figs. 2 and 3). Moreover, nuclear TP53BP1 expression was also significantly decreased in the LN group, relative to the N+ group ($P = 0.019$, Figs. 2 and3).

3.2. Expression of MDC1 in CaP

Nuclear expression of MDC1 was found in 69% of N0, 54% of N+, and in 69% of LN cases. Statistical analysis demonstrated no significant differences between MDC1 levels in groups N0 and N+. The nuclear MDC1 expression was similar in both examined groups. Likewise, no significant differences in MDC1 levels were found between N+ and LN groups (Tables 2 and 3).

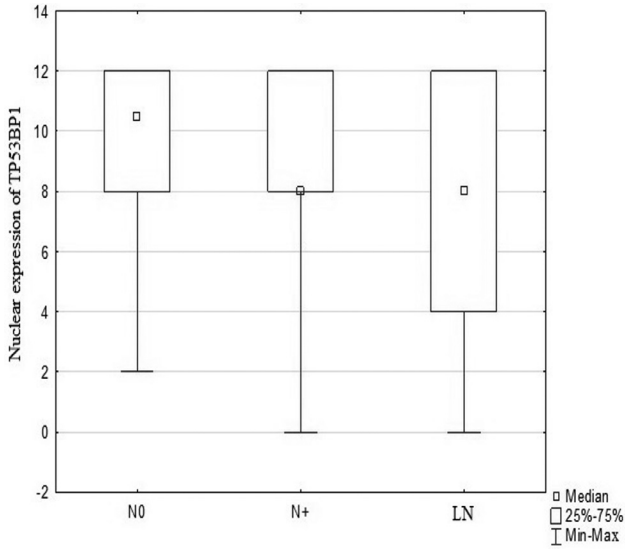


Fig. 3. Median nuclear expression of TP53BP1 for the studied group. N0—CaP without nodal metastases, N+—Prostate cancer with nodal metastases, LN—lymph node metastasis.

Table 2
Summarized 25% percentiles, medians, 75% percentiles and statistical significance of our results of metastatic and nonmetastatic groups.

	N0			N+			P <0.05
	25% Percentile	Median	75% Percentile	25% Percentile	Median	75% Percentile	
TP53BP1	8.00	10.50	12.00	8.00	8.00	12.00	0.026
MDC1	0.00	3.500	8.00	0.00	2.00	6.00	ns
MSH2	4.00	8.000	12.00	6.00	8.00	12.00	ns
MSH6	0.00	0.00	0.00	0.00	0.00	0.00	ns
PMS2	3.00	4.00	8.00	3.00	6.00	8.00	ns
MLH1	5.50	12.00	12.00	3.00	8.00	12.00	0.003

N0—CaP without nodal metastases, N+—CaP with nodal metastases.

3.3. Expression of MSH2 in CaP cells

Nuclear expression of MSH2 was detected in 92% of N0, 100% of N+, and in 92% of LN cases. Statistical analysis demonstrated no differences in MSH2 levels between the N0 and N+ groups. The nuclear MSH2 staining was similar in both investigated groups. Furthermore, no significant changes to the levels of nuclear expression of MSH2 were detected in the LN group (Tables 2 and 3).

3.4. Expression of MSH6 in CaP

Nuclear expression of MSH6 was found in 8% of N0, 4% of N+, and in 15% of LN cases. Statistical analysis showed no significant differences in the nuclear expression of MSH6 which was unaltered between N0 and N+ groups and between groups N+ and LN (Tables 2 and 3, Fig. 4).

3.5. Expression of PMS2 in CaP

Nuclear expression of PMS2 was found in 72% of N0, 75% of N+, and in 80% of LN cases. Statistical analysis showed no significant differences between MDC1 levels in groups N0 and N+. Nuclear PMS2 expression was similar in both examined groups. Similarly, PMS2 expression exhibited no significant differences between groups N+ and LN (Tables 2 and 3).

3.6. Expression of MLH1 in CaP

Nuclear expression of MLH1 was found in 92% of N0, 73% of N+, and in 73% of LN cases. Statistical analysis showed significantly decreased nuclear expression ($P = 0.004$, Fig. 3) of MLH1 in the N+ group in comparison to the N0 group. However, no similar significant reduction of nuclear MLH1 staining was observed in the LN group (Tables 2 and 3, Fig. 4).

4. Discussion

Normal cells employ specific molecular pathways to detect DSB, which protects these cells against DNA lesions and against the initiation of carcinogenesis [31,32]. Jäämaa et al. observed the accumulation of TP53BP1 and MDC1 at sites of DNA damage, induced by cytotoxic drugs and ionizing radiation, in nonmalignant human prostate tissue [33]. Yan et al. showed that the expression of TP53B1 is associated with an antiproliferative effect in CaP cells [34]. Other authors have shown that TP53BP1suppresses S-phase entry through a downstream ATM-CHK2-p53 pathway, and through the inhibition of the transcriptional factor E2F1 [35,36]. These observations suggest that DSB repair pathways may protect prostate cells from malignant transformation through the detection of alterations in DNA chains, thus inducing, depending on the severity, repair, or apoptosis.

Table 3
Summarized 25% percentiles, medians, 75% percentiles and statistical significance of our results of primary tumor and nodal metastasis groups.

	N+			LN			P <0.05
	25% Percentile	Median	75% Percentile	25% Percentile	Median	75% Percentile	
TP53BP1	8.00	8.00	12.00	4.00	8.00	12.00	0.019
MDC1	0.00	2.00	6.00	0.00	4.00	8.00	ns
MSH2	6.00	8.00	12.00	6.00	8.00	12.00	ns
MSH6	0.00	0.00	0.00	0.00	0.00	1.00	ns
PMS2	3.00	6.00	8.00	4.00	4.00	8.00	ns
MLH1	3.00	8.00	12.00	4.00	8.00	12.00	ns

N+—Primary CaP with nodal metastasis, LN—Lymph node metastasis.

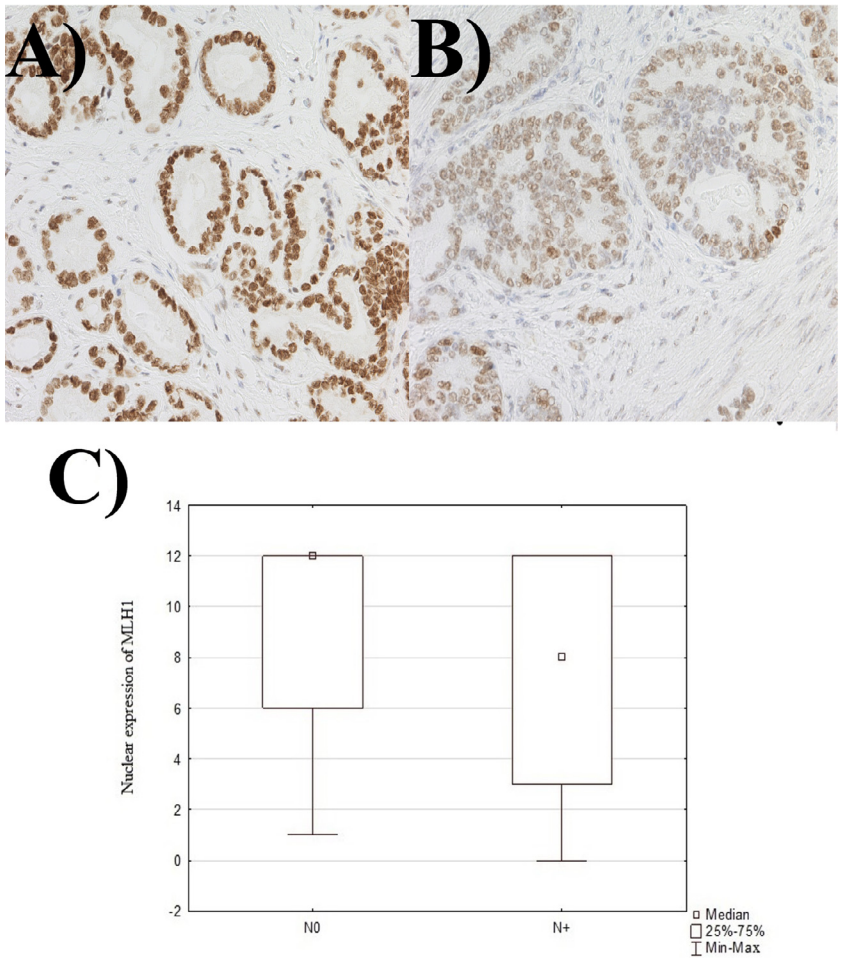


Fig. 4. Expression of MLH1 in CaP cells (A) A representative micrograph of N0 CaP. MLH1 immunostaining. Primary objective magnification 20×. (B) A representative micrograph of N+ CaP. MLH1 immunostaining. Primary objective magnification 20 ×. (C) Median nuclear expression of MLH1 for N0 and N+ group. N0—CaP without nodal metastases, N+—CaP with nodal metastases.

Work by Kurfurstov et al. revealed that TP53BP1 expression deceases during the progression of benign prostatic lesions to cancer [37]. The activation of DSB repair processes inhibits cell division in response to replication stress that is related to overstimulation of the cells [38]. However, the subpopulation of aberrant cells does not undergo permanent cell cycle arrest, which may be due to genetic alternations of *TP53BP1* in CaP, such as missense, synonymous mutations, or single nucleotide polymorphism [38,39].

Errors in DNA DSB repair lead to allelic imbalance in CaP cells, resulting in breakages at common chromosomal fragile sites. The accumulation of chromosomal aberrations leads to the progression of disease and cells with DSB repair dysfunctions become the dominant population [38]. The activation of the TP53BP1 pathway in CaP occurs later and to a lesser extent than is seen in other solid tumors, as there is lower replication stress than in other major malignancies [37]. In our study, we showed that nuclear TP53B1 expression was decreased not only during the acquisition of metastatic capability, but also in the event of metastases to the LNs. Our results suggest that the development of metastatic capability may be related to genomic instability, associated with the loss of TP53BP1 function. Disorders of TP53BP1 may be one of the "driver mutations" in CaP, associated with disease progression.

Our results might also suggest clinically important consequences for the decreased expression of TP53BP1. Studies have revealed that TP53BP1 is one of the most significant markers of radiotherapy response [40–43]. Dysfunction of this protein may prevent the inhibition of cell proliferation in response to injury caused by ionizing radiation [36,44]. Our results suggest that decreased or absent TP53BP1 expression in CaP tissue is related to the formation of nodal metastases. It may be reasonable to determine TP53BP1 levels using IHC examinations of materials obtained through biopsy and, on that basis, to predict the likely biological behavior of each cancer. Owing to insensitivity to radiotherapy, and to metastatic potential in cases of cancer with low TP53BP1 expression, the correct treatment for these patients might be radical prostatectomy with pelvic lymphadenectomy.

MDC1 is another essential component of DBS response [45]. Recent studies have revealed increased expression of

MDC1 in cells of several cancer types in comparison to normal cells [46,47].This may be caused by the fact that MDC1 initiates the primary response mechanism by participating in the DSB detection pathway. In turn, Wang et al. reported that the expression of MDC1 is decreased in CaP. They also showed that MDC1 is an androgen receptor coactivator involved in CaP cell growth and migration [48]. Our results, however, showed the lack of a significant correlation between the levels of MDC1 protein expression in the N0, N+, and LN groups.

The other DNA repair proteins investigated in this study were those implicated in the MMR machinery. The MMR system promotes repair by the excision and resynthesis of incorrectly docked bases during and after DNA replication [49–51]. Because the MMR mechanism ensures protection against DNA alterations in prostate cells, abnormalities of the MMR mechanism may lead to carcinogenesis and disease progression. A recent study showed that loss-of-function mutations of the MutSα complex components MSH2 and MSH6 are more common in CaP than those of other MMR proteins such as MLH1 and PMS2 [52–54].

It has been suggested that chronic exposure to arsenic and estrogen decreases the expression of MSH6 and MLH1 [55]. Abnormalities within the MLH1 gene may underlie carcinogenesis and be the cause of increasingly aggressive prostate tumors [49,56]. CaP cells exhibit down-regulation of MLH1 expression in comparison to normal prostate and benign hyperplasia [57–59]. Consequently, our findings suggest a role for MLH1 alternations in disease progression. We observed a negative correlation between nuclear expression of MLH1 and the metastatic capability of CaP. A study performed on cell lines by Fukuhara et al. demonstrated the significant impact of MLH1 gene re-expression among CaP cells. After re-expression, *MLH1*-deficient CaP cells exhibit decreased potential for invasion, inhibition of cell migration and a reduction in proliferation. Moreover, among MLH1-transfected cells, apoptosis was increased and tumor growth was suppressed [51]. These observations point to the importance of the MLH1 protein in CaP development and metastasis. Reduced expression of the MLH1 protein in primary tumors suggests an increased risk of LN involvement and assessment of its status in biopsy material makes it possible to determine this probability, as is the case with TP53BP1. On the other hand, Wilczek et al. observed significantly higher MLH1 in cancers with nodal metastasis [60]. This finding underlines the high heterogeneity of CaP and the dual nature of MMR alternations.

In contrast to MLH1, our analysis indicated no correlation between PMS2 and the occurrence of nodal metastases. PMS2 is increasingly expressed, independently of its heterodimeric partner MLH1 in both PIN lesions and CaP tissue, in comparison to normal prostatic tissue [61]. Moreover, a study by Norris et al. underlined that prostate tumors with elevated levels of PMS2 were genetically unstable, but that this could be corrected by MLH1 coelevation [61]. Our results suggest that it is possible for PMS2 to promote CaP development from normal tissue, but metastatic capability is acquired by CaP cells shortly after the appearance of MLH1 disorders. However, Fukuhara et al. showed that PMS2 decreases cell proliferation, migration, invasion, and growth while increasing apoptosis through the up-regulation of apoptosis-related TMS1 and down-regulation of the antiapoptotic BCL2A1 [62].

The role of the components of the MutSα complex, MSH2 and MSH6, in CaP is not clear. The rate of MMR defects was higher in metastatic tumors than in primary tumors [52,53,58,63,64]. Studies have shown that rearrangements in the DNA MMR genes MSH2 and MSH6 are a major mechanism underlying hypermutation in advanced CaP [65,66]. Primary CaP with MSH2 loss were more aggressive and associated with a poor prognosis. Furthermore, CaP with MSH2 loss showed concordant MSH6 loss, mainly due to its epigenetic regulation, because the stability of these proteins is only ensured as heterodimers [60,67,68]. Guedes et al. confirmed the loss of MSH2 protein in only 1% of examined primary prostatic adenocarcinomas. They also confirmed that many prostate tumors showing MSH2 loss also showed an increased density of CD8+ lymphocytes. On the other hand, Nighiem et al. observed the absence of this protein in 15% of the cases of CaP they investigated, whilst detecting MSH6 in 78% of their tissue specimens [69]. Other authors have observed MSH6 expression in between 42.1% and 89.5% of CaP cells, and a correlation with presence of nodal metastases [60,70]. While the number of MSH2 positive cells in our study was similar to that found in earlier studies, MSH6 was present in an extremely small group of CaP cells. This may be the result of technical issues during validation, preparation, or assessment of the tissue. Nevertheless, the statistical analysis of our results showed no significant correlations between the components of the MutSα complex and nodal status.

In summary, our study allows for the conclusion that alterations in both MMR and DSB repair mechanisms are involved in the lymphatic spread of locally advanced CaP. Decreased expression of MLH1 and TP53B1 was detected in CaP with nodal metastasis. These results suggest that metastatic ability is associated with altered response to DNA injury. Furthermore, a reduction of TP53BP1 expression was observed in metastatic LN tissue in comparison to primary tumor tissue. It may be assumed that cell subpopulations with reduced TP53BP1 function become dominant during disease progression and are responsible for the development of metastasis. In addition, TP53BP1 dysfunction is known to be associated with a decreased response to injury caused by ionizing radiation and therefore with a reduced response to this treatment modality. It may be possible, through the assessment of MLH1 and TP53B1 status by the immunostaining of biopsy material, to determine the risk of nodal metastasis, and the response to radiotherapy, in cases of locally advanced CaP. We hypothesize that an assessment of the expression of these proteins may be of

assistance in the selection of optimal treatment procedures in cases of locally advanced CaP. In light of the above, follow-up studies to further clarify these findings would be justified and reasonable.

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

For this type of study formal consent is not required.

Informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Malvezzi M, Carioli G, Bertuccio P, Boffetta P, Levi F, La Vecchia C, et al. European cancer mortality predictions for the year 2017, with focus on lung cancer. Ann Oncol 2017;28:1117–23. https://doi.org/10.1093/annonc/mdx033.

[2] Cheng L, Zincke H, Blute ML, Bergstralh EJ, Scherer B, et al. Risk of prostate carcinoma death in patients with lymph node metastasis. Cancer 2001;91:66–73.

[3] Preisser F, Marchioni M, Nazzani S, Bandini M, Tian Z, Pompe RS, et al. The impact of lymph node metastases burden at radical prostatectomy. Eur Urol Focus 2018. https://doi.org/10.1016/j.euf.2017.12.009.

[4] Chang AJ, Autio KA, Roach M, Scher HI, Scher HI. High-risk prostate cancer-classification and therapy. Nat Rev Clin Oncol 2014;11:308–23. https://doi.org/10.1038/nrclinonc.2014.68.

[5] Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18:11–22. https://doi.org/10.1016/j.ccr.2010.05.026.

[6] Oberle C, Blattner C. Regulation of the DNA damage response to DSBs by post-translational modifications. Curr Genomics 2010;1:184–98.

[7] Reyes GX, Schmidt TT, Kolodner RD, Hombauer H, Diego S, Jolla L. New insights into the mechanism of DNA mismatch repair. Chromosoma 2015;124:443–62:10.1007/s00412-015-0514-0.New.

[8] Poulogiannis G, Frayling IM, Arends MJ. DNA mismatch repair deficiency in sporadic colorectal cancer and Lynch syndrome. Histopathology 2010;56:167–79. https://doi.org/10.1111/j.1365-2559.2009.03392.x.

[9] Zhou H, Qin Y, Ji S, Ling J, Fu J, Zhuang Z, et al. SOX9 activity is induced by oncogenic Kras to affect MDC1 and MCMs expression in pancreatic cancer. Oncogene 2018;37:912–23. https://doi.org/10.1038/onc.2017.393.

[10] Zou R, Zhong X, Wang C, Sun H, Wang S, Lin L, et al. MDC1 enhances estrogen receptor-mediated transactivation and contributes to breast cancer Suppression. Int J Biol Sci 2015;11:992–1005. https://doi.org/10.7150/ijbs.10918.

[11] Lai T-C, Chow K-C, Lin T-Y, Chiang I-P, Fang H-Y, Chen C-Y, et al. Expression of 53BP1 as a cisplatin-resistant marker in patients with lung adenocarcinomas. Oncol Rep 2010;24:321–8.

[12] Neboori HJR, Haffty BG, Wu H, Yang Q, Aly A, Goyal S, et al. Low p53 binding protein 1 (53BP1) expression is associated with increased local recurrence in breast cancer patients treated with breast-conserving surgery and radiotherapy. Int J Radiat Oncol 2012;83:e677–83. https://doi.org/10.1016/j.ijrobp.2012.01.089.

[13] Bouwman P, Aly A, Escandell JM, Pieterse M, Bartkova J, van der Gulden H, et al. 53BP1 loss rescues BRCA1 deficiency and is associated with triple-negative and BRCA-mutated breast cancers. Nat Struct Mol Biol 2010;17:688–95. https://doi.org/10.1038/nsmb.1831.

[14] Yao J, Huang A, Zheng X, Liu T, Lin Z, Zhang S, et al. 53BP1 loss induces chemoresistance of colorectal cancer cells to 5-fluorouracil by inhibiting the ATM−CHK2−P53 pathway. J Cancer Res Clin Oncol 2017;143:419–31. https://doi.org/10.1007/s00432-016-2302-5.

[15] Yeon SY, Jo YS, Choi EJ, Kim MS, Yoo NJ, Lee SH. Frameshift mutations in repeat sequences of ANK3, HACD4, TCP10L, TP53BP1, MFN1, LCMT2, RNMT, TRMT6, METTL8 and METTL16 genes in colon cancers. Pathol Oncol Res 2017. https://doi.org/10.1007/s12253-017-0287-2.

[16] De Gregoriis G, Ramos JA, Fernandes PV, Vignal GM, Brianese RC, Carraro DM, et al. DNA repair genes *PAXIP1* and *TP53BP1* expression is associated with breast cancer prognosis. Cancer Biol Ther 2017;18:439–49. https://doi.org/10.1080/15384047.2017.1323590.

[17] Vasen HFA. Review article: the Lynch syndrome (hereditary nonpolyposis colorectal cancer)*. Aliment Pharmacol Ther 2007;26:113–26. https://doi.org/10.1111/j.1365-2036.2007.03479.x.

[18] Jaworski D, Szylberg Ł, Gzil A, Stawinski P, Kasperska A, Marszałek A. Diagnostic difficulties in cases of papillary urothelial neoplasm of low malignant potential, urothelial proliferation of uncertain malignant potential, urothelial dysplasia and urothelial papilloma: a review of current literature. Ann Diagn Pathol 2017. https://doi.org/10.1016/j.anndiagpath.2017.12.007.

[19] Goldberg M, Bell K, Aronson M, Semotiuk K, Pond G, Gallinger S, et al. Association between the Lynch syndrome gene MSH2 and breast cancer susceptibility in a Canadian familial cancer registry. J Med Genet 2017;54:742–6. https://doi.org/10.1136/jmedgenet-2017-104542.

[20] Tangjitgamol S, Kittisiam T, Tanvanich S. Prevalence and prognostic role of mismatch repair gene defect in endometrial cancer patients. Tumor Biol 2017;39:101042831772583. https://doi.org/10.1177/1010428317725834.

[21] Zhao C, Li S, Zhao M, Zhu H, Zhu X. Prognostic values of DNA mismatch repair genes in ovarian cancer patients treated with platinum-based chemotherapy. Arch Gynecol Obstet 2018;297:153–9. https://doi.org/10.1007/s00404-017-4563-x.

[22] Urakami S, Inoshita N, Oka S, Miyama Y, Nomura S, Arai M, et al. Clinicopathological characteristics of patients with upper urinary tract urothelial cancer with loss of immunohistochemical expression of the DNA mismatch repair proteins in universal screening. Int J Urol 2018;25:151–6. https://doi.org/10.1111/iju.13481.

[23] Cloyd JM, Chun YS, Ikoma N, Vauthey JN, Aloia TA, Cuddy A, et al. Clinical and genetic implications of DNA mismatch repair deficiency in biliary tract cancers associated with Lynch syndrome. J Gastrointest Cancer 2018;49:93–6. https://doi.org/10.1007/s12029-017-0040-9.

[24] Sanguedolce F, Cormio A, Massenio P, Pedicillo MC, Cagiano S, Fortunato F, et al. Altered expression of HER-2 and the mismatch repair genes MLH1 and MSH2 predicts the outcome of T1 high-grade bladder cancer. J Cancer Res Clin Oncol 2018;144:637–44. https://doi.org/10.1007/s00432-018-2593-9.

[25] Rodríguez-Hernández I, Garcia JL, Santos-Briz A, Hernández-Laín A, González-Valero JM, Gómez-Moreta JA, et al. Integrated analysis of mismatch repair system in malignant astrocytomas. PLoS One 2013;8:1–10. https://doi.org/10.1371/journal.pone.0076401.

[26] Su Y, Yin L, Liu R, Sheng J, Yang M, Wang Y, et al. Promoter methylation status of MGMT, hMSH2, and hMLH1 and its relationship to corresponding protein expression and TP53 mutations in human esophageal squamous cell carcinoma. Med Oncol 2014;31:784. https://doi.org/10.1007/s12032-013-0784-4.

[27] Schröder FH, Hermanek P, Denis L, Fair WR, Gospodarowicz MK, Pavone-Macaluso M. The TNM classification of prostate cancer. Prostate 1992;21:129–38. https://doi.org/10.1002/pros.2990210521.
[28] Smyth EC, Verheij M, Allum W, Cunningham D, Cervantes A, Arnold D, et al. Gastric cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol 2016;27:v38–49. https://doi.org/10.1093/annonc/mdw350.
[29] Edge SB, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. Ann Surg Oncol 2010;17:1471–4. https://doi.org/10.1245/s10434-010-0985-4.
[30] Bodnar M, Szylberg L, Kazmierczak W, Marszalek A. Differentiated expression of membrane type metalloproteinases (MMP-14, MMP-15) and pro-MMP2 in laryngeal squamous cell carcinoma. A novel mechanism. J Oral Pathol Med 2013;42:267–74. https://doi.org/10.1111/jop.12000.
[31] Shibata A. Regulation of repair pathway choice at two-ended DNA double-strand breaks. Mutat Res - Fundam Mol Mech Mutagen 2017:51–5. https://doi.org/10.1016/j.mrfmmm.2017.07.011:803–805.
[32] Lukas J, Lukas C, Bartek J. More than just a focus: the chromatin response to DNA damage and its role in genome integrity maintenance. Nat Cell Biol 2011;13:1161–9. https://doi.org/10.1038/ncb2344.
[33] Jäämaa S, Af Hällström TM, Sankila A, Rantanen V, Koistinen H, Stenman UH, et al. DNA damage recognition via activated ATM and p53 pathway in nonproliferating human prostate tissue. Cancer Res 2010;70:8630–41. https://doi.org/10.1158/0008-5472.CAN-10-0937.
[34] Yan D, Wen Y, Spohn B, Choubey D, Gutterman JU. Reduced growth rate and transformation phenotype of the prostate cancer cells by an interferon-inducible protein, p202. Oncogene 1999;18:807–11.
[35] Choubey D, Li SJ, Datta B, Gutterman JU, Lengyel P. Inhibition of E2F-mediated transcription by p202. EMBO J 1996;15:5668–78.
[36] Squatrito M, Brennan CW, Helmy K, Huse JT, Petrini JH, Holland EC. Loss of ATM/Chk2/p53 pathway components accelerates tumor development and contributes to radiation resistance in gliomas. Cancer Cell 2010;18:619–29. https://doi.org/10.1016/j.ccr.2010.10.034.
[37] Kurfurstova D, Bartkova J, Vrtel R, Mickova A, Burdova A, Majera D, et al. DNA damage signalling barrier, oxidative stress and treatment-relevant DNA repair factor alterations during progression of human prostate cancer. Mol Oncol 2016;10:879–94. https://doi.org/10.1016/j.molonc.2016.02.005.
[38] Gorgoulis VG, Vassiliou L-VF, Karakaidos P, Zacharatos P, Kotsinas A, Liloglou T, et al. Activation of the DNA damage checkpoint and genomic instability in human precancerous lesions. Nature 2005;434:907–13. https://doi.org/10.1038/nature03485.
[39] Du R, Zheng L, Huang W, Zhang H, Jiang Z. Correlation of 53BP1 gene mutation with prostatic adenocarcinoma. Zhonghua Bing Li Xue Za Zhi Chinese J Pathol 2011;40:449–53.
[40] Xiao Y, Zheng X, Huang A, Liu T, Zhang T, Ma H. Deficiency of 53BP1 inhibits the radiosensitivity of colorectal cancer. Int J Oncol 2016;49:1600–8. https://doi.org/10.3892/ijo.2016.3629.
[41] Gou Q, Xie Y, Liu L, Xie K, Wu Y, Wang Q, et al. Downregulation of MDC1 and 53BP1 by short hairpin RNA enhances radiosensitivity in laryngeal carcinoma cells. Oncol Rep 2015;34:251–7. https://doi.org/10.3892/or.2015.3980.
[42] Squatrito M, Vanoli F, Schultz N, Jasin M, Holland EC. 53BP1 is a haploinsufficient tumor suppressor and protects cells from radiation response in glioma. Cancer Res 2012;72:5250–60. https://doi.org/10.1158/0008-5472.CAN-12-0045.
[43] Hu B, Wu L, Han W, Zhang L, Chen S, Xu A, et al. The time and spatial effects of bystander response in mammalian cells induced by low dose radiation. Carcinogenesis 2006;27:245–51. https://doi.org/10.1093/carcin/bgi224.
[44] Xiao Y, Zheng X, Huang A, Liu T, Zhang T, Ma H. Deficiency of 53BP1 inhibits the radiosensitivity of colorectal cancer. Int J Oncol 2016;49:1600–8. https://doi.org/10.3892/ijo.2016.3629.
[45] Stewart GS, Wang B, Bignell CR, Taylor AMR, Elledge SJ. MDC1 is a mediator of the mammalian DNA damage checkpoint. Nature 2003;421:961–6. https://doi.org/10.1038/nature01446.
[46] Liu X, Dong R, Jiang Z, Wei Y, Li Y, Wei L, et al. MDC1 promotes ovarian cancer metastasis by inducing epithelial-mesenchymal transition. Tumor Biol 2015;36:4261–9. https://doi.org/10.1007/s13277-015-3063-5.
[47] Yang Z, Bu Y, Wang C, Liu G, Song F. Growth inhibition, morphology change, and cell cycle alterations in NFBD1-depleted human esophageal cancer cells. Mol Cell Biochem 2010;342:1–6. https://doi.org/10.1007/s11010-010-0460-3.
[48] Wang C, Sun H, Zou R, Zhou T, Wang S, Sun S, et al. MDC1 functionally identified as an androgen receptor co-activator participates in suppression of prostate cancer. Nucleic Acids Res 2015;43:4893–908. https://doi.org/10.1093/nar/gkv394.
[49] Reyes GX, Schmidt TT, Kolodner RD, Hombauer H. New insights into the mechanism of DNA mismatch repair. Chromosoma 2015;124:443–62. https://doi.org/10.1007/s00412-015-0514-0.
[50] Fishel R. Mismatch repair. J Biol Chem 2015;290:26395–403. https://doi.org/10.1074/jbc.R115.660142.
[51] Fukuhara S, Chang I, Mitsui Y, Chiyomaru T, Yamamura S, Majid S, et al. DNA mismatch repair gene MLH1 induces apoptosis in prostate cancer cells. Oncotarget 2014;5:11297–307. https://doi.org/10.18632/oncotarget.2315.
[52] Pritchard CC, Morrissey C, Kumar A, Zhang X, Smith C, Coleman I, et al. Complex MSH2 and MSH6 mutations in hypermutated microsatellite unstable advanced prostate cancer. Nat Commun 2014;5:4988. https://doi.org/10.1038/ncomms5988.
[53] Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015;161:1215–28. https://doi.org/10.1016/j.cell.2015.05.001.
[54] Abeshouse A, Ahn J, Akbani R, Ally A, Amin S, Andry CD, et al. The molecular taxonomy of primary prostate cancer. Cell 2015;163:1011–25. https://doi.org/10.1016/j.cell.2015.10.025.
[55] Treas J, Tyagi T, Singh KP. Chronic exposure to arsenic, estrogen, and their combination causes increased growth and transformation in human prostate epithelial cells potentially by hypermethylation-mediated silencing of MLH1. Prostate 2013;73:1660–72. https://doi.org/10.1002/pros.22701.
[56] Supek F, Lehner B. Differential DNA mismatch repair underlies mutation rate variation across the human genome. Nature 2015;521:81–4. https://doi.org/10.1038/nature14173.
[57] Soni A, Bansal A, Singh LC, Mishra AK, Majumdar M, Regina T, et al. Gene expression profile and mutational analysis of DNA mismatch repair genes in carcinoma prostate in Indian population. Omi A J Integr Biol 2011;15:319–24. https://doi.org/10.1089/omi.2010.0110.
[58] Chen Y, Wang J, Fraig MM, Henderson K, Bissada NK, Watson DK, et al. Alterations in PMS2, MSH2 and MLH1 expression in human prostate cancer. Int J Oncol 2003;22:1033–43.
[59] Chen Y, Wang J, Fraig MM, Metcalf J, Turner WR, Bissada NK, et al. Defects of DNA mismatch repair in human prostate cancer. Cancer Res 2001;61:4112–21.
[60] Wilczak W, Rashed S, Hube-Magg C, Kluth M, Simon R, Büscheck F, et al. Up-regulation of mismatch repair genes MSH6, PMS2 and MLH1 parallels development of genetic instability and is linked to tumor aggressiveness and early PSA recurrence in prostate cancer. Carcinogenesis 2017;38:19–27. https://doi.org/10.1093/carcin/bgw116.
[61] Norris AM, Woodruff RD, D'Agostino RB, Clodfelter JE, Scarpinato KD. Elevated levels of the mismatch repair protein PMS2 are associated with prostate cancer. Prostate 2007;67:214–25. https://doi.org/10.1002/pros.20522.
[62] Fukuhara S, Chang I, Mitsui Y, Chiyomaru T, Yamamura S, Majid S, et al. Functional role of DNA mismatch repair gene PMS2 in prostate

cancer cells. Oncotarget 2015;6:16341–51. https://doi.org/10.18632/oncotarget.3854.

[63] Guedes L, Antonarakis ES, Schweizer MT, Mirkheshti N, Almutairi F, Park JC, et al. MSH2 loss in primary prostate cancer. Clin Cancer Res 2017. https://doi.org/10.1158/1078-0432.CCR-17-0955:clincanres.0955.2017.

[64] Dominguez-Valentin M, Joost P, Therkildsen C, Jonsson M, Rambech E, Nilbert M. Frequent mismatch-repair defects link prostate cancer to Lynch syndrome. BMC Urol 2016;16:15. https://doi.org/10.1186/s12894-016-0130-1.

[65] Schweizer MT, Cheng HH, Tretiakova MS, Vakar-Lopez F, Klemfuss N, Konnick EQ, et al. Mismatch repair deficiency may be common in ductal adenocarcinoma of the prostate. Oncotarget 2016;7:82504–10. https://doi.org/10.18632/oncotarget.12697.

[66] Pritchard CC, Morrissey C, Kumar A, Zhang X, Smith C, Coleman I, et al. Complex MSH2 and MSH6 mutations in hypermutated microsatellite unstable advanced prostate cancer. Nat Commun 2014;5. https://doi.org/10.1038/ncomms5988.

[67] Guedes LB, Antonarakis ES, Schweizer MT, Mirkheshti N, Almutairi F, Park JC, et al. MSH2 loss in primary prostate cancer. Clin Cancer Res 2017;23:6863–74. https://doi.org/10.1158/1078-0432.CCR-17-0955.

[68] Ngollo M, Lebert A, Daures M, Judes G, Rifai K, Dubois L, et al. Global analysis of H3K27me3 as an epigenetic marker in prostate cancer progression. BMC Cancer 2017;17. https://doi.org/10.1186/s12885-017-3256-y.

[69] Nghiem B, Zhang X, Lam HM, True LD, Coleman I, Higano CS, et al. Mismatch repair enzyme expression in primary and castrate resistant prostate cancer. Asian J Urol 2016;3:223–8. https://doi.org/10.1016/j.ajur.2016.09.002.

[70] Albero-González R, Hernández-Llodrà S, Juanpere N, Lorenzo M, Lloret A, Segalés L, et al. Immunohistochemical expression of mismatch repair proteins (MSH2, MSH6, MLH1, and PMS2) in prostate cancer: correlation with grade groups (WHO 2016) and ERG and PTEN status. Virchows Arch 2019. https://doi.org/10.1007/s00428-019-02591-z.

4.2 Artykuł 2

Jaworski Damian, Gzil Arkadiusz, Antosik Paulina, Zarębska Izabela, Dominiak Joanna, Neska-Długosz Izabela, Kasperska Anna, Grzanka Dariusz, Szylberg Łukasz, Expression differences between proteins responsible for DNA damage repair according to the Gleason grade as a new heterogeneity marker in prostate cancer, Arch. Med. Sci.; 2023 :Vol. 19, nr 2. s. 499-506. DOI: 10.5114/aoms.2019.89773

Expression differences between proteins responsible for DNA damage repair according to the Gleason grade as a new heterogeneity marker in prostate cancer

Damian Jaworski[1], Arkadiusz Gzil[1], Paulina Antosik[1], Izabela Zarębska[1], Joanna Dominiak[1], Izabela Neska-Długosz[1], Anna Kasperska[1], Dariusz Grzanka[1], Łukasz Szylberg[1,2]

[1]Department of Clinical Pathomorphology, *Collegium Medicum* in Bydgoszcz, Nicolaus Copernicus University in Torun, Poland
[2]Department of Pathomorphology, Military Clinical Hospital, Bydgoszcz, Poland

Submitted: 12 March 2019
Accepted: 4 August 2019

Arch Med Sci
DOI: https://doi.org/10.5114/aoms.2019.89773

Corresponding author:
Damian Jaworski
Department of Clinical Pathomorphology
CM UMK
9 Sklodowskiej-Curie Str.
85-094 Bydgoszcz, Poland
Phone: +48 52 585 42 00
Fax: +48 52 585 40 49
E-mail:
jaworskiresearch@gmail.com

Abstract

Introduction: The purpose of this research was to explore the correlation between Gleason score and pattern and the expression of the MLH1, MSH2, MDC1, TP53BP1 proteins in prostate cancer (PC). Prostate cancer development is related to errors in DNA, among others double-strand breaks (DSB) and changes in the base sequence of the DNA. These errors should be repaired through mismatch (MMR) or DSB repair proteins such as MSH2, MLH1, MDC1 and TP53BP1.

Material and methods: A total of 500 prostate cancer specimens were recruited in this study. From among all gathered specimens the 52 most suitable cases were selected. The expression of examined proteins was detected by immunohistochemistry, and its correlation with the Gleason score and pattern were further analyzed through standard statistical algorithms.

Results: The results show a significant correlation between Gleason pattern and the nuclear expression of the MSH2 protein and the cytoplasmic expression of the MLH1 protein. Gleason score significantly correlates with the nuclear and the cytoplasmic expression of the MSH2 protein and the cytoplasmic expression of the MDC1 protein. There is no correlation between the nuclear or cytoplasmic expression of the TP53BP1 protein and Gleason pattern or score.

Conclusions: Our study suggests that the aberration in the MMR repair mechanism may be significantly more important regarding the grading among PC cells in comparison to the impact of alterations in the DSB repair mechanism. The lack of correlation between expression of the TP53BP1 protein and Gleason pattern and Gleason score suggests that the radiation resistance of PC is independent of alterations connected with TP53BP1.

Key words: radiotherapy, mismatch repair genes, double-strand breaks.

Introduction

Prostate adenocarcinoma (PC) is the most common cancer among men, with the cancer mortality approximately 16% per year [1]. The mortality risk after radical prostatectomy could be predicted using a grading system based on histopathological examination of postoperative material [2]. The histologic grading of PC is still based on the standard Gleason score (GS) [3] and the prognosis is closely negatively correlated

with this grading. In the 2014 International Society of Urological Pathology proposed a modification of the aforementioned scale, reorganizing the previous GS into a 5-grade system [4]. However, the proper grading may be difficult, because of the multifocal growth of PC.

Prostate adenocarcinoma development is related to various errors in DNA. One of the major DNA error types arises because of single or double-strand breaks (DSBs). These errors are repaired by specific molecular pathways in which DSB repair proteins such as MDC1 and TP53 are involved. In this process, the TP53BP1 is involved by the MDC1 protein, which causes activation of downstream effector molecules and the initiation of repair [5]. BRCA1 and BRCA2 proteins are also involved in DSB repair. Studies show that loss of their specific functions in this pathway causes genomic instability and is connected with higher risk of breast, ovarian and pancreas cancers and PCs [6–9].

Another type of DNA errors stems from changes in the base sequence of the DNA and is recognized by the DNA mismatch repair (MMR) pathway. It results from the incorrect DNA replication made by DNA polymerase, wrong linked bases or the impact of drugs [10, 11]. Mistakes in DNA are detected by the MutSα complex. This complex is a homodimer and consists of MSH2 and MSH6 proteins [10]. The assembly of the MutSα complex with the hMutLα complex, which consists of MLH1 and PMS2 proteins, creates the hMutLα-hMutSα-heteroduplex complex. A formed complex initiates repair of the mismatch defects [11].

Conventionally, the loss of MMR proteins is associated with the development of adenocarcinomas, mostly colorectal cancers and also squamous cell carcinomas [11, 12]. The germline mutation in one of the MMR genes, such as *MLH1*, *MSH2*, *MSH6* or *PMS2*, leads to hereditary nonpolyposis colon carcinoma (Lynch syndrome) [13–15].

The accumulation of DNA mutations could be caused by dysfunction of DNA repair pathways. An interesting question is whether there is a correlation between MMR protein alterations and histological grade of cancer. The grading of PC is specific and well defined, due to the fact that the authors of the current study wanted to investigate the association between MLH1, MSH2, MDC1 and TP53BP1 and Gleason pattern (GP) and GS.

Moreover, the results of this study could indicate the relevance of this protein expression assessment as valuable information in deciding on follow-up or adjuvant treatment, e.g. adjuvant radiotherapy. On the basis of this study we supposed that alterations in TP53BP1 might have significant value for selection of the treatment after prostatectomy, e.g. between immediate adjuvant radiotherapy and hormone therapy.

Material and methods

Patients and tissue samples

The study included 500 prostates with lymph nodes from patients who underwent radical prostatectomy for prostate carcinoma. The patients were aged between 52 and 78. All the material was fixed in 10% buffered formalin and processed according to the standard protocol. Finally, paraffin blocks were prepared. The inclusion criteria for material used in this study were the clear-cut diagnosis of PC that fits the Gleason classification criteria, and the presence of sufficient material for further work. Moreover, we specified the group and selected 26 patients in whom lymphadenectomy was performed during prostatectomy, N status was available and there were metastases to lymph nodes. Afterwards we selected a group of 26 patients with N status described as N0. Finally we created a group of the 52 most suitable cases. Subsequently, two independent pathologists verified specimens of those cases. We divided chosen specimens into 3 groups. The current study was focused on alterations of protein expression between different GS and GP, whereas for the further part of this study we needed a group with metastases to lymph nodes.

The first group included 29 specimens of GS 7, the second group 29 specimens of GS 8, and the third group 18 specimens of GS 9. Furthermore, we evaluated separately areas with the highest protein expression (hot spots) for GP 3, 4 and 5. For expression of the MDC1 protein, we evaluated 72 hot spots for GP3, 149 hot spots for GP4, and 13 hot spots for GP5. For expression of the TP53BP1 protein, we evaluated 62 hot spots for GP3, 116 hot spots for GP4, and 14 hot spots for GP5. For expression of the MLH1 protein, we evaluated 69 hot spots for GP3, 155 hot spots for GP4, and 10 hot spots for GP5. For expression of the MSH2 protein, we evaluated 77 hot spots for GP3, 133 hot spots for GP4, and 18 hot spots for GP5.

Selected places of specimens were evaluated by Remmele-Stegner immunoreactive score [16].

The expression of each examined protein among specimens was evaluated in reference to GS and GP.

Methods

The formalin-fixed, paraffin-embedded (FFPE) tissue specimens were cut into 3 µm paraffin sections, using a rotary microtome (Accu-Cut SRM 200; Sakura, Torrance, CA, USA). The sections were mounted on microscopic slides providing superior adhesion (SuperFrost Plus; Menzel-Glaser, Braunschweig, Germany). For deparaffinization, rehydration, and antigen retrieval, paraffin sec-

tions were pre-treated with a high-pH buffer (Epitope Retrieval Solution) in an automated PT-link system (Dako; Agilent Technologies, Inc., Santa Clara, CA, USA). Thereafter, immunohistochemical staining was performed using rabbit polyclonal anti-MDC1 (1 : 200, Sigma-Aldrich; HPA006915), rabbit polyclonal anti-53BP1 (1 : 300, Novus Biologicals; NB100-304), rabbit monoclonal anti-MLH1 (1 : 100, Abcam; ab92312), mouse monoclonal anti-MSH2 (1 : 200, BD Pharmingen; G219-1129) and using the visualization system EnVision FLEX + HRP (Dako; Agilent Technologies, Inc. Inc., Santa Clara, CA, USA) on an Autostainer Link 48 platform according to well-known protocols [17, 18]. Finally, the slides were counterstained with hematoxylin, dehydrated in an alcohol gradient, cleared in xylene, and mounted (Dako; Agilent Technologies, Inc.).

Antigen expression for each studied antibody was evaluated by the Remmele-Stegner immunoreactive score [16].

The expression of each examined protein among specimens was evaluated in reference to the GS and GP. Three sections for every case were chosen, after which 3 different fields of view were evaluated and the average of those results was calculated. Positive controls were performed by immunohistochemical staining on every specimen on its own tissue without cancer involvement. The power of magnification was ×20.

Statistical analysis

All statistical analyses were performed using Statistica version 13 (StatSoft) and Microsoft Excel 2007. The comparative studies were analyzed statistically using the nonparametric Kruskal-Wallis test. The p value < 0.05 was considered statistically significant. The expression values of analyzed proteins were presented as the median and 25th and 75th percentiles.

Results

Association of MSH2 expression with Gleason score and Gleason pattern

In 97% of cases nuclear expression and in 68% of cases cytoplasmic expression of the MSH2 was revealed. Statistical analysis demonstrated a significant correlation between GP and nuclear expression of MSH2 (p = 0.004) (Figure 1A).

Statistical analysis did not show any significant correlation between cytoplasmic MSH2 level and GP (Table I).

Statistical analysis showed a significant correlation between nuclear and cytoplasmic expression of MSH2 and GS (p = 0.044, p = 0.045 respectively) (Figure 2A and 2B respectively).

Association of MLH1 expression with Gleason score and Gleason pattern

In 93% of cases nuclear expression and in 74% of cases cytoplasmic expression of MLH1 was revealed. Statistical analysis demonstrated no significant correlation between the level of MLH1 in nuclei with reference to GP.

Statistical analysis demonstrated a significant correlation between cytoplasmic expression of MLH1 and GP (p = 0.0255) (Figure 1B).

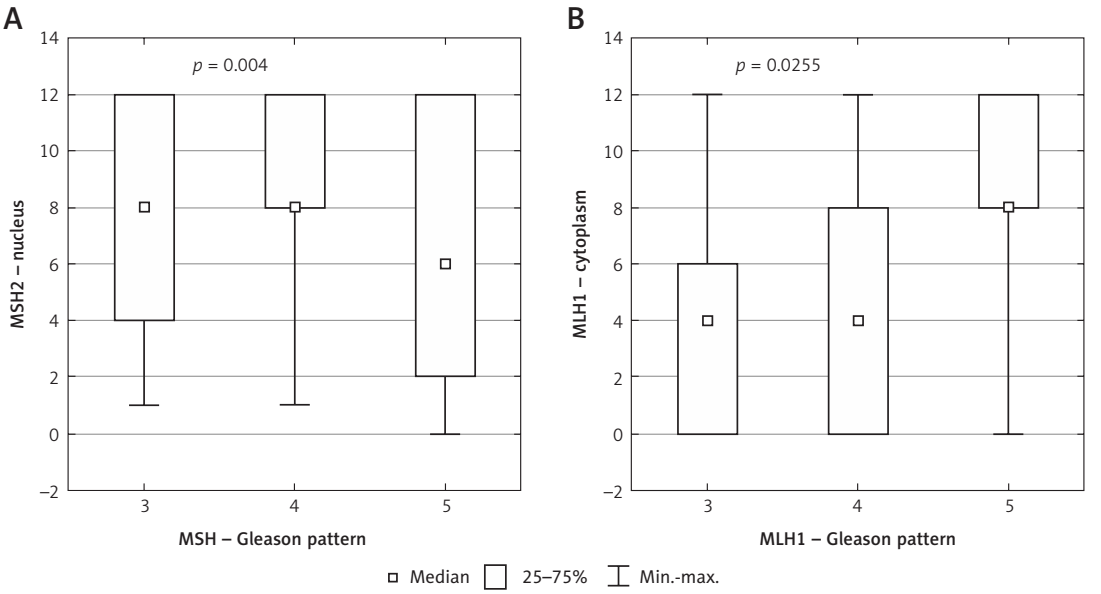


Figure 1. Box plot of data with correlation between Gleason pattern and protein expression. **A** – Correlation between Gleason pattern and nuclear expression of MSH2 protein. **B** – Correlation between Gleason pattern and cytoplasmic expression of MLH1 protein

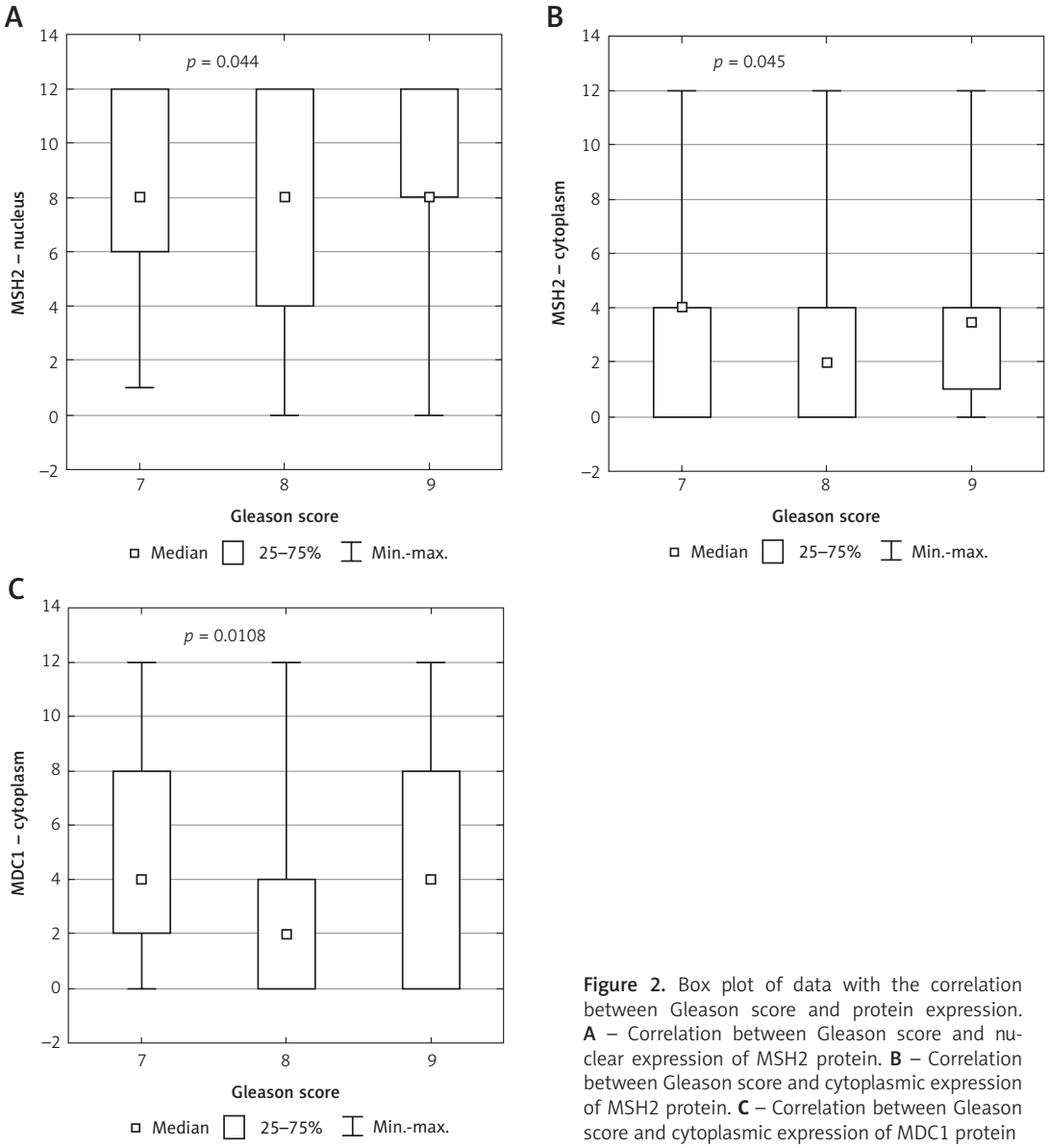


Figure 2. Box plot of data with the correlation between Gleason score and protein expression. **A** – Correlation between Gleason score and nuclear expression of MSH2 protein. **B** – Correlation between Gleason score and cytoplasmic expression of MSH2 protein. **C** – Correlation between Gleason score and cytoplasmic expression of MDC1 protein

Statistical analysis demonstrated no significant correlation between the level of MLH1 in nuclei and cytoplasm with reference to GS (Table II).

Association of TP53BP1 expression with Gleason score and Gleason pattern

In 95% of cases nuclear expression and in 96% of cases cytoplasmic expression of TP53BP1 was revealed. Statistical analysis demonstrated no correlation between cytoplasmic or nuclear expression of the TP53BP1 and GS and GP (Table II and I respectively).

Association of MDC1 expression with Gleason score and Gleason pattern

In 71% of cases nuclear expression and in 69% of cases cytoplasmic expression of MDC1 was revealed. Statistical analysis demonstrated no significant correlation between the level of MDC1 in nuclei and cytoplasm with reference to GP (Table I).

Statistical analysis showed a significant correlation between cytoplasmic expression of MDC1 and GS ($p = 0.0108$) (Figure 2C).

Statistical analysis showed no significant correlation between the level of MDC1 in nuclei and with reference to GS (Table II).

Discussion

In the normal cell, the MMR system promotes repair during and after DNA replication mainly through the excision-repair reaction. This MMR mechanism engages numerous proteins, including MSH2 and MLH1, which were a part of this study [19–21]. MSH2 gene inactivation and the loss of MSH2 protein cause insufficient DNA repair

Table I. Summarized medians and statistical significance of our results in reference to Gleason pattern

Protein	Gleason pattern 3			Gleason pattern 4			Gleason pattern 5			$p < 0.05$
	Median	Percentile		Median	Percentile		Median	Percentile		
		25%	75%		25%	75%		25%	75%	
TP53BP1 – n	8.00	4.00	12.00	8.00	8.00	12.00	10.00	3.00	12.00	ns
TP53BP1 – c	8.00	4.00	8.00	8.00	4.00	8.00	8.00	3.00	12.00	ns
MDC1 – n	3.00	0.00	6.00	3.00	0.00	7.00	6.00	4.50	8.00	0.0591
MDC1 – c	2.50	0.00	7.50	3.00	0.00	6.00	8.00	2.50	8.00	ns
MSH2 – n	8.00	4.00	12.00	8.00	8.00	12.00	3.00	0.25	8.75	0.004
MSH2 – c	3.00	0.00	4.00	3.00	0.50	4.00	0.00	0.00	2.50	ns
MLH1 – n	8.00	4.000	12.00	8.00	3.00	12.00	12.00	8.75	12.00	ns
MLH1 – c	4.00	0.00	7.00	8.00	4.00	12.00	8.00	6.25	12.00	0.0255

n – nuclear staining, c – cytoplasmic staining.

Table II. Summarized medians and statistical significance of our results in reference to Gleason score

Protein	Gleason score 7			Gleason score 8			Gleason score 9			$p < 0.05$
	Median	Percentile		Median	Percentile		Median	Percentile		
		25%	75%		25%	75%		25%	75%	
TP53BP1 – n	8.00	6.00	12.00	8.00	6.00	12.00	8.00	6.00	12.00	ns
TP53BP1 – c	8.00	4.00	8.00	8.00	4.00	8.00	8.00	4.00	12.00	ns
MDC1 – n	4.00	1.00	6.00	2.00	0.00	6.00	5.00	0.00	8.00	0.046
MDC1 – c	4.00	2.00	8.00	2.00	0.00	4.00	4.00	0.00	8.00	0.0108
MSH2 – n	8.00	6.00	12.00	8.00	3.00	12.00	8.00	8.00	12.00	0.044
MSH2 – c	4.00	0.00	4.00	2.00	0.00	4.00	3.50	1.00	4.00	0.045
MLH1 – n	8.00	4.00	12.00	8.00	4.00	12.00	8.00	2.00	12.00	ns
MLH1 – c	4.00	2.00	8.00	4.00	0.00	8.00	4.00	0.00	8.00	ns

n – nuclear staining, c – cytoplasmic staining.

and the development of tumors with high levels of microsatellite instability [22–27]. Furthermore, a recent study showed that loss-of-function mutations of MSH2 were most common in PCa compared to another MMR protein, i.e. MLH1 [28–30].

Dominguez-Valentin *et al*. followed a similar approach in their study which was focused on patients with Lynch syndrome and PC. Their results showed that defect of MSH2 was associated with high GS (GS of ≥ 8) and more aggressive biological behavior of the prostatic carcinomas [31]. Another study that addressed a similar subject was that of Guedes *et al*., which revealed that the highest rates of MSH2 gene inactivation and loss MSH2 protein occurred among the most aggressive high-grade prostatic adenocarcinomas, especially among tumors with GP5 [32]. However, in our study the nuclear and cytoplasmic level of MSH2 according to GS did not support this concept. This fact may suggest other significant alterations in MSH2 expression among PCs in patients with and without Lynch syndrome.

Based on our results, we can suspect that in cases with GS of ≥ 7 there were significantly more incorrectly paired nucleotides, which caused increased nuclear expression of MSH2 to conduct sufficient DNA repair [33, 34]. The corresponding cytoplasmic and protein expression of the MSH2 in specimens with GS ≥ 7 could be a result of increased expression of the MSH2 gene caused by intensified DNA repair because of an increased level of mutations. Moreover, our study showed a decreased overall level of nuclear MSH2 according to GP. It revealed that MSH2 loss was more common among high grade prostatic cancer. In the group with GP4 the level of nuclear MSH2 was increased compared to samples with GP3. The greater number of incorrectly paired nucleotides might have been connected with higher demand of the MSH2. However, a decreased level of the nuclear MSH2 in GP5 might have resulted from a higher amount of mutations in the MSH2 gene, which prevented adequate DNA repair. The high GS in cancers with MSH2 deficien-

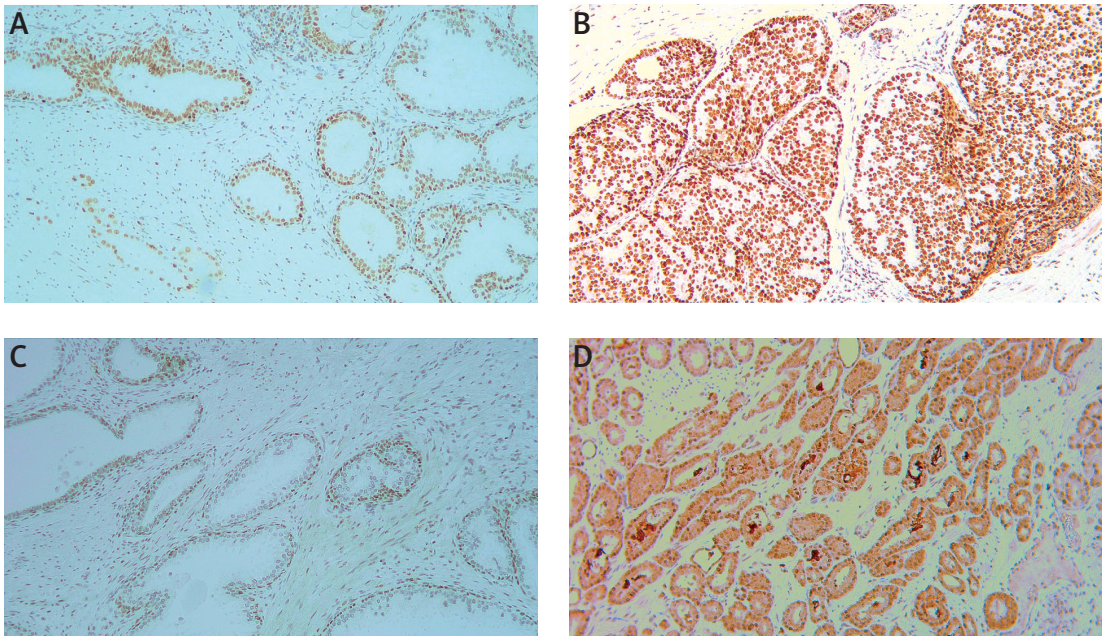


Figure 3. Collage of representative area photographs with protein expression. **A** – Photograph of area with MSH2 protein expression. **B** – Photograph of area with MLH1 protein expression. **C** – Photograph of area with MDC1 protein expression. **D** – Photograph of area with TP53BP1 protein expression

cy suggested more aggressive behavior compared to prostatic tumors without MSH2 defects. It might have been connected with a subset of prostatic carcinoma called "hypermutated" [35]. The Pritchard *et al*. study revealed that all hypermutated prostatic cancers had mutations in MMR genes and MSI. Complex structural rearrangements in the MSH2 gene (for example MSH2-KCNK12 inversion) were an important mechanism determining hypermutation in advanced PC. Thus, hypermutated prostatic cancers showed complete loss of MSH2 protein, as we observed in our study. According to Pritchard *et al*. prostatic cancers without hypermutation were microsatellite stable and had valid MSH2 protein [28]. In our study we observed the increase of MSH2 in cancers with higher GS and its decrease in cases with higher GP. This observation may result from intratumor heterogeneity in PC.

Another investigated protein of the MMR mechanism was MLH1. Studies suggested that MLH1 abnormalities could increase prostate tumor aggressiveness and indicated that expression of MLH1 among PC cells was significantly downregulated in comparison to normal prostate or benign hyperplasia [19, 36–39]. Numerous studies revealed the impact of alteration within the MLH1 gene on PC stage, but the results of those studies were diverse. Studies showed that simultaneously with growth of the GS, MLH1 gene expression declined [39, 40]. However, other studies showed the rising trend of the MLH1 gene expression among PC with the higher GS [41, 42]. The findings in our study could help to clarify the reason for these inconsistencies among studies. Our study showed a relevant dependency for the cytoplasmic MLH1 expression among PCs with a higher GP, but no significant correlation among PCs regarding the GS.

We assumed that it could have resulted from the PC heterogeneity and the intratumoral heterogeneity of MLH1 gene expression, which could be the reason for these discrepancies between the results for the GS and GP.

The other investigated proteins engaged in DNA repair were TP53BP1 and MDC1. These proteins are involved in specific molecular pathways to detect DSB, which protects cells against DNA alterations and the initiation of carcinogenesis [43, 44]. Jäämaa *et al*. observed the accumulation of TP53BP1 and MDC1 at places of DNA damage induced by cytotoxic drugs and ionizing radiation in nonmalignant human prostate tissue, which implied a protective function of the DSB repair pathway against malignant transformation [45].

The current research is the first to center on TP53BP1 level according to histopathological grade. However, our results demonstrated no significant correlation between this protein level in all evaluated localizations and GP and GS.

As a consequence, we supposed that the TP53BP1 function does not undergo disorders during a process of PC dedifferentiation.

Different studies revealed only that TP53BP1 decreased during cancer clinical progression [46–48]. These observations suggested the independence of disease risk factors related to clinical progression and the factors leading to cancer progression.

An interesting fact is that other studies showed that alterations in TP53BP1 function resulted in insensitivity to radiotherapy [49–52].

However, an earlier clinical study has demonstrated that the radiotherapy relapse rate increases in the case of prostatic cancer with an increasing GS value [53]. Our results suggest nevertheless that the mechanism of this radiation resistance might not arise from alterations of TP53BP1. Moreover, there is a lack of studies about another clinical context and further research is needed in this field.

MDC1 was another investigated component of the DNA damage response that participates in the DNA damage checkpoint and protects the integrity of the genome [54]. The latest study showed that there was overexpression of MDC1 in cells of several cancer types in comparison to normal cells [55, 56]. Zou *et al*. found that MDC1 was a positive co-activator of the estrogen receptor α (ERα) in breast cancer [55]. They detected that down-regulation of MDC1 decreased the expression of the endogenous estrogen responsive genes and, therefore, the growth of the tumor [55]. Similar correlations have been described by Wang *et al*. for PC. They proved that MDC1 was an androgen receptor co-activator involved in PC suppression. Moreover, they showed that MDC1 participated in suppression of PCa cell growth and migration [56]. However, our results showed no significant correlation between the level of MDC1 in nuclei and cytoplasm with reference to GP. On the other hand, we demonstrated a significant decrease of the cytoplasmic expression of MDC1 in cases with GS 8. This process may be caused by PC heterogeneity and the results may differ according to the PC group, which is examined [57].

In conclusion, our study suggested that the aberration in the MMR repair mechanism may be significantly more crucial regarding grading among PC cells in comparison to the impact of alterations in the DSB repair mechanism. Moreover, the present study indicated divergences among expression of the respective proteins in GP and GS. There was a significant positive correlation between GS and nuclear expression of MSH2, but a negative correlation between GP and MSH2 nuclear expression. According to this, there was no relevant correlation between MLH1 cytoplasmic expression and GS, whereas there was a significant positive correlation between cytoplasmic expression of MLH1 and GP. This may indicate significant heterogeneity among PC. Furthermore, we concluded due to the lack of a correlation between expression of the TP53BP1 protein and GP and GS that the radiation resistance of prostate cancer seems to be independent of alterations connected with TP53BP1.

Conflict of interest

The authors declare no conflict of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017; 67: 7-30.
2. Ham WS, Chalfin HJ, Feng Z, et al. New prostate cancer grading system predicts long-term survival following surgery for Gleason score 8-10 prostate cancer. Eur Urol 2017; 71: 907-12.
3. Humphrey PA, Moch H, Cubilla AL, Ulbright TM, Reuter VE. The 2016 WHO classification of tumours of the urinary system and male genital organs – part B: prostate and bladder tumours. Eur Urol 2016; 70: 106-19.
4. Epstein J, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA; Grading Committee. The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma: definition of grading patterns and proposal for a new grading system. Am J Surg Pathol 2016; 40: 244-52.
5. Oberle C, Blattner C. Regulation of the DNA damage response to DSBs by post-translational modifications. Curr Genomics 2010; 11: 184-98.
6. Breast Cancer Linkage Consortium. Cancer risks in BRCA2 mutation carriers. J Natl Cancer Inst 1999; 91: 1310-6.
7. O'Donovan PJ, Livingston DM. BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair. Carcinogenesis 2010; 31: 961-7.
8. Tirkkonen M, Johannsson O, Agnarsson BA, et al. Distinct somatic genetic changes associated with tumor progression in carriers of BRCA1 and BRCA2 germ-line mutations. Cancer Res 1997; 57: 1222-7.
9. Thompson D, Easton DF; Breast Cancer Linkage Consortium. Cancer Incidence in BRCA1 mutation carriers. J Natl Cancer Inst 2002; 94: 1358-65.
10. Reyes GX, Schmidt TT, Kolodner RD, Hombauer H, Diego S, Jolla L. New insights into the mechanism of DNA mismatch repair. Chromosoma 2015; 124: 443-62.
11. Poulogiannis G, Frayling IM, Arends MJ. DNA mismatch repair deficiency in sporadic colorectal cancer and Lynch syndrome. Histopathology 2010; 56: 167-79.
12. Chui MH, Ryan P, Radigan J, et al. The histomorphology of Lynch syndrome-associated associated ovarian carcinomas: toward a subtype-specific screening strategy. Am J Surg Pathol 2014; 38: 1173-1181.
13. Montenegro YM, Ramírez AT MC. Hereditary colo-rectal cancer. Rev Colomb Cir 2002; 17: 31-6.
14. Hashmi AA, Ali R, Hussain ZF, et al. Mismatch repair deficiency screening in colorectal carcinoma by a four-antibody immunohistochemical panel in Pakistani population and its correlation with histopathological parameters. World J Surg Oncol 2017; 15: 4-11.
15. Yoshioka Y, Togashi Y, Chikugo T, et al. Clinicopathological and genetic differences between low-grade and high-grade colorectal mucinous adenocarcinomas. Cancer 2015; 121: 4359-68.
16. Remmele W, Stegner HE. Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue. Pathologe 1987; 8: 138-140.
17. Kowalewski A, Szylberg Ł, Tyloch J, et al. Caspase 3 as a novel marker to distinguish chromophobe renal cell carcinoma from oncocytoma. Pathol Oncol Res 2019; 25: 1519-24.

18. Sadlecki P, Jóźwicki J, Antosik P, Grabiec M. Expression of selected epithelial-mesenchymal transition transcription factors in serous borderline ovarian tumors and type I ovariancancers.TumourBiol2018;40:1010428318784807.
19. Reyes GX, Schmidt TT, Kolodner RD, Hombauer H. New insights into the mechanism of DNA mismatch repair. Chromosoma 2015; 124: 443-62.
20. Fishel R. Mismatch repair. J Biol Chem 2015; 290: 26395-403.
21. Fukuhara S, Chang I, Mitsui Y, et al. DNA mismatch repair gene MLH1 induces apoptosis in prostate cancer cells. Oncotarget 2014; 5: 11297-307.
22. Peltomaki P, Lothe RA, Aaltonen LA, et al. Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. Cancer Res 1993; 53: 5853-5.
23. Aaltonen L, Peltomäki P, Leach F, et al. Clues to the pathogenesis of familial colorectal cancer. Science 1993; 260: 812-6.
24. Papadopoulos N, Lindblom A. Molecular basis of HNPCC: mutations of MMR genes. Hum Mutat 1997; 10: 89-99.
25. Peltomäki P, Vasen HF. Mutations predisposing to hereditary nonpolyposis colorectal cancer: database and results of a collaborative study. The International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer. Gastroenterology 1997; 113: 1146-58.
26. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med 2003; 348: 919-32.
27. Nagasaka T, Rhees J, Kloor M, et al. Somatic hypermethylation of MSH2 is a frequent event in Lynch syndrome colorectal cancers. Cancer Res 2010; 70: 3098-108.
28. Pritchard CC, Morrissey C, Kumar A, et al. Complex MSH2 and MSH6 mutations in hypermutated microsatellite unstable advanced prostate cancer. Nat Commun 2014; 5: 4988.
29. Abeshouse A, Ahn J, Akbani R, et al. The molecular taxonomy of primary prostate cancer. Cell 2015; 163: 1011-25.
30. Robinson D, Van Allen EM, Wu YM, et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015; 161: 1215-28.
31. Dominguez-Valentin M, Joost P, Therkildsen C, Jonsson M, Rambech E, Nilbert M. Frequent mismatch-repair defects link prostate cancer to Lynch syndrome. BMC Urol 2016; 16: 15; doi: 10.1186/s12894-016-0130-1.
32. Guedes LB, Antonarakis ES, Schweizer MT, et al. MSH2 loss in primary prostate cancer. Clin Cancer Res 2017; 23: 6863-74.
33. Geng H, Sakato M, DeRocco V, et al. Biochemical analysis of the human mismatch repair proteins hMutSα MSH2(G674A)-MSH6 and MSH2-MSH6(T1219D). J Biol Chem 2012; 287: 9777-91.
34. Heinen CD, Cyr JL, Cook C, et al. Human MSH2 (hMSH2) protein controls ATP processing by hMSH2-hMSH6. J Biol Chem 2011; 286: 40287-95.
35. Kumar A, White TA, MacKenzie AP, et al. Exome sequencing identifies a spectrum of mutation frequencies in advanced and lethal prostate cancers. Proc Natl Acad Sci 2011; 108: 17087-92.
36. Supek F, Lehner B. Differential DNA mismatch repair underlies mutation rate variation across the human genome. Nature 2015; 521: 81-4.
37. Soni A, Bansal A, Singh LC, et al. Gene expression profile and mutational analysis of DNA mismatch repair genes in carcinoma prostate in Indian population. Omi A J Integr Biol 2011; 15: 319-24.
38. Chen Y, Wang J, Fraig MM, et al. Alterations in PMS2, MSH2 and MLH1 expression in human prostate cancer. Int J Oncol 2003; 22: 1033-43.
39. Chen Y, Wang J, Fraig MM, et al. Defects of DNA mismatch repair in human prostate cancer. Cancer Res 2001; 61: 4112-21.
40. Burger M, Denzinger S, Hammerschmied CG, et al. Elevated microsatellite alterations at selected tetranucleotides (EMAST) and mismatch repair gene expression in prostate cancer. J Mol Med 2006; 84: 833-41.
41. Wilczak W, Rashed S, Hube-Magg C, et al. Up-regulation of mismatch repair genes MSH6, PMS2 and MLH1 parallels development of genetic instability and is linked to tumor aggressiveness and early PSA recurrence in prostate cancer. Carcinogenesis 2017; 38: 19-27.
42. Norris AM, Gentry M, Peehl DM, D'Agostino R, Scarpinato KD. The elevated expression of a mismatch repair protein is a predictor for biochemical recurrence after radical prostatectomy. Cancer Epidemiol Biomarkers Prev 2009; 18: 57-64.
43. Shibata A. Regulation of repair pathway choice at two-ended DNA double-strand breaks. Mutat Res 2017; 803-805: 51-55.
44. Lukas J, Lukas C, Bartek J. More than just a focus: the chromatin response to DNA damage and its role in genome integrity maintenance. Nat Cell Biol 2011; 13: 1161-9.
45. Jäämaa S, Af Hällström TM, Sankila A, et al. DNA damage recognition via activated ATM and p53 pathway in nonproliferating human prostate tissue. Cancer Res 2010; 70: 8630-41.
46. Kurfurstova D, Bartkova J, Vrtel R, et al. DNA damage signalling barrier, oxidative stress and treatment-relevant DNA repair factor alterations during progression of human prostate cancer. Mol Oncol 2016; 10: 879-94.
47. Du R, Zheng L, Huang W, Zhang H, Jiang Z. Correlation of 53BP1 gene mutation with prostatic adenocarcinoma. Zhonghua Bing Li Xue Za Zhi 2011; 40: 449-53.
48. Lewinska A, Jarosz P, Czech J, et al. Capsaicin-induced genotoxic stress does not promote apoptosis in A549 human lung and DU145 prostate cancer cells. Mutat Res 2015; 779: 23-34.
49. Xiao Y, Zheng X, Huang A, Liu T, Zhang T, Ma H. Deficiency of 53BP1 inhibits the radiosensitivity of colorectal cancer. Int J Oncol 2016; 49: 1600-8.
50. Gou Q, Xie Y, Liu L, et al. Downregulation of MDC1 and 53BP1 by short hairpin RNA enhances radiosensitivity in laryngeal carcinoma cells. Oncol Rep 2015; 34: 251-7.
51. Squatrito M, Vanoli F, Schultz N, Jasin M, Holland EC. 53BP1 is a haploinsufficient tumor suppressor and protects cells from radiation response in glioma. Cancer Res 2012; 72: 5250-60.
52. Wang W, Song Z, Zhang Y. Efficacy of brain radiotherapy plus EGFR-TKI for EGFR-mutated NSCLC patients who develop brain metastasis. Arch Med Sci 2018; 14: 1298-307.
53. Brawer MK. Radiation therapy failure in prostate cancer patients: risk factors and methods of detection. Rev Urol 2002; 4 Suppl 2: S2-S11.
54. Stewart GS, Wang B, Bignell CR, Taylor AMR, Elledge SJ. MDC1 is a mediator of the mammalian DNA damage checkpoint. Nature 2003; 421: 961-6.
55. Zou R, Zhong X, Wang C, et al. MDC1 enhances estrogen receptor-mediated transactivation and contributes to breast cancer suppression. Int J Biol Sci 2015; 11: 992-1005.
56. Wang C, Sun H, Zou R, et al. MDC1 functionally identified as an androgen receptor co-activator participates in suppression of prostate cancer. Nucleic Acids Res 2015; 43: 4893-908.
57. Tolkach Y, Kristiansen G. The heterogeneity of prostate cancer: a practical approach. Pathobiology 2018; 85: 108-16.

4.3 Artykuł 3

Jaworski Damian, Brzoszczyk Bartosz, Szylberg Łukasz, Recent research advances in double-strand break and mismatch repair defects in prostate cancer and potential clinical applications, Cells; 2023 :Vol. 12, nr 10, s. 1-20; 1375, DOI: 10.3390/cells12101375

cells

MDPI

Review

Recent Research Advances in Double-Strand Break and Mismatch Repair Defects in Prostate Cancer and Potential Clinical Applications

Damian Jaworski [1,2,*], Bartosz Brzoszczyk [3] and Łukasz Szylberg [4,5]

[1] Department of Clinical Pathomorphology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, 85-067 Bydgoszcz, Poland
[2] Division of Ophthalmology and Optometry, Department of Ophthalmology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, 85-067 Bydgoszcz, Poland
[3] Department of Urology, University Hospital No. 2 im. Dr. Jan Biziel in Bydgoszcz, 85-067 Bydgoszcz, Poland
[4] Department of Obstetrics, Gynaecology and Oncology, Chair of Pathomorphology and Clinical Placentology, Collegium Medicum in Bydgoszcz, Nicolaus Copernicus University in Torun, 85-067 Bydgoszcz, Poland
[5] Department of Tumor Pathology and Pathomorphology, Oncology Centre-Prof. Franciszek Łukaszczyk Memorial Hospital, 85-796 Bydgoszcz, Poland
* Correspondence: jaworskiresearch@gmail.com

Abstract: Prostate cancer remains a leading cause of cancer-related death in men worldwide. Recent research advances have emphasized the critical roles of mismatch repair (MMR) and double-strand break (DSB) in prostate cancer development and progression. Here, we provide a comprehensive review of the molecular mechanisms underlying DSB and MMR defects in prostate cancer, as well as their clinical implications. Furthermore, we discuss the promising therapeutic potential of immune checkpoint inhibitors and PARP inhibitors in targeting these defects, particularly in the context of personalized medicine and further perspectives. Recent clinical trials have demonstrated the efficacy of these novel treatments, including Food and Drugs Association (FDA) drug approvals, offering hope for improved patient outcomes. Overall, this review emphasizes the importance of understanding the interplay between MMR and DSB defects in prostate cancer to develop innovative and effective therapeutic strategies for patients.

Keywords: prostate cancer; double-strand break; mismatch repair; PARP inhibitors

Citation: Jaworski, D.; Brzoszczyk, B.; Szylberg, Ł. Recent Research Advances in Double-Strand Break and Mismatch Repair Defects in Prostate Cancer and Potential Clinical Applications. *Cells* **2023**, *12*, 1375. https://doi.org/10.3390/cells12101375

Academic Editors: Manuela Pellegrini, Maria Laura Falchetti, Maria Patrizia Mongiardi and Andrea Levi

Received: 17 April 2023
Revised: 9 May 2023
Accepted: 10 May 2023
Published: 12 May 2023

1. Introduction

Among all cancers in men, prostate cancer (PC) is the most common non-cutaneous cancer and the second most common cancer worldwide, with approximately 366,000 deaths and 1,600,000 cases annually [1]. PC is characterized by a variable disease course with either aggressive development and metastasis or slow progression without metastasis. These tumors are graded using the Gleason score [2].

The underlying cause of PC is still under investigation, but studies have shown that both differentiated and stem/progenitor cells have the potential to initiate PC of either the luminal or basal phenotype [3], although there is no conclusive result regarding the clinical and biological relevance of the PC phenotype [4–6].

Nevertheless, it is widely accepted that chronic inflammation plays a critical role in the development of PC. Prolonged exposure to oxidative stress and reactive oxygen species can cause DNA damage, leading to the selection of mutated cells and the progression to prostate intraepithelial neoplasia and malignancy [7].

Although numerous studies have identified multiple genetic alternations associated with PC, the genetic and epigenetic features of PC in relation to DNA repair are currently poorly understood. Impaired DNA repair pathways are considered to be the cause of several types of cancers, including XP gene loss in skin cancer, BRCA1/2 defect in ovarian

and breast cancers, and mismatch repair (MMR) deficiency in colorectal cancer (Lynch syndrome) [8].

While most PCs occur sporadically, approximately 5–15% are associated with hereditary factors. Studies suggest that mutations in DNA damage repair (DDR) genes, including DNA MMR and double-strand break repair (DSBR) genes, may be used as biomarkers for hereditary PC (HPC), which is characterized by a more aggressive disease course [9–11]. DDR is involved in tumorigenesis, but is also an important factor in treatment. The identification of impaired DNA repair pathways in specific cancers is crucial to the exploration of new treatment protocols that exploit DDR defects. This approach has been applied to numerous cancers, including BRCA1/2-deficient ovarian and breast cancers, where the inhibition of poly(ADP-ribose) polymerase 1 (PARP) has been proven to be synthetically lethal in other tumors with PTEN deficiency, and, more recently, in patients with homologous recombination (HR) repair gene-mutated metastatic castration-resistant PC (mCRPC) [8,12,13].

Moreover, DDR is also known to be useful in predicting drug resistance in cancer. In the treatment of O(6)-methylguanine DNA methyltransferase-deficient glioblastomas, increased sensitivity to temozolomide has been observed [14]. In addition, PARP inhibition has been shown to restore temozolomide sensitivity in MMR-deficient tumors [15].

Recent studies have also highlighted the potential role of immune checkpoint inhibitors, such as CTLA-4 and PD-L1 inhibitors, in the context of DDR-deficient tumors [16,17]. These agents unleash the immune system to identify and target cancer cells by exploiting the increased mutational burden and neoantigen load present in DDR-deficient tumors [16,17].

In this review, we have summarized recent studies focused on two of the DDR pathways, MMR and DSBR, in PC and their implications for cancer therapy, particularly in regard to prostate cancer. The aim is to provide a comprehensive overview of the current state of the field and to highlight areas for future research and development.

2. Mechanisms of the DNA Damage Repair

DDR is a vital mechanism that cells use to maintain the integrity of their genetic material. DDR is activated in response to various types of DNA damage, such as single-strand breaks (SSBs) repaired by mechanisms such as MMR, base excision repair (BER), nucleotide excision repair (NER), and double-strand breaks (DSBs) that can be repaired by homologous recombination (HR) or non-homologous end joining (NHEJ). By initiating the appropriate repair mechanisms, DDR helps cells to effectively repair DNA damage, thereby preventing replication errors and maintaining genomic stability [18].

3. Immune Checkpoint Molecules: PD-L1 and CTLA-4

PD-L1 and CTLA-4 belong to the immune checkpoint molecules that are crucial in the regulation of the immune response against cancer cells. Immune checkpoint inhibitors targeting the CLTA-4 and PD-L1/PD-1 pathways have been proven to enhance anti-tumor immune responses, which indicates a promising strategy for cancer treatment, including for PC [16,17].

Cells with deficiencies in the main DDR pathways, such as MMR and DSBR, accumulate DNA mutations and chromosomal rearrangements, resulting in tumorigenesis and cancer progression [19]. Cancer cells harboring MMR and DSBR defects are characterized by higher mutational burden and increased genomic instability [20]. Furthermore, the aforementioned increased mutational burden results in the generation of tumor-specific neoantigens that could be recognized by the immune system. Consequently, T-cells are activated, which initiates an ani-tumor immunological response. The immune response is stronger in tumors with high mutational burden, which increases the infiltration of such immune cells, such as natural killer cells and T-cells [21,22]. Cancers with MMR and DSBR deficiencies show increased sensitivity to immune checkpoint inhibitors targeting the CTLA-4 and PD-L1/PD-1 pathways as a result of their increased immunogenicity. These cancers, due to the accumulation of mutations, are more susceptible to immune-mediated

attack, especially after the inhibition of immune checkpoint molecules [23,24]. Moreover, a combined therapy of PARP inhibitors with immune checkpoint inhibitors could have synergistic effects in cancer treatment [8,25–27].

4. Implications of the MMR on Tumorigenesis and Its Alterations in Prostate Cancer

MMR is a highly conserved DNA repair that corrects errors in the replication, recombination, and repair processes, preserving genomic stability by correcting mismatched bases, insertion-deletion loop-type mismatches (IDLs), and SSBs [28]. Mutations in MMR genes cause a high frequency of microsatellite instability (MSI), which is characterized by an increased rate of insertion-deletion mutations in repetitive DNA sequences. The severity of this process can be assessed by examining the repetitive sizes of the selected microsatellite markers. Cancers can be defined as MSI-high (MSI-H) (multiple marker instability) or MSI-low (MSI-L) (only one marker instability). MSI is a hallmark of several hereditary and sporadic cancers, including colorectal, endometrial, gastric, ovarian, and pancreatic cancers [29]. Deficient MMR activity leads to the accumulation of DNA damage, genomic instability, and the emergence of cancer-promoting mutations.

MMR defects can occur as a result of germline mutations in MMR genes, somatic mutations in MMR genes, or the epigenetic silencing of MMR gene expression. Germline mutations in MMR genes cause hereditary cancer syndromes, such as Lynch syndrome, which predisposes individuals to the development of colorectal, endometrial, ovarian, prostate, and other cancers [30].

Somatic mutations and the epigenetic silencing of MMR genes occur in sporadic cancers and contribute to the development and progression of cancer. The loss of MMR activity leads to the accumulation of mutations in oncogenes and tumor suppressor genes, as well as the activation of the oncogenic signaling pathways that promote cancer cell survival, proliferation, and invasion [28,31]. This high mutational burden can produce novel tumor-specific antigens, making MMR-deficient tumors more immunogenic and susceptible to immune checkpoint blockade therapies [32]. MMR deficiency has also been associated with an increased sensitivity to certain DNA-damaging agents and PARP inhibitors, as these agents exploit the impaired DNA repair capacity of MMR-deficient cells, leading to synthetic lethality [33,34].

The incidence of MMR defects in PC ranges between 3% and 5% and mainly affects the MSH2 and MSH6 genes [35,36]. In addition to Lynch syndrome, ductal subtypes of PC show a higher number of MMR mutations. This is associated with poorer histopathological differentiation, according to the Gleason score, and a worse prognosis [37,38]. Loss of function alterations in the MMR genes (MLH1, MSH2, MSH6, and PMS2) define a subgroup of patients with a high potential response to immune checkpoint blockade, which together with MSI and a higher expression of tumor neoantigens, facilitates immunological diagnosis. These observations served as the biological basis for testing pembrolizumab (anti-PD1) in high MMR/MSI solid tumors and led to the Food and Drug Administration (FDA) approval of pembrolizumab [39]. However, some patients with the MMR mutations have a high immune resistance, so not all men benefit equally from this treatment [23,40]. Intratumoral DNA sensing deficiency is one mechanism of low T-cell recruitment that can impair the response to immune checkpoint blockade. In MMR tumors lacking a pathway to detect pro-inflammatory cytosolic DNA, tumor growth was accelerated and the immune checkpoint blockade response was lost during treatment with pembrolizumab [41].

Mutations in the MMR genes have been reported in several studies of PC patients. The most commonly altered MMR genes in PC are MLH1, MSH2, MSH6, and PMS2, although other MMR genes, such as MSH3 and PMS1, have also been implicated in some cases [42–45].

4.1. MLH1

MLH1 is an essential component of the MMR system, and its alterations have been associated with MSI and the development of various cancers [46], including increased

PC risk [47]. In PC, MLH1 alterations are relatively rare, with a reported loss of MLH1 expression in 0–0.9% of PCs [48,49]. However, in a study by Khan HM et al. in men aged over 75, PCs with a MLH1 mutation had a cumulative incidence of 13.8% [50]. MLH1 alterations in PC have been associated with higher Gleason scores, aggressive tumor behavior, and a poor prognosis [51]. One study suggests that MLH1 deficiency may contribute to resistance to radiotherapy in PC cells, indicating that a patient's MLH1 status may influence their treatment response [52]. In addition, a study by Rodrigues et al. showed that MLH1-deficient PC cells were more sensitive to the PARP inhibitor olaparib, suggesting a potential therapeutic strategy for patients with MLH1 alterations [53]. Moreover, studies show that a lower expression of MLH1 protein in PC correlates with a higher prevalence of lymph nodes metastases. In addition, these studies suggest a positive correlation between the Gleason pattern and MLH1 protein expression [54,55].

4.2. MSH2

MSH2 alterations have also been identified in PC patients. The reported prevalence of MSH2 alterations is estimated to be 1–2% in mCRPC [32,42,56], and a loss of MSH2 expression is estimated to occur in 2.7–12.2% of PCs [48,49]. These alterations include point mutations, deletions, and rearrangements, resulting in a loss of MSH2 protein expression and impaired MMR function [57]. In the study by Hiba et al., patients at the age of 75 with MSH2 mutations had a cumulative PC incidence of 23.8% [50]. Interestingly, Jaworski et al., in their study, indicate a negative correlation between MSH2 nuclear expression in PC and the Gleason pattern, as well as a positive correlation between nuclear and cytoplasmic expression with the Gleason score. [54] MSH2 mutations are associated with a higher risk of developing PC and are implicated in disease aggressiveness and progression [50,58].

4.3. MSH6

MSH6 mutations in PC have been reported in several studies. MSH6 alterations have been reported in approximately 1% of PC cases [42,59–61]. A loss of the immunohistochemical expression of MSH6 was found in 2.7–16.8% of PCs [48,49]. However, Alberto-Gonzalez et al., in their study, revealed MSH6 overexpression in 42.1% of the cases [62]. In a study by Pritchard et al., MSH6 mutations were found in 0.14% of men with metastatic PC [42]. MSH6 alterations were associated with higher Gleason scores, an advanced stage, and a poor prognosis [62].

4.4. PMS2

PMS2 alterations are less common in PC compared to other MMR genes. In a study by Pritchard et al., PMS2 mutations were found in 0.29% of men with metastatic PC [42]. Another study found PMS2 mutations in 0.4% of metastatic PC patients [59]. However, Sharma M. et al. and Javeed S. et al., in their studies, revealed a loss of PMS2 expression in 12.3% and 12.2% of PCs, respectively [48,49]. Although MMR gene alterations are associated with a worse prognosis in PC patients, there are no exact data correlating the PC grade with PMS2 expression [63].

4.5. Clinical Implications of MMR Alterations in Prostate Cancer

MMR alterations in PC have significant clinical implications. Patients with germline MMR mutations are at a higher risk of developing PC and other cancers, such as colorectal and endometrial cancers [64–66]. MMR alterations are also associated with aggressive tumor behavior, a higher Gleason scores, an advanced stage, and a poor prognosis [67,68]. MMR alterations have been identified as potential biomarkers for treatment response. In a study by Abida et al., patients with mCRPC harboring MMR alterations were more likely to respond to immune checkpoint inhibitors, such as pembrolizumab, compared to those without MMR alterations [36]. MMR deficiency has also been associated with an increased sensitivity to PARP inhibitors, such as olaparib [69].

4.5.1. Impact of MMR Alterations on Treatment Strategies in Prostate Cancer

The presence of MMR alterations in PC has led to the development of targeted therapies and personalized treatment strategies. Two main classes of targeted therapies have shown promise in the treatment of PC patients with MMR alterations: immune checkpoint inhibitors and PARP inhibitors.

4.5.2. Immune Checkpoint Inhibitors

MMR-deficient tumors are characterized by MSI-H and an increased neoantigen load, which makes them more susceptible to immune checkpoint inhibitors [23]. Several clinical trials have reported the efficacy of pembrolizumab and nivolumab, the PD-1 inhibitors, in MMR-deficient PC patients. The KEYNOTE-028 trial demonstrated an objective response rate of 27% in patients with PD-L1-positive mCRPC [70]. The KEYNOTE-199 trial reported a 50% overall response rate in patients with MSI-H- or MMR-deficient mCRPC [71]. Based on these findings, pembrolizumab has been approved by the FDA for the treatment of MSI-H- or MMR-deficient mCRPC patients who have progressed in prior treatment. A summary of the drugs that have been studied thus far in the treatment of prostate cancer with the immune checkpoint inhibitors group can be found in Table 1.

Table 1. Summary of all completed and selected ongoing clinical trials investigating immune checkpoint inhibitors in the treatment of prostate cancer. Summarized outcomes for all phase 3 clinical trials and selected phase 2 and 1 clinical trials.

Drug Name	Clinical Trial Number	Efficacy/Results		Annotation
Pembrolizumab	NCT02054806 [70] Phase 1	ORR: 17.4%		Investigated in locally advanced and/or metastatic PC
		PFS: 3.5 months		
		OS: 7.9 months		
	NCT03093428 [72] (active) Phase 2	Pembrolizumab + Radium 223	Radium 223	Pembrolizumab + Radium 223 vs. Radium 223 alone in mCRPC
		OS: 16.9 m	OS: 16.0 m	
		PFS: 6.1 m	PFS: 5.7 m	
	NCT02787005 [73] Phase 2	Full description of the results available in the citation		mCRPC patients divided into 5 cohorts. Accelerated FDA-approval in May 2017 for unresectable/metastatic, MSI-H or MMR-deficient solid tumors
	NCT03658447 NCT03582475 NCT04148937 NCT03849469 Phase 1			
Nivolumab	NCT02601014 [74] Phase 2	Nivolumab + Ipilimumab	Enzalutamide + Nivolumab + Ipilimumab	Investigated in mCRPC
		ORR: 25%	ORR: 0%	
		OS: 8.2%	OS: 14.2%	
		PFS: 3.7 m	PFS: 2.9 m	
	NCT03554317 NCT00441337 NCT03532217 NCT03835533 Phase 1			

Table 1. *Cont.*

Drug Name	Clinical Trial Number	Efficacy/Results		Annotation
Atezolizumab	NCT03016312 [75] Phase 3	Atezolizumab + enzalutamide	Enzalutamide	Investigated in combination with enzalutamide in mCRPC
		OS: 15.2 m	OS: 16.6 m	
		rPFS: 4.2 m	rPFS: 4.1 m	
		OR: 13.7%	OR: 7.4%	
	NCT04404140 NCT03024216 NCT02814669 NCT02655822 Phase 1			
Durvalumab	NCT04089553 [76] (active) Phase 2	AZD4635 + Durvalumab	AZD4635 + Oleclumab	Investigated in combination with AZD4635 in mCRPC
		% of patients with rPRFS at 6 months: 8.8%	% of patients with rPRFS at 6 months: 11.1%	
	NCT03204812 [77] Phase 2	rPFS: 3.7 m OS: 28.1 m		Durvalumab + Tremelimumab in naive patients with mCRPC
	NCT04495179 Phase 2, NCT02643303 Phase 1			
Ipilimumab	NCT00861614 [78] Phase 3	Ipilimumab + RTH	Placebo + RTH	Investigated as monotherapy or in combination with RTH in mCRPC
		OS: 11.04 m	OS: 10.02 m	
		OSR at year 5: 7.9%	OSR at year 5: 2.7%	
		PFS: 4.01 m	PFS: 3.06 m	
	NCT01057810 [79] Phase 3	Ipilimumab	Placebo	
		OS: 28.65 m	OS: 29.73 m	
		PFS: 5.59 m	PFS: 3.81 m	
	NCT01194271 NCT02279862 NCT00323882 NCT00170157 NCT00050596 NCT02601014 NCT01498978 NCT01804465 Phase 2 NCT03532217 NCT00064129 NCT02113657 NCT00323882 NCT01832870 Phase 1			

Abbreviations: ORR: Overall response rate; OS: overall survival; OSR: overall survival rate; rPFS: Radiographic Progression-Free Survival (in months); PFS: Progression-Free Survival (in months); m: months; PC: prostate cancer; mCRPC: Metastatic Castration-Resistant Prostate Cancer.

4.5.3. PARP Inhibitors

PARP inhibitors, such as olaparib and rucaparib, both of which are approved by the FDA in mCRPC, target the PARP enzyme involved in DNA repair. PARP inhibitors have shown efficacy in MMR-deficient cancers by exploiting the synthetic lethality, where two independent DNA repair pathways are disrupted, leading to cell death [80]. Although initially developed for BRCA-mutated and homologous recombination repair-deficient tumors, PARP inhibitors have also shown potential in MMR-deficient cancers [81]. Furthermore, it has been proposed that the clinical application of PARP inhibitors in prostate cancer could be broadened by combining them with androgen receptor inhibitors, which have been found to suppress the expression of numerous HR genes [27].

The TOPARP-A trial (NCT01682772) [61] and the TRITON2 trial (NCT02952534) [82] evaluated olaparib and rucaparib, respectively, in patients with mCRPC harboring DNA repair gene alterations, including MMR gene alterations. Both trials demonstrated antitumor activity in patients with MMR-deficient PCs, suggesting a potential role for PARP inhibitors in this patient population. A summary of the drugs that have been studied to date in the treatment of prostate cancer with the PARP inhibitors can be found in Table 2.

Table 2. List of all completed and selected ongoing clinical trials investigating PARP inhibitors in the treatment. Summarized outcomes for phase 2 and 3 clinical trials if available (NCT02987543, NCT02952534, NCT02854436, NCT03148795, and NCT01576172).

Drug Name	Clinical Trial Number/ Phase	Efficacy/Results					Annotation
Olaparib	NCT02987543 [83] Phase 3	Cohort A with olaparib	Cohort A with Investigators Choice of NHA	Cohort B with olaparib	Cohort B with Investigators Choice of NHA		Approved by FDA for mCRPC with HRR gene alterations, including BRCA1/2, ATM (PROfound clinical trial). Cohort A: mCRPC with either BRCA1/2/ATM mutation Cohort B: 12 other genes involved in the HRR. Investigators Choice of NHA: enzalutamide or abiraterone acetate
		ORR: 33.3%	ORR: 2.3%	n/a	n/a		
		OS: 56.2%	OS: 68.7%	n/a	n/a		
		RPFS: 7.39 m	RPFS: 3.55 m	n/a	n/a		
		RPFS in cohort A + B with olaparib: 5.82 m		RPFS in cohort A + B with Investigators Choice of NHA: 3.52 m			
	NCT03205176, NCT02324998 Phase 1 NCT03434158 Phase 2						
Rucaparib	NCT02952534 [84] Phase 2	BRCA	ATM	CDK12	CHEK2	Others	Approved by FDA for mCRPC with BRCA1/2 mutations that have been previously treated with androgen receptor-directed therapy and a taxane-based chemotherapy (TRITON2 clinical trial) mCRPC patients divided into 5 groups with: either BRCA, ATM, CDK12, CHEK or other HRR gene mutation.
		ORR: 45.7%	ORR: 0%	ORR: 0%	ORR: 0%	ORR: 41.2%	
		RPFS: 10.7 m	RPFS: 5.3 m	RPFS:3.7 m	RPFS: 9.4 m	RPFS: 11.6 m	
		OS: 17.2 m	OS: 14.6 m	OS: 13.9 m	OS: 11.1 m	OS: 11.6 m	
	NCT03840200 Phase 1						

Table 2. *Cont.*

Drug Name	Clinical Trial Number/ Phase	Efficacy/Results		Annotation
Niraparib	NCT02854436 (active) Phase 2	ORR: 50%; mPFS: 11 months [85]		Investigated in combination with abiraterone and prednisone in mCRPC with HRR gene alterations, including BRCA1/2, ATM
	NCT02924766 Phase 1b NCT03076203, NCT00749502 Phase 1			
Talazoparib	NCT03148795 Phase 2 (active) [86]	ORR: 29.8% PFS: 5.6 m		Investigated in mCRPC who previously received taxane-based chemotherapy and progressed on at least 1 novel hormonal agent
	NCT03330405 Phase 2	Investigated in solid tumors including PC		
	NCT01286987 Phase 1			
Veliparib	NCT01576172 [87] Phase 2	Abiraterone Acetate + Prednisone	Abiraterone Acetate + Prednisone + Veliparib	Investigated in combination with abiraterone in mCRPC
		ORR: 45%	ORR: 52.2%	
		PFS: 10.1 m	PFS: 11.0 m	
	NCT01085422, NCT00892736 Phase 1			

Abbreviations: NHA: new hormonal agent; ORR: Overall response rate; RPFS: Radiographic Progression-Free Survival (in months); PFS: Progression-Free Survival (in months); M: months; PC: prostate cancer; mCRPC: Metastatic Castration-Resistant Prostate Cancer; HRR: homologous recombination repair; n/a: not applicable.

4.5.4. Novel Treatment Strategies Aiming MMR Genes

As research progresses, new drugs and strategies targeting MMR genes in PC may emerge. Combining immune checkpoint inhibitors with other immunotherapies, radiation or chemotherapy could potentially enhance their efficacy in MMR-deficient PCs [88]. Additionally, novel small molecules targeting MMR proteins, such as MSH2-MSH6 inhibitors [89], could be developed and tested in PC.

Moreover, biomarker-driven patient selection will be critical in identifying the most appropriate treatment options for individual patients. Comprehensive genomic profiling can help identify MMR-deficient PCs and guide personalized therapy [90].

5. Implications of the DSBR on Tumorigenesis and Its Alterations in PC

DSBs are a severe form of DNA damage that can arise from endogenous factors during DNA replication or can be induced by exogenous agents, such as ionizing radiation and chemotherapeutic agents. To maintain genomic integrity, cells have evolved two main pathways for repairing DSBs: HR and NHEJ. These pathways involve a complex interplay of proteins, including DNA damage sensors, signal transducers, mediators, and effectors. [18,29] Moreover, there are three additional mechanisms involved in DSBs repair: alternative NHEJ (alt-NHEJ), break-induced replication (BIR), and single-strand annealing (SSA) [91,92].

BRCA1 plays a critical role in regulating the balance between HR and NHEJ, with a loss of BRCA1 resulting in a shift towards NHEJ and increased sensitivity to DNA damaging agents. 53BP1 and its downstream effector RIF1 are key factors in promoting NHEJ and suppressing HR, with a loss of 53BP1 leading to increased HR and reduced NHEJ. MDC1 acts as a scaffold for the recruitment of DNA damage response proteins,

including NBS1, 53BP1, RNF8, the MRN complex, and RNF168, which ubiquitylate histone H2AX and promote the accumulation of 53BP1 and BRCA1 at DSBs. The process of the phosphorylation of histone H2AX is catalyzed by the PI3-like kinase ataxia-telangiectasia mutated (ATM) [19,92–94].

The 53BP1/MDC1 axis is a key regulator of the DSB repair pathway choice, with 53BP1 promoting NHEJ and inhibiting HR, while MDC1 promotes HR. This is achieved, in part, by the differential regulation of RPA and RAD51 by the two proteins. 53BP1 inhibits the loading of RAD51 onto the DNA ends, thereby preventing HR, while MDC1 promotes the retention of RPA and the loading of RAD51, thus promoting HR [19,95]. In addition, BRCA1 counteracts 53BP1's inhibition of HR, while BRCA2 plays a role in the loading of RAD51 onto resected DNA ends [96,97]. Mutations in these genes have been associated with an increased risk of developing cancer, particularly breast and ovarian cancers [98].

Defective DSBR main pathways, such as HR, often coexist with MMR defects, leading to the accumulation of DNA damage and genomic instability [99,100]. As a result, the affected cancer cells become more dependent on alternative DNA repair pathways, such as the ssDNA repair mechanisms [101]. Exploiting the vulnerabilities in these alternative DNA repair pathways can lead to synthetic lethality, selectively eliminating cancer cells while sparing normal cells. This method is used by PARP inhibitors in cancers with MMR and HR defects [61]. PARP inhibitors block the repair of ssDNA breaks, leading to the accumulation of DSBs that the affected cells are unable to repair, resulting in the death of the cancer cell [80].

Alterations in the DSB repair pathway have been implicated in PC progression and treatment resistance. Two primary pathways are responsible for repairing DSBs in eukaryotic cells: HR and NHEJ. Both pathways are crucial for maintaining genomic stability, and defects in either pathway can lead to genomic instability and cancer development [102]. Multiple proteins play a critical role in the DSB repair mechanisms, and alterations in these proteins have been observed in PC. In this section, we will discuss the key proteins associated with both the HR and NHEJ pathways.

5.1. Homologous Recombination

The key proteins involved in the HR pathway include BRCA1, BRCA2, RAD51, and the MRN complex (MRE11, RAD50, and NBS1) [94,95,100].

5.1.1. BRCA1 and BRCA2

BRCA1 and BRCA2 are crucial proteins in the HR pathway, and germline and somatic mutations in these genes have been observed in PC [103]. Men with BRCA1 mutations account for 0.9% of PCs and have a 3-fold increased risk, and those with BRCA2 mutations account for 1.2–5.3% of PCs, overall, and have an 8-fold increased risk of developing PC [42,104]. In metastatic PC, the prevalence of BRCA2 mutation is estimated at 13.0% [105]. BRCA1/2 mutations in PC are associated with more aggressive disease, a higher Gleason score, increased metastasis [106], a poor prognosis, and resistance to conventional therapies [42]. As a result of the deficiency in DNA repair, agents such as platinum-based chemotherapy [107] and PARP inhibitors have shown promise in the treatment of BRCA1/2-mutated PCs [33,61,105]. Moreover, two of the PARP inhibitors, rucaparib and olaparib, have received FDA approval for BRCA-mutated mCRPC [108,109].

5.1.2. MDC1

The primary function of MDC1 is involved in the HR pathway of DSBR; it acts as a scaffold protein and recruits other factors at the site of DNA damage in the HR pathway [110]. Studies have revealed the overexpression of MDC1 in various malignancies, although the study by Jaworski et al. shows decreased MDC1 expression in PC with higher GS [54,111,112]. Moreover, MDC1 alteration is associated with the increased radiosensitivity of PC [113], and MDC1 knockdown promotes PC cells' migration and growth [112].

5.1.3. The RAD Family of Genes: RAD51 and RAD54

RAD51, responsible for catalyzing the strand invasion step and playing a crucial role in homologous recombination repair, has been shown to be affected by the deletion of MMS22L, which is commonly observed (up to 14%) in prostate cancer [114]. In a study by Mitra A. et al., RAD51 cytoplasmic staining was observed in 32.5% of PC cases compared with 0.74% of benign prostate tissues and has been associated with aggressive disease [42,115]. Moreover, RAD51 overexpression in PC is associated with the enhanced sensitivity of PC to radiotherapy [116]. Hine et al. indicated that the inhibition of RAD51 sensitizes PC cells to radiotherapy and chemotherapy [117]. RAD54, another key player in HR, has also been implicated in PC. Genetic alterations in RAD54 have been associated with an increased risk of PC [118].

5.1.4. MRN Complex

The MRN complex, consisting of MRE11, RAD50, and NBS1, plays a crucial role in sensing and repairing DSBs during the HR pathway [119]. Alterations in MRN complex proteins have been reported in PC, with potential implications for disease progression and treatment resistance [119]. MRE11 overexpression correlates with a poor outcome and progression of PC [120]. Alterations in MRN complex proteins, such as MRE11 and RAD50, can affect the sensitivity of PC cells to radiotherapy and chemotherapy with PARP inhibitors [121].

5.2. Non-Homologous End Joining

The key proteins involved in the NHEJ pathway include TP53BP1, Ku70, Ku80, DNA-PKcs, and LIG4 [122].

5.2.1. TP53 and TP53BP1

TP53BP1 is a protein that interacts with TP53 and plays an important role in the DSBR pathway, and its primary role is in the NHEJ pathway [123]. This interaction leads to the activation of TP53-dependent cell cycle checkpoints and apoptosis, ensuring the proper cellular response to DNA damage [124]. Alterations in this protein disrupt the function of the NHEJ pathway and promote the utilization of error-prone alt-NHEJ, which can lead to genomic instability and tumorigenesis [125,126]. Studies have shown that mutations in TP53BP1 are present in PC and its expression decreases with cancer progression [127–130]. In addition, the study by Jaworski et al. indicates no correlation between TP53BP1 expression and GS or GP, while Gzil et al. observed decreased TP53BP1 expression in lymph node metastases compared to primary PC [54,55]. Studies indicate that alterations in TP53BP1 were correlated with the insensitivity of PC to radiotherapy [54,131,132]. Moreover, Chipidza FE et al., in their study, described the mutation of the TP53 gene as an independent, unfavorable prognostic factor in PC [133]. The frequency of TP53 mutations in metastatic PC is estimated to be 31.3% [105].

5.2.2. Ku70 and Ku80

Ku70 and Ku80 are essential proteins in the NHEJ pathway, forming a heterodimer that binds to DSB ends. The impact of alterations in Ku70 and Ku80 expression on PC development is significant, not only because of their direct involvement with DSBR, but also because of their interaction with the androgen receptor as a coactivator [134]. A decrease in the expression of Ku70 has been observed in PC cells following neoadjuvant castration therapy, which in turn impairs DNA repair, and it is suggested as an explanation for the increased sensitivity to radiotherapy in PC following castration [135]. Hasewaga T. et al. suggested that radiotherapy combined with androgen deprivation therapy is effective in patients with GS $\leq$ 7 or low Ku70 expression [136].

5.2.3. DNA-Dependent Protein Kinase Catalytic Subunit (DNA-PKcs)

The DNA-PKcs is a key component of the NHEJ pathway and plays a critical role in the DNA damage response. The dysregulation of the DNA-PKcs has been implicated in PC progression, metastasis, and resistance to therapy, and its upregulation correlates with poor patient outcomes [60,137,138]. In a study by Pu J. et al., it was suggested that the downregulation of the Androgen receptor/PARP/DNA-PKcs axis could be used as a potential therapeutic strategy to increase the radiosensitivity of castrate-resistant PCs [139].

5.2.4. LIG4

LIG4 is a critical protein in the NHEJ pathway and is responsible for the ligation step during DSB repair [140]. LIG4 has been implicated in PC progression and therapeutic response, including urogenital radiotoxicity [141], although the precise role and clinical significance of LIG4 alterations in PC remain to be fully elucidated [113,141]. High LIG4 expression correlates with advanced GS and nodal involvement [142].

5.2.5. ATM

The ataxia-telangiectasia mutated (ATM) protein is another key player in the DSB repair process through the HR and NHEJ pathways [19,93]. ATM mutations are associated with aggressive PC and occur in 13.7% of metastatic PC [105,143]. Preclinical studies have shown that ATM inhibitors can sensitize PC cells to radiotherapy [144]. The studies conducted thus far have revealed better progression-free survival in mCRPC patients treated with lutetium-177-prostate-specific membrane antigen-617 compared with cabazitaxel, but without an overall improvement in survival [33]. Moreover, alterations in the ATM gene are associated with improved overall survival in PC patients treated with olaparib [33]. However, in mCRPC patients treated with olaparib, there was no objective radiological response, in contrast to BRCA1/2 patients [145]. Interestingly, ATM alterations were correlated with better outcomes to cisplatin-based chemotherapy in patients with mCRPC, compared to mCRPC with CDK12 defects [146].

5.2.6. XRCC1, XRCC2 and XRCC3

XRCC2 and XRCC3 are essential for the RAD51-mediated HR repair of DSBs [147]. Studies have reported associations between XRCC2 and XRCC3 polymorphisms and PC risk, although the evidence is not entirely consistent [141,148,149]. Moreover, polymorphism in XRCC3 is associated with an increased risk of acute genitourinary toxicity during radiotherapy in PC patients [149]. Further research is needed to understand the precise role of these proteins in PC progression and the response to therapy.

6. Current Role of DDR Mutation in Prostate Cancer Treatment

Due to the prevalence of germline mutations in the DDR genes, the guidelines of the European Association of Urology (EAU) and the National Comprehensive Cancer Network (NCCN) recommend germline testing for all men with metastatic disease and castration-resistant PC [150,151].

On the other hand, the diagnostic process for DDR mutations should begin at the time of PC diagnosis, especially in patients who meet the criteria for active surveillance (AS) but have a history of familial PC: men with high-risk PC and a family member diagnosed with PC at age < 60 years or a family member who died from PC cancer or familial syndromes, such as hereditary breast and ovarian cancer and Lynch syndrome [152]. The results of the study of 1211 men under AS showed that carriers of the BRCA2 and five ATM mutations were significantly more likely to be reclassified and to progress to clinical disease, requiring exclusion from observation [153]. Interestingly, germline DNA repair gene mutations are not only found in high-risk cancers. The short-term outcomes of AS for low-risk PC showed that at a median follow up of 28 months (IQR 8.5–42), 80% of patients on AS with low-risk PC were free from upgrading or radical treatment [154]. Therefore, patients with

DDR mutations included in AS should be carefully monitored until more reliable data are available [151].

The incidence of HR repair mutations in men with PC is significantly higher in the presence of metastases (11% vs. 33% M0/M1) [155]. Furthermore, the Profound study showed that in patients with mCRPC, the number of DDR mutations was lower in the primary tumor (27%) than in the metastatic tissue (32%) [156]. Additionally, the outcomes of this clinical trial revealed a notably longer PFS and a higher ORR for men treated with olaparib compared to the control group, with 7.4 months versus 3.6 months and 33% versus 1%, respectively [157]. In PROREPAIR-B, 68 mCRPC patients with germline BRCA2 mutations had half the CSS compared to non-carriers (17.4 vs. 33.2 months, $p = 0.027$). Importantly, ATM or BRCA1 mutations showed no difference in the CSS in this group of patients [158]. The ability of a cancer cell to repair double-stranded DNA breaks with a BRCA2 mutation is impaired; however, further repair of damage is possible through the activity of PARP. In May 2020, the FDA approved the oral PARP inhibitors rucaparib (Rubraca) and olaparib (Lynparza) for the treatment of mCRPC; talazoparib, niraparib, and veliparib are under investigation. A recent study indicates that PARP inhibitors may be effective not only in BRCA1/2-defficient tumors, but also in tumors with other DDR-deficiencies, such as MMS22L deletion [114]. The efficacy of PARP inhibitors in PC is highest when the number of mutations in the HR repair genes and DSBR is high [159]. In TOPARP-A and TOPARP-B, patients with BRCA1, ATM, PALB2, and FANCA mutations treated with 400 mg of olaparib twice daily achieved clinical benefit (including radiological response, decrease in PSA, and/or reduction in circulating tumor cell count) [61,160]. Moreover, patients with mCRPC and alterations in the DDR genes are more sensitive to platinum chemotherapy, and this is also the case after progression on PARP inhibitors [82]. Importantly, men previously treated with both docetaxel and at least one androgen receptor pathway inhibitor (ARPI) whose tumors had homozygous deletions or deleterious mutations in the DNA repair genes had an 88% response rate to olaparib [61]. A phase III, randomized, double-blind study (PROpel) of abiraterone (1000 mg once daily) plus prednisone 5 mg/twice daily (AAP) and olaparib (300 mg twice daily) in patients with mCRPC showed that the imaging-based progression-free survival (ibPFS) may have been dependent on the number of mutations in the homologous recombination repair gene [161]. Patients who qualified for mCRPC treatment with olaparib must have a mutation in one of the 14 genes, including: BRCA1, BRCA2, ATM, CHEK2, PALB2, and CDK12. Despite the positive results of the treatment of patients with mCRPRC and the ATM mutation in the TRITON2, TRITON3, and GALAHAD preclinical trials, rucaparib cannot be recommended for patients with mutations other than BRCA [82,109,162].

The discovery of an aggressive clinical course, resistance to hormonal treatment, and the occurrence of histological forms with a worse prognosis in patients with mCRPC and the CDK12 mutation prompted researchers to search for a link between MMR deficiency and immune characteristics [163]. Moreover, CDK12 mutations have been observed to occur much less frequently in BRCA2 mutations than in homologous recombination deficiency mutations. Therefore, a different mechanism of association of MMR mutations and high MSI with increased T-cell association with immune checkpoints has been noted [164]. It should be emphasized that the efficacy of using the anti-PD-1/PD-L1 antibody in patients with mCRPC following prior hormonal therapy depends on the number of biallelic CDK12 mutations [163] as both Phase III IMbassador 250 (atezolizumab + enzalutamide) and Phase II STARVE-PC (nivolumab + ipilimumab), which did not test CDK12 expression in patients with mCRPC, failed to meet the primary endpoint of improved overall survival in the unselected patients [71,165]. Interestingly, in the KEYNOTE-199 study, the median OS and disease control rate (DCR) after pembrolizumab were highest in the group of patients with mCRPC with dominant bone metastases, regardless of PD-L1 expression, compared to the selected group of patients with a high expression of these proteins [166]. The lack of conclusive data on the efficacy of the use of pembrolizumab in men with PC has led

clinicians to conclude that new treatment strategies are needed to improve the efficacy of CDK12 mutation checkpoint blockade in patients with MMR [167].

7. Conclusions

Undoubtedly, the role of the clinical geneticist is becoming increasingly important at the current stage of mCRPC management, and due to the prognostic value of the homologous recombination repair number, the indications for somatic and germline mutation testing in high-risk cancer will expand [168]. DDR mutations can be identified through the analysis of peripheral whole blood testing or tumor tissue. The current objective advantage of tissue testing is the simultaneous analysis of both genomic and somatic mutations. On the other hand, the multifocal and heterogeneous nature of PC in the context of tissue testing may result in the analyzed core biopsy not representing a clone of metastatic disease [169]. Therefore, taking into account the invasive nature of the material collection (visceral, bone metastases) and the 20% false negative rate due to the quality of the material collected, the improvement of blood assessment methods seems promising [161]. The use of liquid biopsy achieves 93% concordance between BRCA 1/2 mutations detected in tissue biopsy and those identified by ctDNA, 100% concordance for germline variants, and the detection of alterations in Tp53, RA, BRCA2/1, PI3K/AKT/mTOR, WNT/β-catenin pathway genes, RAS/RAF/MEK, and MSI-H is also possible [170]. In addition to their predictive value, ctDNA, PacBioScience, and Oxford Nanopore may have a predictive value for patients in active surveillance and salvage therapy; however, the cost and wide availability of genomic profiling tools continue to limit the development of this technology [171,172].

It seems that PC is a heterogeneous group of diseases, heterogeneous in terms of MMR and DSBR deficiency and PTEN protein mutations, which determine different clinical courses and resistance to treatment. Thanks to the improvement of molecular classification and the detailed analysis of MMR, including personalized therapy and targeted treatment at PD-1/PD-L1, PARP inhibitors and future novel treatment strategies will prove to be more effective [173].

Author Contributions: Conceptualization: D.J.; writing—original draft preparation: D.J., B.B. and Ł.S.; visualization: D.J.; writing—review and editing: D.J., B.B. and Ł.S.; supervision: Ł.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Torre, L.A.; Bray, F.; Siegel, R.L.; Ferlay, J.; Lortet-Tieulent, J.; Jemal, A. Global cancer statistics, 2012. CA. *Cancer J. Clin.* **2015**, *65*, 87–108. [CrossRef] [PubMed]
2. Testa, U.; Castelli, G.; Pelosi, E. Cellular and Molecular Mechanisms Underlying Prostate Cancer Development: Therapeutic Implications. *Medicines* **2019**, *6*, 82. [CrossRef] [PubMed]
3. Sandhu, S.; Moore, C.M.; Chiong, E.; Beltran, H.; Bristow, R.G.; Williams, S.G. Prostate cancer. *Lancet* **2021**, *398*, 1075–1090. [CrossRef] [PubMed]
4. Wang, Z.A.; Mitrofanova, A.; Bergren, S.K.; Abate-Shen, C.; Cardiff, R.D.; Califano, A.; Shen, M.M. Lineage analysis of basal epithelial cells reveals their unexpected plasticity and supports a cell of origin model for prostate cancer heterogeneity. *Nat. Cell Biol.* **2013**, *15*, 274. [CrossRef] [PubMed]
5. Smith, B.A.; Sokolov, A.; Uzunangelov, V.; Baertsch, R.; Newton, Y.; Graim, K.; Mathis, C.; Cheng, D.; Stuart, J.M.; Witte, O.N. A basal stem cell signature identifies aggressive prostate cancer phenotypes. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E6544–E6552. [CrossRef] [PubMed]
6. Wang, G.; Zhao, D.; Spring, D.J.; Depinho, R.A. Genetics and biology of prostate cancer. *Genes Dev.* **2018**, *32*, 1105–1140. [CrossRef]
7. Sfanos, K.S.; Yegnasubramanian, S.; Nelson, W.G.; De Marzo, A.M. The inflammatory microenvironment and microbiome in prostate cancer development. *Nat. Rev. Urol.* **2018**, *15*, 11–24. [CrossRef]

8. Hopkins, J.L.; Lan, L.; Zou, L. DNA repair defects in cancer and therapeutic opportunities. *Genes Dev.* **2022**, *36*, 278. [CrossRef] [PubMed]
9. Thalgott, M.; Kron, M.; Brath, J.M.; Ankerst, D.P.; Thompson, I.M.; Gschwend, J.E.; Herkommer, H. Men with family history of prostate cancer have a higher risk of disease recurrence after radical prostatectomy. *World J. Urol.* **2018**, *36*, 177–185. [CrossRef]
10. Heidegger, I.; Tsaur, I.; Borgmann, H.; Surcel, C.; Kretschmer, A. Hereditary prostate cancer—Primetime for genetic testing? *Cancer Treat. Rev.* **2019**, *81*, 101927. [CrossRef]
11. Das, S.; Salami, S.S.; Spratt, D.E.; Kaffenberger, S.D. Bringing Prostate Cancer Germline Genetics into Clinical Practice. *J. Urol.* **2019**, *202*, 223–230. [CrossRef] [PubMed]
12. Mendes-Pereira, A.M.; Martin, S.A.; Brough, R.; McCarthy, A.; Taylor, J.R.; Kim, J.; Waldman, T.; Lord, C.J.; Ashworth, A. Synthetic lethal targeting of PTEN mutant cells with PARP inhibitors. *EMBO Mol. Med.* **2009**, *1*, 315–322. [CrossRef] [PubMed]
13. Tutt, A.; Robson, M. Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: A proof-of-concept trial. *Lancet* **2020**, *376*, 235–244. [CrossRef]
14. Knizhnik, A.V.; Roos, W.; Nikolova, T.; Quiros, S.; Tomaszowski, K.-H.; Christmann, M.; Kaina, B. Survival and death strategies in glioma cells: Autophagy, senescence and apoptosis triggered by a single type of temozolomide-induced DNA damage. *PLoS ONE* **2012**, *8*, e55665. [CrossRef] [PubMed]
15. Curtin, N.J.; Wang, L.Z.; Yiakouvaki, A.; Kyle, S.; Arris, C.A. Novel poly(ADP-ribose) polymerase-1 inhibitor, AG14361, restores sensitivity to temozolomide in mismatch repair-deficient cells. *Clin. Cancer Res.* **2004**, *10*, 881–889. [CrossRef] [PubMed]
16. Kwon, E.D.; Drake, C.G.; Scher, H.I.; Fizazi, K.; Bossi, A.; Van den Eertwegh, A.J.M.; Krainer, M.; Houede, N.; Santos, R.; Mahammedi, H.; et al. Ipilimumab versus placebo after radiotherapy in patients with metastatic castration-resistant prostate cancer that had progressed after docetaxel chemotherapy (CA184-043): A multicentre, randomised, double-blind, phase 3 trial. *Lancet Oncol.* **2014**, *15*, 700–712. [CrossRef]
17. Graff, J.N.; Alumkal, J.J.; Drake, C.G.; Thomas, G.V.; Redmond, W.L.; Farhad, M.; Cetnar, J.P.; Ey, F.S.; Bergan, R.C.; Slottke, R.; et al. Early evidence of anti-PD-1 activity in enzalutamide-resistant prostate cancer. *Oncotarget* **2016**, *7*, 52810–52817. [CrossRef]
18. Chatterjee, N.; Walker, G.C. Mechanisms of DNA damage, repair and mutagenesis. *Environ. Mol. Mutagen.* **2017**, *58*, 235. [CrossRef]
19. Jackson, S.P.; Bartek, J. The DNA-damage response in human biology and disease. *Nature* **2009**, *461*, 1071–1078. [CrossRef]
20. Lord, C.J.; Ashworth, A. The DNA damage response and cancer therapy. *Nature* **2012**, *481*, 287–294. [CrossRef]
21. Hugo, W.; Zaretsky, J.M.; Sun, L.; Song, C.; Moreno, B.H.; Hu-Lieskovan, S.; Berent-Maoz, B.; Pang, J.; Chmielowski, B.; Cherry, G.; et al. Genomic and Transcriptomic Features of Response to Anti-PD-1 Therapy in Metastatic Melanoma. *Cell* **2016**, *165*, 35–44. [CrossRef]
22. Rizvi, N.A.; Hellmann, M.D.; Snyder, A.; Kvistborg, P.; Makarov, V.; Havel, J.J.; Lee, W.; Yuan, J.; Wong, P.; Ho, T.S.; et al. Mutational landscape determines sensitivity to PD-1 blockade in non–small cell lung cancer. *Science* **2015**, *348*, 124. [CrossRef] [PubMed]
23. Le, D.T.; Durham, J.N.; Smith, K.N.; Wang, H.; Bartlett, B.R.; Aulakh, L.K.; Lu, S.; Kemberling, H.; Wilt, C.; Luber, B.S.; et al. Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science* **2017**, *357*, 409–413. [CrossRef] [PubMed]
24. Sharma, P.; Allison, J.P. The future of immune checkpoint therapy. *Science* **2015**, *348*, 56–61.
25. Ding, L.; Kim, H.-J.; Wang, Q.; Kearns, M.; Jiang, T.; Ohlson, C.E.; Li, B.B.; Xie, S.; Liu, J.F.; Stover, E.H.; et al. PARP Inhibition Elicits STING-Dependent Antitumor Immunity in Brca1-Deficient Ovarian Cancer. *Cell Rep.* **2018**, *25*, 2972–2980.e5. [CrossRef] [PubMed]
26. Voena, C.; Menotti, M.; Mastini, C.; Di Giacomo, F.; Longo, D.L.; Castella, B.; Merlo, M.E.B.; Ambrogio, C.; Wang, Q.; Minero, V.G.; et al. Efficacy of a Cancer Vaccine against ALK-Rearranged Lung Tumors. *Cancer Immunol. Res.* **2015**, *3*, 1333–1343. [CrossRef] [PubMed]
27. Li, L.; Karanika, S.; Yang, G. Enzalutamide-induced "BRCAness" and PARP inhibition is a synthetic lethal therapy for castration-resistant prostate cancer. *Sci. Signal.* **2017**, *10*, eaam7479. [CrossRef]
28. Jiricny, J. Postreplicative mismatch repair. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a012633. [CrossRef]
29. Leach, F.S. Microsatellite instability and prostate cancer: Clinical and pathological implications. *Curr. Opin. Urol.* **2002**, *12*, 407–411. [CrossRef]
30. Hampel, H.; Frankel, W.L.; Martin, E.; Arnold, M.; Khanduja, K.; Kuebler, P.; Nakagawa, H.; Sotamaa, K.; Prior, T.W.; Westman, J.; et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). *N. Engl. J. Med.* **2005**, *352*, 1851–1860. [CrossRef]
31. Kunkel, T.A.; Erie, D.A. Eukaryotic Mismatch Repair in Relation to DNA Replication. *Annu. Rev. Genet.* **2015**, *49*, 291–313. [CrossRef] [PubMed]
32. Le, D.T.; Uram, J.N.; Wang, H.; Bartlett, B.R.; Kemberling, H.; Eyring, A.D.; Skora, A.D.; Luber, B.S.; Azad, N.S.; Laheru, D.; et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N. Engl. J. Med.* **2015**, *372*, 2509–2520. [CrossRef] [PubMed]
33. Yanagisawa, T.; Kawada, T.; Rajwa, P.; Kimura, T.; Shariat, S.F. Emerging systemic treatment for metastatic castration-resistant prostate cancer: A review of recent randomized controlled trials. *Curr. Opin. Urol.* **2023**, *33*, 219–229. [CrossRef] [PubMed]
34. Pommier, Y.; O'Connor, M.J.; De Bono, J. Laying a trap to kill cancer cells: PARP inhibitors and their mechanisms of action. *Sci. Transl. Med.* **2016**, *8*, 362ps17. [CrossRef]

35. Sedhom, R.; Antonarakis, E.S. Clinical implications of mismatch repair deficiency in prostate cancer. *Futur. Oncol.* **2019**, *15*, 2395. [CrossRef] [PubMed]
36. Abida, W.; Cheng, M.L.; Armenia, J.A.; Middha, S.; Autio, K.A. Analysis of the Prevalence of Microsatellite Instability in Prostate Cancer and Response to Immune Checkpoint Blockade. *JAMA Oncol.* **2019**, *5*, 471–478. [CrossRef] [PubMed]
37. Schweizer, M.T.; Antonarakis, E.S.; Bismar, T.A.; Guedes, L.B.; Cheng, H.H.; Tretiakova, M.S.; Vakar-Lopez, F.; Klemfuss, N.; Konnick, E.Q.; Mostaghel, E.A.; et al. Genomic Characterization of Prostatic Ductal Adenocarcinoma Identifies a High Prevalence of DNA Repair Gene Mutations. *JCO Precis. Oncol.* **2019**, *3*, 1–9. [CrossRef]
38. Arce, S.; Athie, A.; Pritchard, C.C.; Mateo, J. Germline and Somatic Defects in DNA Repair Pathways in Prostate Cancer. *Adv. Exp. Med. Biol.* **2019**, *1210*, 279–300.
39. Roth, M.T.; Das, S. Pembrolizumab in unresectable or metastatic MSI-high colorectal cancer: Safety and efficacy. *Expert Rev. Anticancer Ther.* **2021**, *21*, 229. [CrossRef]
40. Ganesh, K.; Stadler, Z.K.; Cercek, A.; Mendelsohn, R.B.; Shia, J.; Segal, N.H.; Diaz, L.A., Jr. Immunotherapy in colorectal cancer: Rationale, challenges and potential. *Nat. Rev. Gastroenterol. Hepatol.* **2019**, *16*, 361–375. [CrossRef]
41. Lu, C.; Guan, J.; Lu, S.; Jin, Q.; Rousseau, B.; Lu, T.; Stephens, D.; Zhang, H.; Zhu, J.; Yang, M.; et al. DNA Sensing in Mismatch Repair-Deficient Tumor Cells Is Essential for Anti-tumor Immunity. *Cancer Cell* **2021**, *39*, 96–108.e6. [CrossRef]
42. Pritchard, C.C.; Mateo, J.; Walsh, M.F.; De Sarkar, N.; Abida, W.; Beltran, H.; Garofalo, A.; Gulati, R.; Carreira, S.; Eeles, R.; et al. Inherited DNA-Repair Gene Mutations in Men with Metastatic Prostate Cancer. *N. Engl. J. Med.* **2016**, *375*, 443–453.
43. Giri, V.N.; Obeid, E.; Gross, L.; Bealin, L.; Hyatt, C.; Hegarty, S.E.; Montgomery, S.; Forman, A.; Bingler, R.; Kelly, W.K.; et al. Inherited Mutations in Men Undergoing Multigene Panel Testing for Prostate Cancer: Emerging Implications for Personalized Prostate Cancer Genetic Evaluation. *JCO Precis. Oncol.* **2017**, *1*, 1–17. [CrossRef]
44. Dominguez-Valentin, M.; Sampson, J.R.; Seppälä, T.T.; ten Broeke, S.W.; Plazzer, J.-P.; Nakken, S.; Engel, C.; Aretz, S.; Jenkins, M.A.; Sunde, L.; et al. Cancer risks by gene, age, and gender in 6350 carriers of pathogenic mismatch repair variants: Findings from the Prospective Lynch Syndrome Database. *Genet. Med.* **2020**, *22*, 15–25. [CrossRef]
45. Zhen, J.T.; Syed, J.; Nguyen, K.A.; Leapman, M.S.; Agarwal, N.; Brierley, K.; Llor, X.; Hofstatter, E.; Shuch, B. Genetic testing for hereditary prostate cancer: Current status and limitations. *Cancer* **2018**, *124*, 3105–3117. [CrossRef] [PubMed]
46. Peltomäki, P. Role of DNA mismatch repair defects in the pathogenesis of human cancer. *J. Clin. Oncol.* **2003**, *21*, 1174–1179. [CrossRef]
47. Cheng, H.H.; Sokolova, A.O.; Schaeffer, E.M.; Small, E.J.; Higano, C.S. Germline and somatic mutations in prostate cancer for the clinician. *JNCCN J. Natl. Compr. Cancer Netw.* **2019**, *17*, 515–521.
48. Javeed, S.; Chughtai, A.; Zafar, G.; Khalid, F.; Batool, A.; Chughtai, A.S. An Evaluation of the Immunohistochemical Expression of Mismatch Repair Proteins (MSH2, MSH6, MLH1, and PMS2) in Prostate Adenocarcinoma. *Cureus* **2022**, *14*. [CrossRef]
49. Sharma, M.; Yang, Z.; Miyamoto, H. Loss of DNA mismatch repair proteins in prostate cancer. *Medicine* **2020**, *99*, e20124. [CrossRef]
50. Khan, H.M.; Cheng, H.H. Germline genetics of prostate cancer. *Prostate* **2022**, *82*, S3–S12. [CrossRef] [PubMed]
51. Marino, F.; Totaro, A.; Gandi, C.; Bientinesi, R.; Moretto, S.; Gavi, F.; Pierconti, F.; Iacovelli, R.; Bassi, P.; Sacco, E. Germline mutations in prostate cancer: A systematic review of the evidence for personalized medicine. *Prostate Cancer Prostatic Dis.* **2022**, 1–10. [CrossRef]
52. Zhang, W.; van Gent, D.C.; Incrocci, L.; van Weerden, W.M.; Nonnekens, J. Role of the DNA damage response in prostate cancer formation, progression and treatment. *Prostate Cancer Prostatic Dis.* **2020**, *23*, 24. [CrossRef] [PubMed]
53. Rodrigues, D.N.; Rescigno, P.; Liu, D.; Yuan, W.; Carreira, S.; Lambros, M.B.; Seed, G.; Mateo, J.; Riisnaes, R.; Mullane, S.; et al. Immunogenomic analyses associate immunological alterations with mismatch repair defects in prostate cancer. *J. Clin. Investig.* **2018**, *128*, 4441–4453. [CrossRef] [PubMed]
54. Jaworski, D.; Gzil, A.; Antosik, P.; Zarębska, I.; Dominiak, J.; Neska-Długosz, I.; Kasperska, A.; Grzanka, D.; Szylberg, L. Expression differences between proteins responsible for DNA damage repair according to the Gleason grade as a new heterogeneity marker in prostate cancer. *Arch. Med. Sci.* **2023**, *19*, 499–506. [CrossRef]
55. Gzil, A.; Jaworski, D.; Antosik, P.; Zarębska, I.; Durślewicz, J.; Dominiak, J.; Kasperska, A.; Neska-Długosz, I.; Grzanka, D.; Szylberg, L. The impact of TP53BP1 and MLH1 on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice. *Urol. Oncol. Semin. Orig. Investig.* **2020**, *38*, 600.e26. [CrossRef]
56. Abida, W.; Armenia, J.; Gopalan, A. Prospective Genomic Profiling of Prostate Cancer Across Disease States Reveals Germline and Somatic Alterations That May Affect Clinical Decision Making. *JCO Precis. Oncol.* **2017**, *2017*, 1–16.
57. Chalmers, Z.R.; Connelly, C.F.; Fabrizio, D.; Gay, L.; Ali, S.M.; Ennis, R.; Schrock, A.; Campbell, B.; Shlien, A.; Chmielecki, J.; et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med.* **2017**, *9*, 1–14. [CrossRef]
58. Risbridger, G.P.; Taylor, R.A.; Clouston, D.; Sliwinski, A.; Thorne, H.; Hunter, S.; Li, J.; Mitchell, G.; Murphy, D.; Frydenberg, M.; et al. Patient-derived xenografts reveal that intraductal carcinoma of the prostate is a prominent pathology in BRCA2 mutation carriers with prostate cancer and correlates with poor prognosis. *Eur. Urol.* **2015**, *67*, 496–503. [CrossRef]
59. Cheng, H.H.; Sokolova, A.O.; Gulati, R.; Bowen, D.; Knerr, S.A.; Klemfuss, N.; Grivas, P.; Hsieh, A.C.; Lee, J.K.; Schweizer, M.T.; et al. Internet-Based Germline Genetic Testing for Men with Metastatic Prostate Cancer. *JCO Precis. Oncol.* **2023**, *7*, e2200104. [CrossRef]

60. Goodwin, J.F.; Schiewer, M.J.; Dean, J.L.; Schrecengost, R.S.; de Leeuw, R.; Han, S.; Ma, T.; Den, R.B.; Dicker, A.P.; Feng, F.Y.; et al. A hormone-DNA repair circuit governs the response to genotoxic insult. *Cancer Discov.* **2013**, *3*, 1254–1271. [CrossRef]
61. Mateo, J.; Carreira, S.; Sandhu, S.; Miranda, S.; Mossop, H.; Perez-Lopez, R.; Nava Rodrigues, D.; Robinson, D.; Omlin, A.; Tunariu, N.; et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. *N. Engl. J. Med.* **2015**, *373*, 1697–1708. [CrossRef]
62. Albero-González, R.; Hernández-Llodrà, S.; Juanpere, N.; Lorenzo, M.; Lloret, A.; Segalés, L.; Duran, X.; Fumadó, L.; Cecchini, L.; Lloreta-Trull, J. Immunohistochemical expression of mismatch repair proteins (MSH2, MSH6, MLH1, and PMS2) in prostate cancer: Correlation with grade groups (WHO 2016) and ERG and PTEN status. *Virchows Arch.* **2019**, *475*, 223–231. [CrossRef]
63. Wilczak, W.; Rashed, S.; Hube-Magg, C. Up-regulation of mismatch repair genes MSH6, PMS2 and MLH1 parallels development of genetic instability and is linked to tumor aggressiveness and early PSA recurrence in prostate cancer. *Carcinogenesis* **2017**, *38*, 19–27. [CrossRef] [PubMed]
64. Fishel, R. Mismatch repair. *J. Biol. Chem.* **2015**, *290*, 26395–26403. [CrossRef] [PubMed]
65. Peltomäki, P. Epigenetic mechanisms in the pathogenesis of Lynch syndrome. *Clin. Genet.* **2014**, *85*, 403–412. [CrossRef] [PubMed]
66. Grindedal, E.M.; Møller, P.; Eeles, R.; Stormorken, A.T.; Bowitz-Lothe, I.M.; Landrø, S.M.; Clark, N.; Kvåle, R.; Shanley, S.; Mæhle, L. Germ-Line Mutations in Mismatch Repair Genes Associated with Prostate Cancer. *Cancer Epidemiol. Biomarkers Prev.* **2009**, *18*, 2460–2467. [CrossRef]
67. Domínguez-Valentin, M.; Joost, P.; Therkildsen, C.; Jonsson, M.; Rambech, E.; Nilbert, M. Frequent mismatch-repair defects link prostate cancer to Lynch syndrome. *BMC Urol.* **2016**, *16*, 15. [CrossRef] [PubMed]
68. Pritchard, C.C.; Morrissey, C.; Kumar, A.; Zhang, X.; Smith, C.; Coleman, I.; Salipante, S.J.; Milbank, J.; Yu, M.; Grady, W.M.; et al. Complex MSH2 and MSH6 mutations in hypermutated microsatellite unstable advanced prostate cancer. *Nat. Commun.* **2014**, *5*, 4988. [CrossRef]
69. Mateo, J.; Boysen, G.; Barbieri, C.E.; Bryant, H.E.; Castro, E.; Nelson, P.S.; Olmos, D.; Pritchard, C.C.; Rubin, M.A.; de Bono, J.S. DNA Repair in Prostate Cancer: Biology and Clinical Implications. *Eur. Urol.* **2017**, *71*, 417–425. [CrossRef]
70. Hansen, A.R.; Massard, C.; Ott, P.A.; Haas, N.B.; Lopez, J.S.; Ejadi, S.; Wallmark, J.M.; Keam, B.; Delord, J.-P.; Aggarwal, R.; et al. Pembrolizumab for advanced prostate adenocarcinoma: Findings of the KEYNOTE-028 study. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* **2018**, *29*, 1807–1813. [CrossRef]
71. Antonarakis, E.S.; Piulats, J.M.; Gross-Goupil, M. Pembrolizumab for Treatment-Refractory Metastatic Castration-Resistant Prostate Cancer: Multicohort, Open-Label Phase II KEYNOTE-199 Study. *J. Clin. Oncol.* **2020**, *38*, 395–405. [CrossRef] [PubMed]
72. Study Record | Beta ClinicalTrials.gov. Available online: https://beta.clinicaltrials.gov/study/NCT03093428?distance=50&cond=pembrolizumabprostate&viewType=Table&limit=100&rank=1&aggFilters=results:with&tab=results (accessed on 15 April 2023).
73. Study Record | Beta ClinicalTrials.gov. Available online: https://beta.clinicaltrials.gov/study/NCT02787005?distance=50&cond=pembrolizumabprostate&viewType=Table&limit=100&rank=2&aggFilters=results:with&tab=results (accessed on 15 April 2023).
74. Study Record | Beta ClinicalTrials.gov. Available online: https://beta.clinicaltrials.gov/study/NCT02601014?distance=50&cond=Nivolumabprostate&viewType=Table&limit=100&rank=3&aggFilters=results:with&tab=results (accessed on 15 April 2023).
75. Study Record | Beta ClinicalTrials.gov. Available online: https://beta.clinicaltrials.gov/study/NCT03016312?distance=50&cond=Atezolizumabprostate&viewType=Table&limit=100&rank=1&aggFilters=results:with&tab=results (accessed on 15 April 2023).
76. Study Record | Beta ClinicalTrials.gov. Available online: https://beta.clinicaltrials.gov/study/NCT04089553?distance=50&cond=durvalumabprostate&viewType=Table&limit=100&page=1&tab=results&rank=8 (accessed on 15 April 2023).
77. Study Record | Beta ClinicalTrials.gov. Available online: https://beta.clinicaltrials.gov/study/NCT03204812?distance=50&cond=durvalumabprostate&viewType=Table&limit=100&rank=1&aggFilters=results:with (accessed on 15 April 2023).
78. Study Record | Beta ClinicalTrials.gov. Available online: https://beta.clinicaltrials.gov/study/NCT00861614?distance=50&cond=ipilimumabprostate&viewType=Table&limit=100&rank=9&aggFilters=results:with&tab=results (accessed on 15 April 2023).
79. Study Record | Beta ClinicalTrials.gov. Available online: https://beta.clinicaltrials.gov/study/NCT01057810?distance=50&cond=ipilimumabprostate&viewType=Table&limit=100&rank=10&aggFilters=results:with&tab=results (accessed on 15 April 2023).
80. Lord, C.J.; Ashworth, A. PARP inhibitors: Synthetic lethality in the clinic. *Science* **2017**, *355*, 1152–1158. [CrossRef] [PubMed]
81. Sandhu, S.K.; Yap, T.A.; de Bono, J.S. The emerging role of poly(ADP-Ribose) polymerase inhibitors in cancer treatment. *Curr. Drug Targets* **2011**, *12*, 2034–2044. [CrossRef] [PubMed]
82. Abida, W.; Patnaik, A.; Campbell, D.; Shapiro, J.; Bryce, A.H.; McDermott, R.; Sautois, B.; Vogelzang, N.J.; Bambury, R.M.; Voog, E.; et al. Rucaparib in Men with Metastatic Castration-Resistant Prostate Cancer Harboring a BRCA1 or BRCA2 Gene Alteration. *J. Clin. Oncol.* **2020**, *38*, 3763–3772. [CrossRef]
83. Study of Olaparib (LynparzaTM) Versus Enzalutamide or Abiraterone Acetate in Men with Metastatic Castration-Resistant Prostate Cancer (PROfound Study)—Study Results—ClinicalTrials.gov. Available online: https://clinicaltrials.gov/ct2/show/results/NCT02987543 (accessed on 15 April 2023).
84. Study Record | Beta ClinicalTrials.gov. Available online: https://beta.clinicaltrials.gov/study/NCT02952534?distance=50&cond=rucaparibprostate&viewType=Table&limit=100&rank=3&aggFilters=results:with&tab=results (accessed on 15 April 2023).
85. Chi, K.N.; Fleshner, N.; Chiuri, V.E. Niraparib with Abiraterone Acetate and Prednisone for Metastatic Castration-Resistant Prostate Cancer: Phase II QUEST Study Results. *Oncologist* **2023**, *28*, e309–e312. [CrossRef]
86. Study Record | Beta ClinicalTrials.gov. Available online: https://beta.clinicaltrials.gov/study/NCT03148795?distance=50&cond=TAlazoparibprostate&viewType=Table&limit=100&rank=2&aggFilters=status:comact&tab=results (accessed on 15 April 2023).

87. Study Record | Beta ClinicalTrials.gov. Available online: https://beta.clinicaltrials.gov/study/NCT01576172?distance=50&cond=Veliparibprostate&viewType=Table&limit=100&rank=2&aggFilters=status:comact&tab=results (accessed on 15 April 2023).
88. Antonarakis, E.S.; Drake, C.G. Combining immunological and androgen-directed approaches: An emerging concept in prostate cancer immunotherapy. *Curr. Opin. Oncol.* **2021**, *24*, 258–265. [CrossRef]
89. Costales, M.G.; Matsumoto, Y.; Velagapudi, S.P.; Disney, M.D. Small Molecule Targeted Recruitment of a Nuclease to RNA. *J. Am. Chem. Soc.* **2018**, *140*, 6741–6744. [CrossRef]
90. Armenia, J.; Wankowicz, S.A.M.; Liu, D.; Gao, J.; Kundra, R.; Reznik, E.; Chatila, W.K.; Chakravarty, D.; Han, G.C.; Coleman, I.; et al. The long tail of oncogenic drivers in prostate cancer. *Nat. Genet.* **2018**, *50*, 645–651. [CrossRef]
91. Kieffer, S.R.; Lowndes, N.F. Immediate-Early, Early, and Late Responses to DNA Double Stranded Breaks. *Front. Genet.* **2022**, *13*. [CrossRef]
92. Caracciolo, D.; Riillo, C.; Di Martino, M.T.; Tagliaferri, P.; Tassone, P. Alternative Non-Homologous End-Joining: Error-Prone DNA Repair as Cancer's Achilles' Heel. *Cancers* **2021**, *13*, 1392. [CrossRef]
93. Lieber, M.R. The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway. *Annu. Rev. Biochem.* **2010**, *79*, 181–211. [CrossRef] [PubMed]
94. Symington, L.S.; Gautier, J. Double-strand break end resection and repair pathway choice. *Annu. Rev. Genet.* **2011**, *45*, 247–271. [CrossRef] [PubMed]
95. Chapman, J.R.; Taylor, M.R.G.; Boulton, S.J. Playing the end game: DNA double-strand break repair pathway choice. *Mol. Cell* **2012**, *47*, 497–510. [CrossRef] [PubMed]
96. Moynahan, M.E.; Chiu, J.W.; Koller, B.H.; Jasint, M. Brca1 controls homology-directed DNA repair. *Mol. Cell* **1999**, *4*, 511–518. [CrossRef]
97. Davies, O.R.; Pellegrini, L. Interaction with the BRCA2 C-terminus Protects RAD51–DNA Filaments from Disassembly by BRC Repeats. *Nat. Struct. Mol. Biol.* **2007**, *14*, 475. [CrossRef]
98. Blanc-Durand, F.; Yaniz-Galende, E.; Llop-Guevara, A.; Genestie, C.; Serra, V.; Herencia-Ropero, A.; Klein, C.; Berton, D.; Lortholary, A.; Dohollou, N.; et al. A RAD51 functional assay as a candidate test for homologous recombination deficiency in ovarian cancer. *Gynecol. Oncol.* **2023**, *171*, 106–113. [CrossRef]
99. Kunkel, T.A.; Erie, D.A. DNA mismatch repair. *Annu. Rev. Biochem.* **2005**, *74*, 681–710. [CrossRef]
100. Ceccaldi, R.; Rondinelli, B.; D'Andrea, A.D. Repair Pathway Choices and Consequences at the Double-Strand Break. *Trends Cell Biol.* **2016**, *26*, 52–64. [CrossRef]
101. Curtin, N.J. DNA repair dysregulation from cancer driver to therapeutic target. *Nat. Rev. Cancer* **2012**, *12*, 801–817. [CrossRef]
102. Ciccia, A.; Elledge, S.J. The DNA Damage Response: Making It Safe to Play with Knives. *Mol. Cell* **2010**, *40*, 179–204. [CrossRef]
103. Castro, E.; Eeles, R. The role of BRCA1 and BRCA2 in prostate cancer. *Asian J. Androl.* **2012**, *14*, 409–414. [CrossRef] [PubMed]
104. Leongamornlert, D.; Mahmud, N.; Tymrakiewicz, M.; Saunders, E.; Dadaev, T.; Castro, E.; Goh, C.; Govindasami, K.; Guy, M.; O'Brien, L.; et al. Germline BRCA1 mutations increase prostate cancer risk. *Br. J. Cancer* **2012**, *106*, 1697. [CrossRef]
105. Zhu, H.; Ding, Y.; Huang, H.; Lin, Q.; Chen, W.; Yu, Z. Prognostic value of genomic mutations in metastatic prostate cancer. *Heliyon* **2023**, *9*, e13827. [CrossRef]
106. Castro, E.; Goh, C. Germline BRCA mutations are associated with higher risk of nodal involvement, distant metastasis, and poor survival outcomes in prostate cancer. *J. Clin. Oncol.* **2013**, *31*, 1748–1757. [CrossRef] [PubMed]
107. Pomerantz, M.M.; Spisák, S.; Jia, L.; Cronin, A.M.; Csabai, I.; Ledet, E.; Sartor, A.O.; Rainville, I.; O'Connor, E.P.; Herbert, Z.T.; et al. The association between germline BRCA2 variants and sensitivity to platinum-based chemotherapy among men with metastatic prostate cancer. *Cancer* **2017**, *123*, 3532–3539. [CrossRef]
108. FDA Grants Accelerated Approval to Rucaparib for BRCA-Mutated Metastatic Castration-Resistant Prostate Cancer | FDA. Available online: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-rucaparib-brca-mutated-metastatic-castration-resistant-prostate (accessed on 15 April 2023).
109. Antonarakis, E.S.; Gomella, L.G.; Petrylak, D.P. When and How to Use PARP Inhibitors in Prostate Cancer: A Systematic Review of the Literature with an Update on On-Going Trials. *Eur. Urol. Oncol.* **2020**, *3*, 594–611. [CrossRef] [PubMed]
110. Stucki, M.; Clapperton, J.A.; Mohammad, D.; Yaffe, M.B.; Smerdon, S.J.; Jackson, S.P. MDC1 directly binds phosphorylated histone H2AX to regulate cellular responses to DNA double-strand breaks. *Cell* **2005**, *123*, 1213–1226. [CrossRef] [PubMed]
111. Zou, R.; Zhong, X.; Wang, C.; Sun, H.; Wang, S.; Lin, L.; Sun, S.; Tong, C.; Luo, H.; Gao, P.; et al. MDC1 Enhances Estrogen Receptor-mediated Transactivation and Contributes to Breast Cancer Suppression. *Int. J. Biol. Sci.* **2015**, *11*, 992–1005. [CrossRef]
112. Wang, C.; Sun, H. MDC1 functionally identified as an androgen receptor co-activator participates in suppression of prostate cancer. *Nucleic Acids Res.* **2015**, *43*, 4893–4908. [CrossRef]
113. Pugh, T.J.; Keyes, M.; Barclay, L.; Delaney, A.; Krzywinski, M.; Thomas, D.; Novik, K.; Yang, C.; Agranovich, A.; McKenzie, M.; et al. Sequence variant discovery in DNA repair genes from radiosensitive and radiotolerant prostate brachytherapy patients. *Clin. Cancer Res.* **2009**, *15*, 5008–5016. [CrossRef]
114. Tsujino, T.; Takai, T.; Hinohara, K.; Gui, F.; Tsutsumi, T.; Bai, X.; Miao, C.; Feng, C.; Bin Gui, B.; Sztupinszki, Z.; et al. CRISPR screens reveal genetic determinants of PARP inhibitor sensitivity and resistance in prostate cancer. *Nat. Commun.* **2023**, *14*, 252. [CrossRef] [PubMed]
115. Mitra, A.; Jameson, C. Over-expression of RAD51 occurs in aggressive prostate cancer. *Histopathology* **2009**, *55*, 696. [CrossRef]

116. Bhattacharya, S.; Srinivasan, K.; Abdisalaam, S.; Su, F.; Raj, P.; Dozmorov, I.; Mishra, R.; Wakeland, E.K.; Ghose, S.; Mukherjee, S.; et al. RAD51 interconnects between DNA replication, DNA repair and immunity. *Nucleic Acids Res.* **2017**, *45*, 4590. [CrossRef]
117. Hine, C.M.; Seluanov, A.; Gorbunova, V. Use of the Rad51 promoter for targeted anti-cancer therapy. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 20810–20815. [CrossRef]
118. Fan, R.; Kumaravel, T.S. Defective DNA strand break repair after DNA damage in prostate cancer cells: Implications for genetic instability and prostate cancer progression. *Cancer Res.* **2004**, *64*, 8526–8533. [CrossRef]
119. McCarthy-Leo, C.; Darwiche, F.; Tainsky, M.A. DNA Repair Mechanisms, Protein Interactions and Therapeutic Targeting of the MRN Complex. *Cancers* **2022**, *14*, 5278. [CrossRef] [PubMed]
120. Wang, J.; Xu, W.-H.; Wei, Y.; Zhu, Y.; Qin, X.-J.; Zhang, H.-L.; Ye, D.-W. Elevated MRE11 expression associated with progression and poor outcome in prostate cancer. *J. Cancer* **2019**, *10*, 4333–4340. [CrossRef]
121. Oplustilova, L.; Wolanin, K.; Mistrik, M.; Korinkova, G.; Simkova, D.; Bouchal, J.; Lenobel, R.; Bartkova, J.; Lau, A.; O'connor, M.J.; et al. Evaluation of candidate biomarkers to predict cancer cell sensitivity or resistance to PARP-1 inhibitor treatment. *Cell Cycle* **2012**, *11*, 3837–3850. [CrossRef]
122. Stinson, B.M.; Loparo, J.J. Repair of DNA Double-Strand Breaks by the Non-homologous End Joining Pathway. *Annu. Rev. Biochem.* **2021**, *90*, 137. [CrossRef] [PubMed]
123. Chapman, J.R.; Barral, P. RIF1 is essential for 53BP1-dependent nonhomologous end joining and suppression of DNA double-strand break resection. *Mol. Cell* **2013**, *49*, 858–871. [CrossRef]
124. Vousden, K.H.; Prives, C. Blinded by the Light: The Growing Complexity of p53. *Cell* **2009**, *137*, 413–431. [CrossRef] [PubMed]
125. Kakarougkas, A.; Jeggo, P.A. DNA DSB repair pathway choice: An orchestrated handover mechanism. *Br. J. Radiol.* **2014**, *87*, 20130685. [CrossRef] [PubMed]
126. Mateos-Gomez, P.A.; Gong, F.; Nair, N.; Miller, K.M.; Lazzerini-Denchi, E.; Sfeir, A. Mammalian polymerase θ promotes alternative NHEJ and suppresses recombination. *Nature* **2015**, *518*, 254–257. [CrossRef]
127. Kurfurstova, D.; Bartkova, J.; Vrtel, R.; Mickova, A.; Burdova, A.; Majera, D.; Mistrik, M.; Kral, M.; Santer, F.R.; Bouchal, J.; et al. DNA damage signalling barrier, oxidative stress and treatment-relevant DNA repair factor alterations during progression of human prostate cancer. *Mol. Oncol.* **2016**, *10*, 879–894. [CrossRef] [PubMed]
128. Kallakury, B.V.; Jennings, T.A.; Ross, J.S.; Breese, K.; Figge, H.L.; Fisher, H.A.; Figge, J. Alteration of the p53 locus in benign hyperplastic prostatic epithelium associated with high-grade prostatic adenocarcinoma. *Diagnostic Mol. Pathol.* **1994**, *3*, 227–232. [CrossRef] [PubMed]
129. Lewinska, A.; Jarosz, P.; Czech, J.; Rzeszutek, I.; Bielak-Zmijewska, A.; Grabowska, W.; Wnuk, M. Capsaicin-induced genotoxic stress does not promote apoptosis in A549 human lung and DU145 prostate cancer cells. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.* **2015**, *779*, 23–34. [CrossRef] [PubMed]
130. Polkinghorn, W.R.; Parker, J.S.; Lee, M.X.; Kass, E.M.; Spratt, D.E.; Iaquinta, P.J.; Arora, V.K.; Yen, W.-F.; Cai, L.; Zheng, D.; et al. Androgen receptor signaling regulates DNA repair in prostate cancers. *Cancer Discov.* **2013**, *3*, 1245–1253. [CrossRef]
131. Gou, Q.; Xie, Y.; Liu, L.; Xie, K.; Wu, Y.; Wang, Q.; Wang, Z.; Li, P. Downregulation of MDC1 and 53BP1 by short hairpin RNA enhances radiosensitivity in laryngeal carcinoma cells. *Oncol. Rep.* **2015**, *34*, 251–257. [CrossRef] [PubMed]
132. Xiao, Y.; Zheng, X.; Huang, A.; Liu, T.; Zhang, T.; Ma, H. Deficiency of 53BP1 inhibits the radiosensitivity of colorectal cancer. *Int. J. Oncol.* **2016**, *49*, 1600–1608. [CrossRef]
133. Chipidza, F.E.; Alshalalfa, M. Development and Validation of a Novel TP53 Mutation Signature That Predicts Risk of Metastasis in Primary Prostate Cancer. *Clin. Genitourin. Cancer* **2021**, *19*, 246–254.e5. [CrossRef]
134. Mayeur, G.L.; Kung, W.-J.; Martinez, A.; Izumiya, C.; Chen, D.J.; Kung, H.-J. Ku is a novel transcriptional recycling coactivator of the androgen receptor in prostate cancer cells. *J. Biol. Chem.* **2005**, *280*, 10827–10833. [CrossRef] [PubMed]
135. Al-Ubaidi, F.L.T.; Schultz, N.; Loseva, O.; Egevad, L.; Granfors, T.; Helleday, T. Castration therapy results in decreased Ku70 levels in prostate cancer. *Clin. Cancer Res.* **2013**, *19*, 1547–1556. [CrossRef] [PubMed]
136. Hasegawa, T.; Masanori Someya, M.D.; Masakazu Hori, M.D. Expression of Ku70 predicts results of radiotherapy in prostate cancer. *Strahlenther. Onkol.* **2017**, *193*, 29–37. [CrossRef]
137. Kothari, V.; Goodwin, J.F.; Zhao, S.G.; Drake, J.M.; Yin, Y.; Chang, S.L.; Evans, J.R.; Wilder-Romans, K.; Gabbara, K.; Dylgjeri, E.; et al. DNA-Dependent Protein Kinase Drives Prostate Cancer Progression through Transcriptional Regulation of the Wnt Signaling Pathway. *Clin. Cancer Res.* **2019**, *25*, 5608–5622. [CrossRef] [PubMed]
138. Dylgjeri, E.; Kothari, V.; Shafi, A.A.; Semenova, G.; Gallagher, P.T.; Guan, Y.F.; Pang, A.; Goodwin, J.F.; Irani, S.; McCann, J.J.; et al. A Novel Role for DNA-PK in Metabolism by Regulating Glycolysis in Castration-Resistant Prostate Cancer. *Clin. Cancer Res.* **2022**, *28*, 1446–1459. [CrossRef]
139. Pu, J.; Li, T. PLCε knockdown enhances the radiosensitivity of castration-resistant prostate cancer via the AR/PARP1/DNA-PKcs axis. *Oncol. Rep.* **2020**, *43*, 1397–1412. [CrossRef]
140. Pan-Hammarström, Q.; Jones, A.M.; Lähdesmäki, A. Impact of DNA ligase IV on nonhomologous end joining pathways during class switch recombination in human cells. *J. Exp. Med.* **2005**, *201*, 189–194. [CrossRef]
141. Damaraju, S.; Murray, D.; Dufour, J. Association of DNA repair and steroid metabolism gene polymorphisms with clinical late toxicity in patients treated with conformal radiotherapy for prostate cancer. *Clin. Cancer Res.* **2006**, *12*, 2545–2554. [CrossRef]
142. Grupp, K.; Roettger, L.; Kluth, M. Expression of DNA ligase IV is linked to poor prognosis and characterizes a subset of prostate cancers harboring TMPRSS2:ERG fusion and PTEN deletion. *Oncol. Rep.* **2015**, *34*, 1211–1220. [CrossRef]

143. Chedgy, E.C.P.; Vandekerkhove, G.; Herberts, C. Biallelic tumour suppressor loss and DNA repair defects in de novo small-cell prostate carcinoma. *J. Pathol.* **2018**, *246*, 244–253. [CrossRef]
144. Chiang, P.-K.; Tsai, W.-K.; Chen, M.; Lin, W.-R.; Chow, Y.-C.; Lee, C.-C.; Hsu, J.-M.; Chen, Y.-J. Zerumbone Regulates DNA Repair Responding to Ionizing Radiation and Enhances Radiosensitivity of Human Prostatic Cancer Cells. *Integr. Cancer Ther.* **2018**, *17*, 292–298. [CrossRef] [PubMed]
145. Dariane, C.; Timsit, M.O. DNA-Damage-Repair Gene Alterations in Genitourinary Malignancies. *Eur. Surg. Res.* **2022**, *63*, 155–164. [CrossRef]
146. Fan, L.; Fei, X.; Zhu, Y. Distinct Response to Platinum-Based Chemotherapy among Patients with Metastatic Castration-Resistant Prostate Cancer Harboring Alterations in Genes Involved in Homologous Recombination. *J. Urol.* **2021**, *206*, 630–637. [CrossRef]
147. Liu, N.; Lamerdin, J.E.; Tebbs, R.S.; Schild, D.; Tucker, J.D.; Shen, M.; Brookman, K.W.; Siciliano, M.J.; Walter, C.A.; Fan, W.; et al. XRCC2 and XRCC3, new human Rad51-family members, promote chromosome stability and protect against DNA cross-links and other damages. *Mol. Cell* **1998**, *1*, 783–793. [CrossRef] [PubMed]
148. Rybicki, B.A.; Conti, D.V.; Moreira, A. DNA repair gene XRCC1 and XPD polymorphisms and risk of prostate cancer. *Cancer Epidemiol. Biomarkers Prev.* **2004**, *13*, 23–29. [CrossRef] [PubMed]
149. Mališić, E.; Petrović, N.; Brengues, M. Association of polymorphisms in TGFB1, XRCC1, XRCC3 genes and CD8 T-lymphocyte apoptosis with adverse effect of radiotherapy for prostate cancer. *Sci. Rep.* **2022**, *12*, 21306. [CrossRef] [PubMed]
150. Mohler, J.L.; Antonarakis, E.S.; Armstrong, A.J. Prostate Cancer, Version 2. 2019, NCCN Clinical Practice Guidelines in Oncology. *J. Natl. Compr. Canc. Netw.* **2019**, *17*, 479–505. [CrossRef]
151. Prostate Cance—INTRODUCTION—Uroweb. Available online: https://uroweb.org/guidelines/prostate-cancer (accessed on 15 April 2023).
152. Clements, M.B.; Vertosick, E.A.; Guerrios-Rivera, L. Defining the Impact of Family History on Detection of High-grade Prostate Cancer in a Large Multi-institutional Cohort. *Eur. Urol.* **2022**, *82*, 163–169. [CrossRef]
153. Carter, H.B.; Helfand, B.; Mamawala, M. Germline Mutations in ATM and BRCA1/2 Are Associated with Grade Reclassification in Men on Active Surveillance for Prostate Cancer. *Eur. Urol.* **2019**, *75*, 743–749. [CrossRef]
154. Halstuch, D.; Ber, Y.; Kedar, D.; Golan, S.; Baniel, J.; Margel, D. Short-Term Outcomes of Active Surveillance for Low Risk Prostate Cancer among Men with Germline DNA Repair Gene Mutations. *J. Urol.* **2020**, *204*, 707–712. [CrossRef]
155. The ICGC/TCGA Pan-Cancer Analysis of Whole Genomes Consortium. Pan-cancer analysis of whole genomes. *Nature* **2020**, *578*, 82–93. [CrossRef] [PubMed]
156. Tosh, J. PROFOUND trial -a new era in targeted therapeutics for prostate carcinoma. *Indian J. Urol.* **2022**, *38*, 73–74. [CrossRef] [PubMed]
157. Thoma, C. Targeting DNA repair defects in prostate cancer. *Nat. Rev. Urol.* **2020**, *17*, 432. [CrossRef] [PubMed]
158. Castro, E.; Romero-Laorden, N.; Del Pozo, A. PROREPAIR-B: A Prospective Cohort Study of the Impact of Germline DNA Repair Mutations on the Outcomes of Patients with Metastatic Castration-Resistant Prostate Cancer. *J. Clin. Oncol.* **2019**, *37*, 490–503. [CrossRef] [PubMed]
159. Stone, L. PARP inhibitor response in prostate cancer. *Nat. Rev. Urol.* **2023**, *20*, 130. [CrossRef] [PubMed]
160. Mateo, J.; Porta, N.; Bianchini, D. Olaparib in patients with metastatic castration-resistant prostate cancer with DNA repair gene aberrations (TOPARP-B): A multicentre, open-label, randomised, phase 2 trial. *Lancet Oncol.* **2020**, *21*, 162–174. [CrossRef]
161. de Bono, J.; Mateo, J.; Fizazi, K. Olaparib for Metastatic Castration-Resistant Prostate Cancer. *N. Engl. J. Med.* **2020**, *382*, 2091–2102. [CrossRef]
162. Smith, M.R.; Sandhu, S.K.; Kelly, W.K.; Scher, H.I.; Efstathiou, E.; Lara, P.N.; Yu, E.Y.; George, D.J.; Chi, K.N.; Saad, F.; et al. Pre-specified interim analysis of GALAHAD: A phase II study of niraparib in patients (pts) with metastatic castration-resistant prostate cancer (mCRPC) and biallelic DNA-repair gene defects (DRD). *Ann. Oncol.* **2019**, *30*, v884–v885. [CrossRef]
163. Antonarakis, E.S.; Velho, P.I.; Fu, W.; Wang, H.; Agarwal, N.; Santos, V.S.; Maughan, B.L.; Pili, R.; Adra, N.; Sternberg, C.N.; et al. CDK12 -Altered Prostate Cancer: Clinical Features and Therapeutic Outcomes to Standard Systemic Therapies, Poly (ADP-Ribose) Polymerase Inhibitors, and PD-1 Inhibitors. *JCO Precis. Oncol.* **2020**, *4*, 370–381. [CrossRef]
164. de Bono, J.; Fizazi, K.; Saad, F.; Shore, N.; Sandhu, S.; Mehra, N.; Kolinsky, M.; Roubaud, G.; Özgüroğlu, M.; Matsubara, N.; et al. Central, prospective detection of homologous recombination repair gene mutations (HRRm) in tumour tissue from >4000 men with metastatic castration-resistant prostate cancer (mCRPC) screened for the PROfound study. *Ann. Oncol.* **2019**, *30*, v328–v329. [CrossRef]
165. Powles, T.; Yuen, K.C.; Gillessen, S.; Kadel, E.E.; Rathkopf, D.; Matsubara, N.; Drake, C.G.; Fizazi, K.; Piulats, J.M.; Wysocki, P.J.; et al. Atezolizumab with enzalutamide versus enzalutamide alone in metastatic castration-resistant prostate cancer: A randomized phase 3 trial. *Nat. Med.* **2022**, *28*, 144–153. [CrossRef]
166. Shenderov, E.; Boudadi, K.; Fu, W. Nivolumab plus ipilimumab, with or without enzalutamide, in AR-V7-expressing metastatic castration-resistant prostate cancer: A phase-2 nonrandomized clinical trial. *Prostate* **2021**, *81*, 326–338. [CrossRef] [PubMed]
167. Sweeney, C.; Bracarda, S.; Sternberg, C.N. Ipatasertib plus abiraterone and prednisolone in metastatic castration-resistant prostate cancer (IPATential150): A multicentre, randomised, double-blind, phase 3 trial. *Lancet* **2021**, *398*, 131–142. [CrossRef] [PubMed]
168. Sciarra, A.; Frisenda, M.; Bevilacqua, G.; Gentilucci, A.; Cattarino, S.; Mariotti, G.; Del Giudice, F.; Di Pierro, G.B.; Viscuso, P.; Casale, P.; et al. How the Analysis of the Pathogenetic Variants of DDR Genes Will Change the Management of Prostate Cancer Patients. *Int. J. Mol. Sci.* **2023**, *24*, 674. [CrossRef]

169. Capoluongo, E.; Ellison, G.; López-Guerrero, J.A. Guidance Statement on BRCA1/2 Tumor Testing in Ovarian Cancer Patients. *Semin. Oncol.* **2017**, *44*, 187–197. [CrossRef]
170. Tukachinsky, H.; Madison, R.W.; Chung, J.H. Genomic analysis of circulating tumor DNA in patients with advanced prostate cancer identifies targetable BRCA alterations and AR resistance mechanisms. *Clin. Cancer Res.* **2021**, *27*, 3094. [CrossRef] [PubMed]
171. Hatano, K.; Nonomura, N. Genomic Profiling of Prostate Cancer: An Updated Review. *World J. Mens. Health* **2022**, *40*, 368. [CrossRef]
172. Catalano, M.; Generali, D.; Gatti, M.; Riboli, B.; Paganini, L.; Nesi, G.; Roviello, G. DNA repair deficiency as circulating biomarker in prostate cancer. *Front. Oncol.* **2023**, *13*, 1115241. [CrossRef]
173. ASCO GU 2023: New Targets, New Concepts for Metastatic Castration-Resistant Prostate Cancer. Available online: https://www.urotoday.com/conference-highlights/asco-gu-2023/asco-gu-2023-prostate-cancer/142464-asco-gu-2023-new-targets-new-concepts-for-metastatic-castration-resistant-prostate-cancer.html (accessed on 15 April 2023).

Rozdział 5. Podsumowanie

Z uwagi na ograniczenia dotychczasowych badań dotyczących wpływu zaburzeń mechanizmów naprawy DNA na rokowanie pacjentów z PCa oraz ich potencjalnego wpływu na dobór strategii terapeutycznych, w niniejszej rozprawie doktorskiej skoncentrowano się na analizie ekspresji białek kluczowych dla szlaków naprawy MMR i DSBR w PCa. Badanie miało również na celu ocenę wpływu tych białek na rozwój przerzutów oraz na skuteczność nowoczesnych terapii, takich jak inhibitory PARP i inhibitory punktów kontrolnych cyklu komórkowego.

Pierwsza publikacja włączona do cyklu koncentrowała się na porównaniu ekspresji białek MLH1, MSH2, MSH6, PMS2, MDC1 i TP53BP1 w tkance guza pierwotnego PCa bez przerzutów do węzłów chłonnych (pN0), w tkance PCa z przerzutami do węzłów chłonnych (pN+) oraz w tkance węzła chłonnego z przerzutem nowotworowym. W przedstawionej pracy wykazano istotnie niższą ekspresję białek TP53BP1 i MLH1 w tkance PCa z przerzutami do węzłów chłonnych w porównaniu do guzów bez przerzutów. Ponadto, ekspresja TP53BP1 była niższa w tkance węzła chłonnego z przerzutem raka w porównaniu z ekspresją tego białka w tkance guza pierwotnego PCa. Wyniki przeprowadzonego badania sugerują, że zaburzenia w obrębie szlaków naprawczych DNA, MMR oraz DSBR mają istotny wpływ na powstawanie przerzutów do węzłów chłonnych. Wnioski te korespondują z wynikami badań Kurfurstova i współautorów, którzy wykazali, że spadek ekspresji TP53BP1 wiąże się z progresją łagodnych zmian prostaty do PCa[41]. Uzyskane rezultaty mogą stanowić podstawę do dalszych badań mających na celu udoskonalenie postępowania terapeutycznego, w tym włączenie pacjentów z zaburzeniami ekspresji badanych białek do bardziej spersonalizowanego leczenia. Ponadto, wykazane zależności pomiędzy ekspresją białek TP53BP1 oraz MLH1, a obecnością przerzutów PCa do węzłów chłonnych mogą w przyszłości posłużyć jako markery prognostyczne dla pacjentów z PCa. Dodatkowo, liczne badania naukowe donoszą, iż podobnie jak w przypadku

raka jasnokomórkowego nerki, PCa wykazuje istotną heterogenność komórkową w obrębie nowotworu[42,43]. Dalsze badania nad modelem ewolucji komórek nowotworowych w kontekście heterogenności oraz w procesie immunoedycji nowotworu mogą rzucić światło na mechanizmy prowadzące do powstawania mCRPC[43,44]. Uzyskane wyniki mogą w konsekwencji stanowić solidną podstawę do dalszych badań nad znaczeniem immunoedycji w progresji PCa, otwierając nowe perspektywy dla terapii celowanej.

Druga oryginalna praca badawcza dotyczyła porównania ekspresji wybranych białek szlaków naprawy uszkodzeń DNA w odniesieniu do stopnia złośliwości histologicznej ocenianej za pomocą GS oraz GP. Wykazano pozytywną korelację pomiędzy ekspresją jądrową i cytoplazmatyczną białka MSH2, a GS. Ponadto, udowodniono negatywną korelację pomiędzy cytoplazmatyczną ekspresją białka MDC1, a GS. Dodatkowo, oceniona została korelacja pomiędzy wartością dominującego GP, a ekspresją wybranych białek. Analiza statystyczna wykazała negatywną korelację pomiędzy ekspresją jądrową białka MSH2, a GP oraz dodatnią korelację pomiędzy cytoplazmatyczną ekspresją białka MLH1, a GP. Różnice korelacji poziomu ekspresji tych białek, pomiędzy GS oraz GP wewnątrz jednego guza są zgodne z dotychczasowymi badaniami podkreślającymi heterogenność PCa[42]. Uzyskane wyniki pozwalają przypuszczać większą rolę zaburzeń w obrębie szlaku MMR (MLH1 oraz MSH2), niż DSBR (TP53BP1 oraz MDC1) w wyższym stopniu złośliwości histologicznej PCa, a tym samym rokowania pacjentów. W badaniu nie wykazano korelacji ekspresji TP53BP1 ze stopniem złośliwości histologicznej, co może warunkować odpowiedź PCa na radioterapię. Zgodnie z doniesieniami naukowymi, w których wykazano częstsze nawroty po radioterapii wraz ze wzrostem stopnia złośliwości histologicznej ocenianej w GS[45,46].

Trzecią pracą włączoną do cyklu jest praca przeglądowa w której dokonano kompleksowego omówienia mechanizmów molekularnych leżących u podstaw zaburzeń DSBR i MMR w PCa, jak również ich klinicznych implikacji.

Scharakteryzowano białka, które dotychczas zostały przebadane w kontekście wpływu na rozwój PCa. Ponadto, omówiono oddziaływanie zaburzeń tych mechanizmów na skuteczność inhibitorów punktów kontrolnych układu odpornościowego oraz inhibitorów PARP w leczeniu PCa oraz dalsze perspektywy rozwoju tych terapii. Przedstawiono podsumowanie dotychczasowych badań klinicznych leków z grup inhibitorów PARP i inhibitorów punktów kontrolnych. Szczegółowo omówiono dwa leki z grupy inhibitorów PARP, takich jak olaparib i rucaparib, które w ostatnich latach uzyskały akceptację FDA w leczeniu mCRPC. Ekspresja białek TP53BP1 oraz MLH1 może w istotny sposób wpływać na zdolność przerzutowania nowotworu, co stanowiłoby wartość predykcyjną dla progresji PCa. W badaniach klinicznych wykazano istotną skuteczność inhibitorów PARP wśród pacjentów z zaburzeniami mechanizmów naprawy DNA na wpływ na przeżycie i zahamowanie progresji choroby nowotworowej. Przeprowadzone w ramach rozprawy doktorskiej badania dostarczają cennych danych na temat roli białek szlaków naprawy DNA w PCa, podkreślając ich potencjalny wpływ na rokowanie oraz skuteczność terapii. Analizy ekspresji białek MMR i DSBR w kontekście korelacji z GS i GP, przewidywanego przebiegu choroby i jej leczenia, wskazują na złożoność mechanizmów molekularnych PCa oraz odpowiedzi na leczenie. Wyniki badań przeprowadzonych przez nas oraz innych naukowców pozwalają wnioskować, iż zaburzenia w obrębie szlaków MMR i DSBR mogą wpływać na stopień złośliwości histologicznej oraz przyczyniać się do rozwoju przerzutów. Badania z zakresu zaburzeń mechanizmów naprawy DNA w PCa przyczyniły się między innymi do rozpoczęcia badań klinicznych nad nowymi terapiami, które w ostatnim czasie poskutkowały zaakceptowaniem przez FDA i wprowadzeniem do leczenia nowych preparatów celujących w zaburzenia mechanizmów naprawy DNA w PCa. Wyniki te otwierają drogę do dalszych badań, które mogą prowadzić do rozwoju nowych biomarkerów i strategii terapeutycznych, bardziej spersonalizowanych i skutecznych w walce z tą chorobą.

Rozdział 6. Wnioski

1. Rak gruczołu krokowego z przerzutami do węzłów chłonnych charakteryzuje się niższą ekspresją białek TP53BP1 i MLH1 niż rak gruczołu krokowego bez przerzutów.
2. Przerzuty raka gruczołu krokowego do węzłów chłonnych cechują się niższą ekspresją białka TP53BP1 niż guzy pierwotne.
3. Wraz ze wzrostem punktacji w skali Gleasona, wzrasta poziom ekspresji jądrowej i cytoplazmatycznej białka MSH2.
4. Wartość wzoru architektonicznego Gleasona negatywnie koreluje z jądrową ekspresją białka MSH2.
5. Wraz ze wzrostem punktacji w skali Gleasona, obniża się ekspresja cytoplazmatyczna białka MDC1.
6. Punktacja architektoniki Gleasona pozytywnie koreluje z cytoplazmatyczną ekspresją białka MLH1.
7. Zaburzenia mechanizmów naprawy DNA spowodowane zmianami ekspresji białek szlaków MMR i DSBR zwiększają skuteczność inhibitorów PARP oraz inhibitorów punktów kontrolnych cyklu komórkowego w leczeniu raka gruczołu krokowego.

Rozdział 7. Bibliografia

1. Nowotwory Złośliwe w Polsce | Krajowy Rejestr Nowotworów Available online: https://onkologia.org.pl/pl/epidemiologia/nowotwory-zlosliwe-w-polsce (accessed on 16 September 2023).

2. Kirby, M.; Hirst, C.; Crawford, E.D. Characterising the Castration-Resistant Prostate Cancer Population: A Systematic Review. *Int. J. Clin. Pract.* **2011**, *65*, 1180–1192, doi:10.1111/J.1742-1241.2011.02799.X.

3. Baretti, M.; Le, D.T. DNA Mismatch Repair in Cancer. *Pharmacol. Ther.* **2018**, *189*, 45–62, doi:10.1016/J.PHARMTHERA.2018.04.004.

4. Gillyard, T.; Davis, J. DNA Double-Strand Breaks Repair in Cancer: A Path to Achieving Precision Medicine. *Int. Rev. Cell Mol. Biol.* **2021**, *364*, 111, doi:10.1016/BS.IRCMB.2021.06.003.

5. Wang, Z.A.; Mitrofanova, A.; Bergren, S.K.; Abate-Shen, C.; Cardiff, R.D.; Califano, A.; Shen, M.M. Lineage Analysis of Basal Epithelial Cells Reveals Their Unexpected Plasticity and Supports a Cell of Origin Model for Prostate Cancer Heterogeneity. *Nat. Cell Biol.* **2013**, *15*, 274, doi:10.1038/NCB2697.

6. Smith, B.A.; Sokolov, A.; Uzunangelov, V.; Baertsch, R.; Newton, Y.; Graim, K.; Mathis, C.; Cheng, D.; Stuart, J.M.; Witte, O.N. A Basal Stem Cell Signature Identifies Aggressive Prostate Cancer Phenotypes. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, E6544–E6552, doi:10.1073/PNAS.1518007112/-/DCSUPPLEMENTAL.

7. Wang, G.; Zhao, D.; Spring, D.J.; Depinho, R.A. Genetics and Biology of Prostate Cancer. *Genes Dev.* **2018**, *32*, 1105–1140, doi:10.1101/GAD.315739.118.

8. Sfanos, K.S.; Yegnasubramanian, S.; Nelson, W.G.; De Marzo, A.M. The Inflammatory Microenvironment and Microbiome in Prostate Cancer Development. *Nat. Rev. Urol.* **2018**, *15*, 11–24, doi:10.1038/NRUROL.2017.167.

9. Zebian, A.; Shaito, A.; Mazurier, F.; Rezvani, H.R.; Zibara, K. XPC beyond Nucleotide Excision Repair and Skin Cancers. *Mutat. Res. Mutat. Res.* **2019**,

782, 108286, doi:10.1016/J.MRREV.2019.108286.

10. Sharma, R.; Lewis, S.; Wlodarski, M.W. DNA Repair Syndromes and Cancer: Insights Into Genetics and Phenotype Patterns. *Front. Pediatr.* **2020**, *8*, doi:10.3389/FPED.2020.570084.

11. Baretti, M.; Le, D.T. DNA Mismatch Repair in Cancer. *Pharmacol. Ther.* **2018**, *189*, 45–62, doi:10.1016/J.PHARMTHERA.2018.04.004.

12. METAXAS, G.I.; TSIAMBAS, E.; MARINOPOULOS, S.; ADAMOPOULOU, M.; SPYROPOULOU, D.; FALIDAS, E.; DAVRIS, D.; MANAIOS, L.; FOTIADES, P.; MASTRONIKOLI, S.; et al. DNA Mismatch Repair System Imbalances in Breast Adenocarcinoma. *Cancer Diagnosis Progn.* **2023**, *3*, 169, doi:10.21873/CDP.10197.

13. McEllin, B.; Camacho, C. V.; Mukherjee, B.; Hahm, B.; Tomimatsu, N.; Bachoo, R.M.; Burma, S. PTEN Loss Compromises Homologous Recombination Repair in Astrocytes: Implications for Glioblastoma Therapy with Temozolomide or Poly(ADP-Ribose) Polymerase Inhibitors. *Cancer Res.* **2010**, *70*, 5457–5464, doi:10.1158/0008-5472.CAN-09-4295.

14. Li, J.; Yen, C.; Liaw, D.; Podsypanina, K.; Bose, S.; Wang, S.I.; Puc, J.; Miliaresis, C.; Rodgers, L.; McCombie, R.; et al. PTEN, a Putative Protein Tyrosine Phosphatase Gene Mutated in Human Brain, Breast, and Prostate Cancer. *Science* **1997**, *275*, 1943–1947, doi:10.1126/SCIENCE.275.5308.1943.

15. Lee, M.; Je, I.G.; Kim, J.E.; Yoo, Y.; Lim, J.H.; Jang, E.; Lee, Y.; Song, D.K.; Moon, A.N.; Kim, J.A.; et al. Venadaparib Is a Novel and Selective PARP Inhibitor with Improved Physicochemical Properties, Efficacy, and Safety. *Mol. Cancer Ther.* **2023**, *22*, 333, doi:10.1158/1535-7163.MCT-22-0068.

16. Mendes-Pereira, A.M.; Martin, S.A.; Brough, R.; McCarthy, A.; Taylor, J.R.; Kim, J.S.; Waldman, T.; Lord, C.J.; Ashworth, A. Synthetic Lethal Targeting of PTEN Mutant Cells with PARP Inhibitors. *EMBO Mol. Med.* **2009**, *1*, 315–322, doi:10.1002/EMMM.200900041.

17. Tutt, A.; Robson, M.; Garber, J.E.; Domchek, S.M.; Audeh, M.W.; Weitzel, J.N.; Friedlander, M.; Arun, B.; Loman, N.; Schmutzler, R.K.; et al. Oral Poly(ADP-Ribose) Polymerase Inhibitor Olaparib in Patients with BRCA1 or BRCA2

Mutations and Advanced Breast Cancer: A Proof-of-Concept Trial. *Lancet (London, England)* **2010**, *376*, 235–244, doi:10.1016/S0140-6736(10)60892-6.

18. Chatterjee, N.; Walker, G.C. Mechanisms of DNA Damage, Repair and Mutagenesis. *Environ. Mol. Mutagen.* **2017**, *58*, 235, doi:10.1002/EM.22087.

19. Jiricny, J. Postreplicative Mismatch Repair. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, 1–23, doi:10.1101/CSHPERSPECT.A012633.

20. Kunkel, T.A.; Erie, D.A. Eukaryotic Mismatch Repair in Relation to DNA Replication. *Annu. Rev. Genet.* **2015**, *49*, 291–313, doi:10.1146/ANNUREV-GENET-112414-054722.

21. Gruber, S.B.; Kohlmann, W. The Genetics of Hereditary Non-Polyposis Colorectal Cancer. *J. Natl. Compr. Canc. Netw.* **2003**, *1*, 137–144, doi:10.6004/JNCCN.2003.0014.

22. Leach, F.S. Microsatellite Instability and Prostate Cancer: Clinical and Pathological Implications. *Curr. Opin. Urol.* **2002**, *12*, 407–411, doi:10.1097/00042307-200209000-00007.

23. Hampel, H.; Frankel, W.L.; Martin, E.; Arnold, M.; Khanduja, K.; Kuebler, P.; Nakagawa, H.; Sotamaa, K.; Prior, T.W.; Westman, J.; et al. Screening for the Lynch Syndrome (Hereditary Nonpolyposis Colorectal Cancer). *N. Engl. J. Med.* **2005**, *352*, 1851–1860, doi:10.1056/NEJMOA043146.

24. Le, D.T.; Uram, J.N.; Wang, H.; Bartlett, B.R.; Kemberling, H.; Eyring, A.D.; Skora, A.D.; Luber, B.S.; Azad, N.S.; Laheru, D.; et al. PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *N. Engl. J. Med.* **2015**, *372*, 2509–2520, doi:10.1056/NEJMOA1500596/SUPPL_FILE/NEJMOA1500596_DISCLOSURES.PDF.

25. Mateo, J.; Carreira, S.; Sandhu, S.; Miranda, S.; Mossop, H.; Perez-Lopez, R.; Nava Rodrigues, D.; Robinson, D.; Omlin, A.; Tunariu, N.; et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. *N. Engl. J. Med.* **2015**, *373*, 1697–1708, doi:10.1056/NEJMOA1506859.

26. Pommier, Y.; O'Connor, M.J.; De Bono, J. Laying a Trap to Kill Cancer Cells: PARP Inhibitors and Their Mechanisms of Action. *Sci. Transl. Med.* **2016**, *8*,

doi:10.1126/SCITRANSLMED.AAF9246.

27. Jasin, M.; Rothstein, R. Repair of Strand Breaks by Homologous Recombination. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, doi:10.1101/CSHPERSPECT.A012740.

28. Symington, L.S.; Gautier, J. Double-Strand Break End Resection and Repair Pathway Choice. *Annu. Rev. Genet.* **2011**, *45*, 247–271, doi:10.1146/ANNUREV-GENET-110410-132435.

29. Jackson, S.P.; Bartek, J. The DNA-Damage Response in Human Biology and Disease. *Nat. 2009 4617267* **2009**, *461*, 1071–1078, doi:10.1038/nature08467.

30. Thadathil, N.; Hori, R.; Xiao, J.; Khan, M.M. DNA Double-Strand Breaks: A Potential Therapeutic Target for Neurodegenerative Diseases. *Chromosom. Res. 2019 274* **2019**, *27*, 345–364, doi:10.1007/S10577-019-09617-X.

31. Caracciolo, D.; Riillo, C.; Di Martino, M.T.; Tagliaferri, P.; Tassone, P. Alternative Non-Homologous End-Joining: Error-Prone DNA Repair as Cancer's Achilles' Heel. *Cancers (Basel).* **2021**, *13*, 1–14, doi:10.3390/CANCERS13061392.

32. Lieber, M.R. The Mechanism of Double-Strand DNA Break Repair by the Nonhomologous DNA End-Joining Pathway. *Annu. Rev. Biochem.* **2010**, *79*, 181–211, doi:10.1146/ANNUREV.BIOCHEM.052308.093131.

33. Carter, H.B.; Helfand, B.; Mamawala, M.; Wu, Y.; Landis, P.; Yu, H.; Wiley, K.; Na, R.; Shi, Z.; Petkewicz, J.; et al. Germline Mutations in ATM and BRCA1/2 Are Associated with Grade Reclassification in Men on Active Surveillance for Prostate Cancer. *Eur. Urol.* **2019**, *75*, 743–749, doi:10.1016/J.EURURO.2018.09.021.

34. Campbell, P.J.; Getz, G.; Korbel, J.O.; Stuart, J.M.; Jennings, J.L.; Stein, L.D.; Perry, M.D.; Nahal-Bose, H.K.; Ouellette, B.F.F.; Li, C.H.; et al. Pan-Cancer Analysis of Whole Genomes. *Nature* **2020**, *578*, 82–93, doi:10.1038/S41586-020-1969-6.

35. Tosh, J. PROFOUND Trial -a New Era in Targeted Therapeutics for Prostate Carcinoma. *Indian J. Urol.* **2022**, *38*, 73–74, doi:10.4103/IJU.IJU_321_21.

36. FDA Grants Accelerated Approval to Rucaparib for BRCA-Mutated Metastatic Castration-Resistant Prostate Cancer | FDA Available online: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-rucaparib-brca-mutated-metastatic-castration-resistant-prostate (accessed on 15 April 2023).

37. Antonarakis, E.S.; Gomella, L.G.; Petrylak, D.P. When and How to Use PARP Inhibitors in Prostate Cancer: A Systematic Review of the Literature with an Update on On-Going Trials. *Eur. Urol. Oncol.* **2020**, *3*, 594–611, doi:10.1016/J.EUO.2020.07.005.

38. Hansen, A.R.; Massard, C.; Ott, P.A.; Haas, N.B.; Lopez, J.S.; Ejadi, S.; Wallmark, J.M.; Keam, B.; Delord, J.P.; Aggarwal, R.; et al. Pembrolizumab for Advanced Prostate Adenocarcinoma: Findings of the KEYNOTE-028 Study. *Ann. Oncol. Off. J. Eur. Soc. Med. Oncol.* **2018**, *29*, 1807–1813, doi:10.1093/ANNONC/MDY232.

39. Study Record | Beta ClinicalTrials.Gov Available online: https://beta.clinicaltrials.gov/study/NCT02601014?distance=50&cond=Nivolumab prostate&viewType=Table&limit=100&rank=3&aggFilters=results:with&tab=results (accessed on 17 April 2023).

40. Chi, K.N.; Fleshner, N.; Chiuri, V.E.; Van Bruwaene, S.; Hafron, J.; McNeel, D.G.; De Porre, P.; Maul, R.S.; Daksh, M.; Zhong, X.; et al. Niraparib with Abiraterone Acetate and Prednisone for Metastatic Castration-Resistant Prostate Cancer: Phase II QUEST Study Results. *Oncologist* **2023**, doi:10.1093/ONCOLO/OYAD008.

41. Kurfurstova, D.; Bartkova, J.; Vrtel, R.; Mickova, A.; Burdova, A.; Majera, D.; Mistrik, M.; Kral, M.; Santer, F.R.; Bouchal, J.; et al. DNA Damage Signalling Barrier, Oxidative Stress and Treatment-Relevant DNA Repair Factor Alterations during Progression of Human Prostate Cancer. *Mol. Oncol.* **2016**, *10*, 879–894, doi:10.1016/J.MOLONC.2016.02.005.

42. Wang, G.; Zhao, D.; Spring, D.J.; Depinho, R.A. Genetics and Biology of Prostate Cancer. *Genes Dev.* **2018**, *32*, 1105, doi:10.1101/GAD.315739.118.

43. Kowalewski, A.; Zdrenka, M.; Grzanka, D.; Szylberg, Ł. Targeting the Deterministic Evolutionary Trajectories of Clear Cell Renal Cell Carcinoma. *Cancers (Basel).* **2020**, *12*, 1–13, doi:10.3390/CANCERS12113300.

44. Sartor, O.; de Bono, J.S. Metastatic Prostate Cancer. *N. Engl. J. Med.* **2018**, *378*, 645–657, doi:10.1056/NEJMRA1701695.

45. Epstein, J.I.; Zelefsky, M.J.; Sjoberg, D.D.; Nelson, J.B.; Egevad, L.; Magi-Galluzzi, C.; Vickers, A.J.; Parwani, A. V.; Reuter, V.E.; Fine, S.W.; et al. A Contemporary Prostate Cancer Grading System: A Validated Alternative to the Gleason Score. *Eur. Urol.* **2016**, *69*, 428, doi:10.1016/J.EURURO.2015.06.046.

46. Brawer, M.K. Radiation Therapy Failure in Prostate Cancer Patients: Risk Factors and Methods of Detection. *Rev. Urol.* **2002**, *4*, S2.

Rozdział 8. Oświadczenia autorów publikacji włączonych do cyklu

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 06.05.2024

Dr n. med. Justyna Durślewicz

Katedra Patomorfologii Klinicznej
Collegium Medicum Uniwersytetu Mikołaja Kopernika
Marii Skłodowskiej Curie 9
85-094 Bydgoszcz

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Gzil Arkadiusz, Jaworski Damian, Antosik Paulina, Zarębska Izabela, Durślewicz Justyna, Dominiak Joanna, Kasperska Anna, Neska-Długosz Izabela, Grzanka Dariusz, Szylberg Łukasz.

The impact of TP53BP1 and MLHI on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice.

Urol. Oncol.-Semin. Orig. Investig.; 2020 : Vol. 38, nr 6. s. 600.e17-600.e26.

DOI: 10.1016/j.urolonc.2020.02.012

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- przygotowaniu manuskryptu
- przygotowaniu preparatów immunohistochemicznych

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

J.Durślewicz
(podpis)

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 14.05.2024

Lek. Anna Kasperska

Zakład Patomorfologii
Specjalistyczny Szpital Miejski
im. Mikołaja Kopernika w Toruniu
Stefana Batorego 17/19,
87-100 Toruń

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Jaworski Damian, Gzil Arkadiusz, Antosik Paulina, Zarębska Izabela, Dominiak Joanna, Neska-Długosz Izabela, Kasperska Anna, Grzanka Dariusz, Szylberg Łukasz.

"Expression differences between proteins responsible for DNA damage repair according to the Gleason grade as a new heterogeneity marker in prostate cancer."

Arch. Med. Sci.; 2023 :Vol. 19, nr 2. s. 499-506. DOI: 10.5114/aoms.2019.89773

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- analizie zebranych preparatów histologicznych i immunohistochemicznych

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

A. Kasperska
(podpis)

Bydgoszcz, dnia 14.05.2024

Lek. Anna Kasperska

Zakład Patomorfologii
Specjalistyczny Szpital Miejski
im. Mikołaja Kopernika w Toruniu
Stefana Batorego 17/19,
87-100 Toruń

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Gzil Arkadiusz, Jaworski Damian, Antosik Paulina, Zarębska Izabela, Durślewicz Justyna, Dominiak Joanna, Kasperska Anna, Neska-Długosz Izabela, Grzanka Dariusz, Szylberg Łukasz.

The impact of TP53BP1 and MLHI on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice.

Urol. Oncol.-Semin. Orig. Investig.; 2020 : Vol. 38, nr 6. s. 600.e17-600.e26.

DOI: 10.1016/j.urolonc.2020.02.012

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- analizie przygotowanych preparatów immunohistochemicznych i histologicznych

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

A. Kasperska
(podpis)

Bydgoszcz, dnia 06.05.2024

Lek. Izabela Neska-Długosz

Katedra Patomorfologii Klinicznej
Uniwersytet Mikołaja Kopernika w Toruniu,
Wydział Lekarski, Collegium Medicum w Bydgoszczy
ul. Curie Skłodowskiej 9,
85-094 Bydgoszcz

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Gzil Arkadiusz, Jaworski Damian, Antosik Paulina, Zarębska Izabela, Durślewicz Justyna, Dominiak Joanna, Kasperska Anna, Neska-Długosz Izabela, Grzanka Dariusz, Szylberg Łukasz.

The impact of TP53BP1 and MLHI on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice.

Urol. Oncol.-Semin. Orig. Investig.; 2020 : Vol. 38, nr 6. s. 600.e17-600.e26.

DOI: 10.1016/j.urolonc.2020.02.012

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- analizie przygotowanych preparatów immunohistochemicznych i histologicznych

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

Neska-Długosz Izabela
(podpis)

Bydgoszcz, dnia 06.05.2024

Lek. Izabela Neska-Długosz

Katedra Patomorfologii Klinicznej
Uniwersytet Mikołaja Kopernika w Toruniu,
Wydział Lekarski, Collegium Medicum w Bydgoszczy
ul. Curie Skłodowskiej 9,
85-094 Bydgoszcz

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Jaworski Damian, Gzil Arkadiusz, Antosik Paulina, Zarębska Izabela, Dominiak Joanna, Neska-Długosz Izabela, Kasperska Anna, Grzanka Dariusz, Szylberg Łukasz.

"Expression differences between proteins responsible for DNA damage repair according to the Gleason grade as a new heterogeneity marker in prostate cancer."

Arch. Med. Sci.; 2023 :Vol. 19, nr 2. s. 499-506. DOI: 10.5114/aoms.2019.89773

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- analizie zebranych preparatów histologicznych i immunohistochemicznych

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

Neska-Długosz Izabela
(podpis)

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 03.06.2024

prof. dr hab. n. med. i n. o zdr. Dariusz Grzanka
Katedra Patomorfologii Klinicznej
Uniwersytet Mikołaja Kopernika w Toruniu,
Wydział Lekarski, Collegium Medicum w Bydgoszczy
ul. Curie Skłodowskiej 9,
85-094 Bydgoszcz

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Gzil Arkadiusz, Jaworski Damian, Antosik Paulina, Zarębska Izabela, Durślewicz Justyna, Dominiak Joanna, Kasperska Anna, Neska-Długosz Izabela, Grzanka Dariusz, Szylberg Łukasz.

The impact of TP53BP1 and MLHI on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice.

Urol. Oncol.-Semin. Orig. Investig.; 2020 : Vol. 38, nr 6. s. 600.e17-600.e26.

DOI: 10.1016/j.urolonc.2020.02.012

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- nadzorze merytorycznym manuskryptu
- koordynowaniu klinicznych aspektów pracy

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

.....
(podpis)

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 03.06.2024

prof. dr hab. n. med. i n. o zdr. Dariusz Grzanka
Katedra Patomorfologii Klinicznej
Uniwersytet Mikołaja Kopernika w Toruniu,
Wydział Lekarski, Collegium Medicum w Bydgoszczy
ul. Curie Skłodowskiej 9,
85-094 Bydgoszcz

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Jaworski Damian, Gzil Arkadiusz, Antosik Paulina, Zarębska Izabela, Dominiak Joanna, Neska-Długosz Izabela, Kasperska Anna, Grzanka Dariusz, Szylberg Łukasz.

"Expression differences between proteins responsible for DNA damage repair according to the Gleason grade as a new heterogeneity marker in prostate cancer."

Arch. Med. Sci.; 2023 :Vol. 19, nr 2. s. 499-506. DOI: 10.5114/aoms.2019.89773

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- nadzorze merytorycznym manuskryptu
- koordynowaniu klinicznych aspektów pracy

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

.....
(podpis)

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 08.05.2024

Lek. Arkadiusz Gzil
Klinik für Hämatologie, Medizinische Onkologie und Palliativmedizin
Marien Kliniken Siegen
Kampenstraße 51
57072 Siegen

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Gzil Arkadiusz, Jaworski Damian, Antosik Paulina, Zarębska Izabela, Durślewicz Justyna, Dominiak Joanna, Kasperska Anna, Neska-Długosz Izabela, Grzanka Dariusz, Szylberg Łukasz.

The impact of TP53BP1 and MLHI on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice.

Urol. Oncol.-Semin. Orig. Investig.; 2020 : Vol. 38, nr 6. s. 600.e17-600.e26.
DOI: 10.1016/j.urolonc.2020.02.012

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- przygotowaniu manuskryptu, analizie literatury.
- analizie zebranych preparatów histologicznych i immunohistochemicznych

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

Arkadiusz Gzil
(podpis)

Bydgoszcz, dnia 05.05.2024

Lek. Arkadiusz Gzil
Klinik für Hämatologie, Medizinische Onkologie und
Palliativmedizin
Marien Kliniken Siegen
Kampenstraße 51
57072 Siegen

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Jaworski Damian, Gzil Arkadiusz, Antosik Paulina, Zarębska Izabela, Dominiak Joanna, Neska-Długosz Izabela, Kasperska Anna, Grzanka Dariusz, Szylberg Łukasz.

"Expression differences between proteins responsible for DNA damage repair according to the Gleason grade as a new heterogeneity marker in prostate cancer."

Arch. Med. Sci.; 2023 :Vol. 19, nr 2. s. 499-506. DOI: 10.5114/aoms.2019.89773

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- przygotowaniu manuskryptu, analizie literatury
- analizie zebranych preparatów histologicznych i immunohistochemicznych

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

Arkadiusz Gzil
(podpis)

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 06.05.24

Dr n. med. Paulina Antosik

Katedra Patomorfologii Klinicznej
Collegium Medicum Uniwersytetu Mikołaja Kopernika
Marii Skłodowskiej Curie 9
85-094 Bydgoszcz

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Jaworski Damian, Gzil Arkadiusz, Antosik Paulina, Zarębska Izabela, Dominiak Joanna, Neska-Długosz Izabela, Kasperska Anna, Grzanka Dariusz, Szylberg Łukasz.

"Expression differences between proteins responsible for DNA damage repair according to the Gleason grade as a new heterogeneity marker in prostate cancer."

Arch. Med. Sci.; 2023 :Vol. 19, nr 2. s. 499-506. DOI: 10.5114/aoms.2019.89773

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- przygotowaniu manuskryptu
- przygotowaniu preparatów immunohistochemicznych

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

Paulina Antosik
(podpis)

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 06.05.24

Dr n. med. Paulina Antosik

Katedra Patomorfologii Klinicznej
Collegium Medicum Uniwersytetu Mikołaja Kopernika
Marii Skłodowskiej Curie 9
85-094 Bydgoszcz

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Gzil Arkadiusz, Jaworski Damian, Antosik Paulina, Zarębska Izabela, Durślewicz Justyna, Dominiak Joanna, Kasperska Anna, Neska-Długosz Izabela, Grzanka Dariusz, Szylberg Łukasz.

The impact of TP53BP1 and MLHI on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice.

Urol. Oncol.-Semin. Orig. Investig.; 2020 : Vol. 38, nr 6. s. 600.e17-600.e26.

DOI: 10.1016/j.urolonc.2020.02.012

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- przygotowaniu manuskryptu
- przygotowaniu preparatów immunohistochemicznych

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

Paulina Antosik
(podpis)

Bydgoszcz, dnia 03.06.2024

Prof. dr hab. Łukasz Szylberg

Zakład Patomorfologii, Placentologii i Hematopatologii Klinicznej
Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy
Uniwersytetu Mikołaja Kopernika w Toruniu

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Gzil Arkadiusz, Jaworski Damian, Antosik Paulina, Zarębska Izabela, Durślewicz Justyna, Dominiak Joanna, Kasperska Anna, Neska-Długosz Izabela, Grzanka Dariusz, Szylberg Łukasz.

"The impact of TP53BP1 and MLHI on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice." Urol. Oncol.-Semin. Orig. Investig.; 2020 : Vol. 38, nr 6. s. 600.e17-600.e26. DOI: 10.1016/j.urolonc.2020.02.012

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- opracowaniu koncepcji badań
- nadzorze merytorycznym manuskryptu i koordynowaniu klinicznych aspektów pracy

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:
- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

.....
(podpis)

Bydgoszcz, dnia 03.06.2024

Prof. dr hab. Łukasz Szylberg
Zakład Patomorfologii, Placentologii i Hematopatologii Klinicznej
Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy
Uniwersytetu Mikołaja Kopernika w Toruniu

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Jaworski Damian, Gzil Arkadiusz, Antosik Paulina, Zarębska Izabela, Dominiak Joanna, Neska-Długosz Izabela, Kasperska Anna, Grzanka Dariusz, Szylberg Łukasz.

"Expression differences between proteins responsible for DNA damage repair according to the Gleason grade as a new heterogeneity marker in prostate cancer."

Arch. Med. Sci.; 2023 :Vol. 19, nr 2. s. 499-506. DOI: 10.5114/aoms.2019.89773

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- opracowaniu koncepcji badań
- nadzorze merytorycznym manuskryptu i koordynowaniu klinicznych aspektów pracy

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

.....
(podpis)

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 03.06.2024

Lek. Damian Jaworski

Klinika Okulistyki i Optometrii
Katedra Chorób Oczu CM UMK

Szpital Uniwersytecki nr 2 im. Jana Biziela w Bydgoszczy

Kornela Ujejskiego 75,
85-168 Bydgoszcz

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Gzil Arkadiusz, Jaworski Damian, Antosik Paulina, Zarębska Izabela, Durślewicz Justyna, Dominiak Joanna, Kasperska Anna, Neska-Długosz Izabela, Grzanka Dariusz, Szylberg Łukasz.

"The impact of TP53BP1 and MLHI on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice."

Urol. Oncol.-Semin. Orig. Investig.; 2020 : Vol. 38, nr 6. s. 600.e17-600.e26.

DOI: 10.1016/j.urolonc.2020.02.012

Mój udział w powstaniu pracy wynosi 55%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje mój indywidualny wkład w powstanie pracy polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

.....
(podpis)

Bydgoszcz, dnia 03.06.2024

Lek. Damian Jaworski

Klinika Okulistyki i Optometrii,
Katedra Chorób Oczu CM UMK

Szpital Uniwersytecki nr 2 im. Jana Biziela w Bydgoszczy

Kornela Ujejskiego 75,
85-168 Bydgoszcz

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Jaworski Damian, Gzil Arkadiusz, Antosik Paulina, Zarębska Izabela, Dominiak Joanna, Neska-Długosz Izabela, Kasperska Anna, Grzanka Dariusz, Szylberg Łukasz.

"Expression differences between proteins responsible for DNA damage repair according to the Gleason grade as a new heterogeneity marker in prostate cancer."

Arch. Med. Sci.; 2023 :Vol. 19, nr 2. s. 499-506. DOI: 10.5114/aoms.2019.89773

Mój udział w powstaniu pracy wynosi 60%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje mój indywidualny wkład w powstanie pracy polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

Jaworski
(podpis)

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 28.V.24

Lek. Izabela Zarębska
Oddział Kliniczny Radioterapii
Centrum Onkologii im. prof. F. Łukaszczyka
w Bydgoszczy

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Gzil Arkadiusz, Jaworski Damian, Antosik Paulina, Zarębska Izabela, Durślewicz Justyna, Dominiak Joanna, Kasperska Anna, Neska-Długosz Izabela, Grzanka Dariusz, Szylberg Łukasz.

The impact of TP53BP1 and MLHI on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice.

Urol. Oncol.-Semin. Orig. Investig.; 2020 : Vol. 38, nr 6. s. 600.e17-600.e26.

DOI: 10.1016/j.urolonc.2020.02.012

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- przygotowaniu manuskryptu.
- analizie zebranych preparatów histologicznych i immunohistochemicznych

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

Izabela Zarębska
.....
(podpis)

Bydgoszcz, dnia 28 V 2024 r.

Lek. Izabela Zarębska
Oddział Kliniczny Radioterapii
Centrum Onkologii im. prof. F. Łukaszczyka
w Bydgoszczy

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Jaworski Damian, Gzil Arkadiusz, Antosik Paulina, Zarębska Izabela, Dominiak Joanna, Neska-Długosz Izabela, Kasperska Anna, Grzanka Dariusz, Szylberg Łukasz.

"Expression differences between proteins responsible for DNA damage repair according to the Gleason grade as a new heterogeneity marker in prostate cancer."

Arch. Med. Sci.; 2023 :Vol. 19, nr 2. s. 499-506. DOI: 10.5114/aoms.2019.89773

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- przygotowaniu manuskryptu.
- analizie zebranych preparatów histologicznych i immunohistochemicznych

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

Izabela Zarębska
(podpis)

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 11.05.2024

Lek. Joanna Saganek (Dominiak)
Klinika Gastroenterologii, Hepatologii, Zaburzeń
Odżywiania i Pediatrii
Instytut „Pomnik-Centrum Zdrowia Dziecka"
Al. Dzieci Polskich 20
04-730 Warszawa

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Gzil Arkadiusz, Jaworski Damian, Antosik Paulina, Zarębska Izabela, Durślewicz Justyna, Dominiak Joanna, Kasperska Anna, Neska-Długosz Izabela, Grzanka Dariusz, Szylberg Łukasz.

The impact of TP53BP1 and MLHI on metastatic capability in cases of locally advanced prostate cancer and their usefulness in clinical practice.

Urol. Oncol.-Semin. Orig. Investig.; 2020 : Vol. 38, nr 6. s. 600.e17-600.e26.

DOI: 10.1016/j.urolonc.2020.02.012

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- przygotowaniu manuskryptu.
- analizie zebranych preparatów histologicznych i immunohistochemicznych

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

Joanna Saganek
(podpis)

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 11.05.2024

Lek. Joanna Saganek (Dominiak)
Klinika Gastroenterologii, Hepatologii, Zaburzeń Odżywiania i Pediatrii
Instytut „Pomnik-Centrum Zdrowia Dziecka"
Al. Dzieci Polskich 20
04-730 Warszawa

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Jaworski Damian, Gzil Arkadiusz, Antosik Paulina, Zarębska Izabela, Dominiak Joanna, Neska-Długosz Izabela, Kasperska Anna, Grzanka Dariusz, Szylberg Łukasz.

"Expression differences between proteins responsible for DNA damage repair according to the Gleason grade as a new heterogeneity marker in prostate cancer."

Arch. Med. Sci.; 2023 :Vol. 19, nr 2. s. 499-506. DOI: 10.5114/aoms.2019.89773

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- przygotowaniu manuskryptu.
- analizie zebranych preparatów histologicznych i immunohistochemicznych

Mój udział w powstaniu pracy wynosi 5%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- przygotowaniu manuskryptu
- interpretacji wyników
- analizie zebranych preparatów histologicznych i immunohistochemicznych, przygotowaniu zdjęć

Joanna Saganek
(podpis)

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 12.04.2024 r.

Dr n. med. Bartosz Brzoszczyk
Klinika Urologii
Szpital Uniwersytecki nr 2 im. Jana Biziela w
Bydgoszczy

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Jaworski D, Brzoszczyk B and Szylberg Ł: Recent Research Advances in Double-Strand Break and Mismatch Repair Defects in Prostate Cancer and Potential Clinical Applications. Cells 12, 2023. PMID: 37408208. DOI: 10.3390/CELLS12101375.

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- przygotowaniu manuskryptu.

Mój udział w powstaniu pracy wynosi 10%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- opracowaniu rycin i tabel
- przygotowaniu manuskryptu

dr n. med. Bartosz Brzoszczyk
specjalista urologii

.....
(podpis)

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 27.05.2024

Lek. Damian Jaworski
Klinika Okulistyki i Optometrii
Szpital Uniwersytęcki nr 2 im. Jana Biziela
w Bydgoszczy

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Jaworski D, Brzoszczyk B and Szylberg Ł: Recent Research Advances in Double-Strand Break and Mismatch Repair Defects in Prostate Cancer and Potential Clinical Applications. Cells 12, 2023. PMID: 37408208. DOI: 10.3390/CELLS12101375.

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- konceptualizacji pracy
- analizie literatury
- opracowaniu rycin i tabel
- przygotowaniu manuskryptu

Mój udział w powstaniu pracy wynosi 80%.

Jaworski
.....
(podpis)

Załącznik nr 5 do uchwały Nr 38 Senatu UMK z dnia 26 września 2023 r.
w sprawie postępowania o nadanie stopnia doktora
na Uniwersytecie Mikołaja Kopernika w Toruniu

Bydgoszcz, dnia 27.05.2024

Prof. dr hab. Łukasz Szylberg

Zakład Patomorfologii, Placentologii i Hematopatologii
Klinicznej
Collegium Medicum im. Ludwika Rydygiera
w Bydgoszczy
Uniwersytetu Mikołaja Kopernika w Toruniu

Rada Dyscypliny Nauki Medyczne
Uniwersytetu Mikołaja Kopernika
w Toruniu

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy:

Jaworski D, Brzoszczyk B and Szylberg Ł: Recent Research Advances in Double-Strand Break and Mismatch Repair Defects in Prostate Cancer and Potential Clinical Applications. Cells 12, 2023. PMID: 37408208. DOI: 10.3390/CELLS12101375.

mój udział merytoryczny w przygotowanie, przeprowadzenie i opracowanie badań oraz ich przedstawienie w formie publikacji polegał na:

- nadzorze merytorycznym i przygotowaniu manuskryptu.

Mój udział w powstaniu pracy wynosi 10%.

Oświadczam, że samodzielna i możliwa do wyodrębnienia część wyżej wymienionej pracy wykazuje indywidualny wkład lek. Damiana Jaworskiego polegający na:

- konceptualizacji pracy
- analizie literatury
- opracowaniu rycin i tabel
- przygotowaniu manuskryptu

.....
(podpis)

Rozdział 9. Zgoda Komisji Bioetycznej

Uniwersytet Mikołaja Kopernika w Toruniu

Collegium Medicum im L. Rydygiera w Bydgoszczy

KOMISJA BIOETYCZNA

Ul. M. Skłodowskiej-Curie 9, 85-094 Bydgoszcz, tel.(052) 585-35-63, fax.(052) 585-38-11

KB 75/2018

Bydgoszcz, 27.02.2018 r.

Działając na podstawie art.29 Ustawy z dnia 5 grudnia 1996 roku o zawodzie lekarza (Dz.U. z 1997 r. Nr 28 poz. 152 (wraz z późniejszymi zmianami), zarządzenia Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. w sprawie szczegółowych zasad powoływania i finansowania oraz trybu działania komisji bioetycznych (Dz.U.Nr 47 poz.480) oraz Zarządzeniem Nr 21 Rektora UMK z dnia 4 marca 2009 r. z późn. zm. w sprawie powołania oraz zasad działania Komisji Bioetycznej Uniwersytetu Mikołaja Kopernika w Toruniu przy Collegium Medicum im Ludwika Rydygiera w Bydgoszczy oraz zgodnie z zasadami zawartymi w ICH – GCP

Komisja Bioetyczna przy UMK w Toruniu, Collegium Medicum w Bydgoszczy

(skład podano w załączeniu), na posiedzeniu w dniu **27.02.2018 r.** przeanalizowała wniosek, który złożył kierownik badania:

dr hab. n. med. Dariusz Grzanka
Katedra i Zakład Patomorfologii Klinicznej
Collegium Medicum w Bydgoszczy

z zespołem w składzie:

- dr n. med. Łukasz Szylberg, dr n. med. Anna Klimaszewska - Wiśniewska, dr hab. n.med. Dariusz Grzanka, dr n. med. Piotr Jarzemski, dr n. med. Maciej Gagat, lek. Bartosz Brzoszczyk, lek. Izabela Neska-Długosz, mgr Paulina Antosik, Arkadiusz Gzil, Damian Jaworski, Izabela Zarębska, Joanna Dominiak,

w sprawie badania:

„Określenie wpływu zaburzonej ekspresji białek w mechanizmie powstawania przerzutów raka gruczołu krokowego."

Po zapoznaniu się ze złożonym wnioskiem i w wyniku przeprowadzonej dyskusji oraz głosowania Komisja podjęła:

Uchwałę o pozytywnym zaopiniowaniu wniosku

w sprawie przeprowadzenia badań w zakresie określonym we wniosku pod warunkiem zachowania tajemnicy wszystkich danych, w tym danych osobowych pacjenta umożliwiających ich identyfikację w ewentualnych publikacjach. Zgoda obejmuje tylko materiał biologiczny pobrany w okresie 2010-2017r. od pacjentów, którzy nie wyrazili stosownego sprzeciwu w Centralnym Rejestrze Sprzeciwów.

Zgoda obowiązuje od daty posiedzenia (27.02.2018 r.) do końca 2020 r.

Wydana opinia dotyczy tylko rozpatrywanego wniosku z uwzględnieniem przedstawionego projektu; każda zmiana i modyfikacja wymaga uzyskania odrębnej opinii

Prof. dr hab. med. Karol Śliwka

Przewodniczący Komisji Bioetycznej

Otrzymuje:
dr hab. n. med. Dariusz Grzanka
Katedra i Zakład Patomorfologii Klinicznej
Collegium Medicum w Bydgoszczy

Lista obecności

na posiedzeniu Komisji Bioetycznej

w dniu 27.02.2018 r.

Lp.	Imię i nazwisko	Funkcja	Podpis
1.	Prof. dr hab. med. Karol Śliwka	przewodniczący	
2.	Prof. dr hab. Adam Buciński	z-ca przewodniczącego	
3.	Prof. dr hab. med. Anna Balcar-Boroń		
4.	Prof. dr hab. med. Mieczysława Czerwionka-Szaflarska		
5.	Prof. dr hab. med. Marek Grabiec		
6.	Prof. dr hab. med. Zbigniew Włodarczyk		
7.	Dr hab. n. med. Katarzyna Pawlak-Osińska, prof. UMK		
8.	Ks. dr hab. Wojciech Szukalski, prof. UAM		
9.	Dr n. med. Radosława Staszak-Kowalska		
10.	Dr hab. n med. Maria Kłopocka		
11.	Mgr prawa Patrycja Brzezicka		
12.	Mgr prawa Joanna Połetek-Żygas		
13.	Mgr piel. Hanna Ziemniarska		

Rozdział 10. Streszczenie w języku polskim

Stały rozwój wiedzy naukowej dotyczący podłoża molekularnego nowotworów,
w tym raka gruczołu krokowego pozwolił na coraz powszechniejsze stosowanie terapii celowanej w leczeniu pacjentów onkologicznych, co przekłada się na dłuższą przeżywalność tych pacjentów. Dotychczasowe doniesienia w odniesieniu do biologii raka gruczołu krokowego potwierdzały istotny wpływ zaburzeń w obrębie mechanizmów naprawy genów, w tym mechanizmów naprawy błędnie sparowanych nukleotydów (MMR) oraz naprawy podwójnego pęknięcia nici DNA (DSBR) na rokowanie pacjentów, a także na dobór proponowanej terapii, w tym terapii celowanej. Doniesienia znalazły dotychczas bezpośrednie zastosowanie w praktyce klinicznej w postaci akceptacji przez FDA leków
z grupy inhibitorów PARP takich jak olaparib i rucaparib oraz inhibitora punktu kontrolnego, pembrolizumabu w leczeniu określonych przypadków raka gruczołu krokowego. Niniejszy projekt miał na celu określenie zależności pomiędzy poziomem ekspresji wybranych białek szlaków MMR oraz DSBR - MDC1, TP53BP1, MLH1, MSH2, MSH6 i PMS2, a zaawansowaniem raka gruczołu krokowego.

W pierwszej pracy przy pomocy badań immunohistochemicznych porównaliśmy ekspresję badanych białek pomiędzy tkankami guza pierwotnego raka gruczołu krokowego bez przerzutów do węzłów chłonnych, guza pierwotnego z przerzutami do węzłów chłonnych oraz tkanki przerzutowej raka gruczołu krokowego do węzła chłonnego. Wykazaliśmy, iż ekspresja białek MLH1 i TP53BP1 istotnie różniła się pomiędzy tymi tkankami. Rak gruczołu krokowego z przerzutami do węzłów chłonnych cechuje się niską ekspresją białek TP53BP1 i MLH1. Przerzuty raka gruczołu krokowego do węzłów chłonnych cechują się niższą ekspresją białka TP53BP1 niż guzy pierwotne. W drugiej z prac, skupiliśmy się na analizie ekspresji białek MDC1, TP53BP1, MLH1 i MSH2 w odniesieniu do złośliwości histologicznej raka gruczołu krokowego i heterogenności wewnętrznej raka gruczołu

krokowego. Wyniki naszej analizy wykazały, że ekspresje białek MSH2, MDC1 oraz MLH1 w istotny sposób różniły się w zależności od stopnia złośliwości histologicznej określanego w skali Gleasona. Wyniki badań wskazują, iż wraz ze wzrostem punktacji w skali Gleasona, wzrasta poziom ekspresji jądrowej i cytoplazmatycznej białka MSH2. Ponadto, wartość wzoru architektonicznego Gleasona negatywnie koreluje z ekspresją jądrową białka MSH2. Wraz ze wzrostem punktacji w skali Gleasona, obniża się ekspresja cytoplazmatyczna białka MDC1. Punktacja architektoniki Gleasona pozytywnie koreluje z cytoplazmatyczną ekspresją białka MLH1.

Wyniki badań wskazują nie tylko na potencjalne możliwości zastosowania szlaków MMR i DSBR lub też konkretnych białek jako biomarkera, ale także punkt uchwytu dla potencjalnych terapii celowanych. Ponadto, wyniki te są zgodne z założeniami dotychczasowych badań nad rakiem gruczołu krokowego, podkreślającymi heterogenność raka gruczołu krokowego, a także z teorią immunoedycji nowotworów. Zebrane dane literaturowe dotyczące wpływu mechanizmów naprawy DNA otwierają drogę do dalszych badań, które mogą prowadzić do rozwoju nowych biomarkerów i strategii terapeutycznych w raku gruczołu krokowego.

Rozdział 11. Streszczenie w języku angielskim

The continuous advancement in scientific knowledge regarding the molecular basis of cancers, including prostate cancer, has led to the increasingly widespread use of targeted therapies in treating oncology patients, resulting in improved survival rates for these patients. Current research on the biology of prostate cancer has confirmed the significant impact of disruptions in gene repair mechanisms, such as mismatch repair (MMR) and double-strand break repair (DSBR), on patient prognosis and the selection of proposed therapies, including targeted therapies.

These findings have already been applied in clinical practice, with the FDA approving PARP inhibitors like olaparib and rucaparib, as well as the checkpoint inhibitor pembrolizumab, for treating specific cases of prostate cancer. The aim of this project was to determine the relationship between the expression levels of selected proteins in the MMR and DSBR pathways —MDC1, TP53BP1, MLH1, MSH2, MSH6, and PMS2—and the progression of prostate cancer.

In one study, we used immunohistochemical analyses to compare the expression of these proteins in primary prostate cancer tissues without lymph node metastases, primary tumors with lymph node metastases, and metastatic prostate cancer tissues in lymph nodes. We demonstrated that the expression of MLH1 and TP53BP1 proteins significantly differed among these tissues. Prostate cancer with lymph node metastases is characterized by low expression of TP53BP1 and MLH1 proteins. Metastatic prostate cancer in lymph nodes exhibits lower TP53BP1 protein expression than primary tumors. In another study, we focused on analyzing the expression of MDC1, TP53BP1, MLH1, and MSH2 proteins in relation to the histological malignancy of prostate cancer and its intratumoral heterogeneity. Our analysis revealed that the expression levels of MSH2, MDC1, and MLH1 proteins significantly varied with the degree of histological malignancy, as determined by Gleason score. The results of our analysis showed that the expression levels of MSH2, MDC1, and MLH1 proteins significantly differed depending on

the histological grade of malignancy determined by Gleason score.
Our findings indicate that as the Gleason score increases, the nuclear and cytoplasmic expression of MSH2 protein increases.
Additionally, Gleason pattern negatively correlates with nuclear expression of MSH2 protein. As Gleason score increases, the cytoplasmic expression of MDC1 protein decreases. Gleason pattern positively correlates with the cytoplasmic expression of MLH1 protein.

Our findings suggest not only the potential use of MMR and DSBR pathways or specific proteins as biomarkers but also as targets for potential targeted therapies. Moreover, these results align with current research on prostate cancer, emphasizing the heterogeneity of the disease and supporting the theory of cancer immunoediting. The data collected from global literature on the impact of DNA repair mechanisms, particularly MMR and DSBR, on the development of prostate cancer, highlighted in our review article, underscore their usefulness and potential application in future studies aimed at improving the diagnosis and treatment of prostate cancer patients.
The collected literature data on the impact of DNA repair mechanisms open the way for further research, which may lead to the development of new biomarkers and therapeutic strategies for prostate cancer.