Title: Prostate Carcinogenesis: Actionable Inflammatory Storms?

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ABSTRACT

Prostate cancer (PCa) is a major cause of cancer morbidity and mortality. Intra-prostatic inflammation is a risk factor for prostate carcinogenesis, with diet, chemical injury, and an altered microbiome being causally implicated. Inflammation can expand intra-prostatic myeloid cells that promote immunosuppression, DNA double-strand breaks, and activate androgen receptor (AR) target genes. AR signalling and free radicals mediate further DNA damage, thereby driving the chronic activation of DNA repair genes (DRG) and tumour suppressor genes, rendering them more susceptible to endogenous and exogenous mutagens. These processes are accelerated in the context of germline DRG defects. We provide an update on recent advances in our understanding of the mechanisms through which inflammation and immune-dysregulation facilitate prostate carcinogenesis. We explore novel therapeutic and prevention strategies harnessing these discoveries.
1. Introduction

Prostate cancer (PCa) is a leading cause of morbidity and mortality in men\(^1\). Although recent treatment advances extend overall survival (OS), men with advanced PCa invariably develop metastatic castration-resistant prostate cancer (mCRPC)\(^2\). In addition to risk factors such as age, ethnicity, and a family history of PCa, potentially modifiable risk factors including infection, obesity, and diet have been implicated in causing prostate inflammation and carcinogenesis\(^3\)–\(^12\) (Table 1). Recent advances in this field are providing novel mechanistic insights regarding the environmental, genomic, and immunologic factors driving the inflammatory ‘storms’ that results in carcinogenesis and progression to lethal PCa. This complex biology highlights the need for an integrative approach to PCa research and drug development.

2. Inflammation and carcinogenesis.

Chronic inflammation, commonly observed in pre-neoplastic and malignant prostates, is implicated as a driver of prostate carcinogenesis and progression\(^13\)–\(^19\). Tumour progenitor cells of an intermediate phenotype expand in the context of inflammation to perpetuate this process\(^13\),\(^18\),\(^20\),\(^21\). While the exact stimuli required to initiate and maintain prostatic inflammation are not fully understood, bacterial or viral infection (perhaps from urinary microbiota), chemical irritation, physical trauma, obesity, and dietary factors are all postulated to play a role\(^3\),\(^6\),\(^10\)–\(^12\),\(^18\),\(^22\),\(^23\) (Table 1).

2.1. The Microbiome and inflammation.

The microbiome (the collective communities of microbes that live in and on our bodies) has been implicated in prostate carcinogenesis, tumour progression, and impact on response to cancer treatment\(^4\),\(^24\). Epidemiological data link prostatic inflammation to bacterial and viral infection, and have been reviewed elsewhere\(^3\),\(^4\). Several groups have used culture-independent methods to identify microorganisms in prostatectomy samples and detected DNA and RNA from bacteria, viruses, fungi, and parasites\(^25\),\(^26\). Additional evidence for the role of host-microbiome interactions in prostatic carcinogenesis arises from the discovery of the urinary microbiome and associated species that may contribute to prostate inflammation. Profiling of the urinary microbiome of 135 men with or without biopsy-proven PCa showed Prostate Carcinogenesis: Actionable Inflammatory Storms?
significantly and differentially abundant pro-inflammatory uropathogens between the two groups, as well as between men with low-grade versus high-grade tumours\textsuperscript{27}. Urinary reflux may be a source of prostatic infection as well as chemical injury\textsuperscript{3,4,23,28}. Once established, prostatic injury is postulated to impair intrinsic antimicrobial and epithelial defences leading to a vicious cycle of bacterial infiltration, outgrowth, epithelial disruption with loss of barrier function, and proliferation resulting in chronic, persistent inflammation\textsuperscript{3,4}.

At a mechanistic level, bacterial prostatitis can lead to prostatic epithelial injury/cell death with compensatory proliferation leading to hyperplasia and perhaps dysplasia\textsuperscript{7,29}. Chronic bacterial prostatitis has been associated with reduced expression of Nkx3.1, a key transcriptional regulator of prostatic epithelial growth\textsuperscript{30}. The loss of Nkx3.1, perhaps due to cytokine-induced proteasomal degradation, leads to dysregulated expression of free radicals, thus exacerbating further oxidative DNA damage\textsuperscript{31,32}. In murine models, acute prostatic inflammation due to bacterial infection or induction of IL-1\textbeta was associated with reduced Nkx3.1 expression and epithelial transformation akin to proliferative inflammatory atrophy (PIA)\textsuperscript{7,33}. PIA is hypothesised to be a precursor of prostatic intraepithelial neoplasia (PIN)\textsuperscript{13}. To date, no single organism has emerged as the primary driver of prostatic inflammation.

Studying how microbes contribute to prostatic inflammation and tumorigenesis has been challenging. The human prostate is probably chronically exposed to a multitude of organisms that can elicit inflammation. Microbes of interest are localized to specific areas of the prostate and false negatives can occur due to inadequate sampling. Culture independent techniques detecting microbial DNA or RNA are subject to the persistent threat of exogenously introduced nucleic acids from the surgical environment, pathology processing, extraction, and sequencing reagents. Longitudinal studies profiling the urinary microbiome, and potential prostatic microbes, with meticulous sample collection to minimise contamination, and orthogonal methods of microbial identification are now required to decipher the role of the microbiome in prostate carcinogenesis. In addition to methods such as shotgun metagenomic sequencing and 16S ribosomal RNA sequencing, techniques such as the sequencing of circulating microbial DNA may allow the detection of the microbiome in

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inaccessible anatomical niches of the body\textsuperscript{34}. Importantly, since inflammation is likely to be triggered by several species over time, there is a need to identify shared mechanisms that can be therapeutically targeted.

There is also emerging evidence supporting the role of the gastrointestinal (GI) microbiome in prostate carcinogenesis. There are several mechanisms through which GI microbes may be implicated\textsuperscript{35-39}. A case control study of 20 men revealed a higher abundance of \textit{Bacteroides massiliensis} in the faecal samples of men with PCa compared to those with benign prostatic conditions. \textit{Faecalibacterium prausnitzii} and \textit{Eubacterium rectalie} were more common in faecal samples from men without PCa\textsuperscript{39}. In contrast, prospective studies involving 133 rectal swabs taken 2-weeks before transrectal prostate biopsies showed an enrichment of \textit{Bacteroides} and \textit{Streptococcus} species in men with PCa. Interestingly, the activation of microbiome metabolic pathways for folate and arginine were significantly reduced in men with PCa compared to those without\textsuperscript{35}. Additional studies supporting the impact of microbiota on prostate carcinogenesis suggest that specific human gut microbes have the potential to synthesise steroids and convert them into androgens\textsuperscript{36,40}. Whether bacterial steroid synthesis then supports PCa growth is unknown.

Further evidence for the role of GI microbes in prostate carcinogenesis comes from a study which showed GI infection with \textit{Helicobacter hepaticus} in mice with a predilection for Wnt signalling dysregulation can lead to systemic inflammation, increased PIN and microadenocarcinoma formation compared to uninfected mice. The transmission of this tumorigenic effect from infected to uninfected animals via lymph node cell transplantation, and the abrogation of tumorigenesis by TNF-\textit{\alpha} blockade, suggest an immunologic basis\textsuperscript{37}. These findings are important since tumour formation in the context of Wnt dysregulation generally requires a second event, such as \textit{Pten} loss\textsuperscript{41}. Collectively, these studies demonstrate a potential intersection between genetic risk factors, microbial risk factors, hormonal, and immunologic processes in prostate carcinogenesis and the development of therapeutic resistance.

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2.2. Diet, the microbiome, obesity, and prostatic inflammation.

The GI microbiota, diet, and obesity are all inter-related and have been implicated in the development of aggressive PCa with diet likely altering the microbiome\textsuperscript{9,42-47}. Some epidemiologic data link diets high in red meat, charred meat, and saturated fats with the development of PCa\textsuperscript{9,10,42}. However, whether fat consumption per se (saturated or unsaturated) increases the risk of prostate cancer development has remained difficult to accurately determine and a controversial subject\textsuperscript{48}. Nevertheless, there is considerable evidence that PCa disease recurrence, progression, and mortality are impacted by diet and specifically saturated fat intake\textsuperscript{49}. Furthermore, diet can impact microbiota composition\textsuperscript{46}, which in turn is linked with obesity\textsuperscript{47}.

Whilst there is controversy over whether obesity increases the risk of developing PCa overall, obesity, weight gain, and the metabolic syndrome have been significantly associated with increased biochemical recurrence risk after primary prostatectomy, more aggressive disease, and increased PCa-specific mortality\textsuperscript{8,45,50,51}. This is of particular clinical relevance since a main side effect of androgen deprivation therapy (ADT) is a metabolic syndrome with associated weight gain. A causal relationship between obesity and prostate cancer growth is difficult to determine based on heterogenous epidemiologic studies of predominantly Caucasian populations, where the associations may be confounded by factors including dietary factors and exercise. The definition of obesity and metabolic syndrome also varied across studies.

Nevertheless, a growing number of preclinical studies and studies of human prostate tumour samples provide mechanistic insights into the links between adiposity and PCa. These appear to be underscored by metabolic, hormonal and inflammatory processes\textsuperscript{44,52-57}. Whilst testosterone levels are decreased in obese and castrate men, adipocytes produce steroid hormones that can activate androgen receptor (AR) or glucocorticoid receptor signaling that can fuel PCa growth\textsuperscript{57,58}. In several studies evaluating the effect of dietary fat on PCa development in a murine model driven by c-MYC (Hi-Myc mice) and \textit{Pten}-loss, a high-fat diet (HFD) increased the rate of development of invasive adenocarcinoma\textsuperscript{54,55,59}. In a murine

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model with prostate-specific Pten-loss, obesity led to hyperinsulinenia which enhanced the expression of pro-inflammatory genes, such as Cxcl1, Ccl20, Mmp7, Il-1β, and Il-6 downstream of the pro-inflammatory cytokine IL-17. In this model, hyperinsulinenia suppressed glycogen synthase kinase-3, thereby preventing the phosphorylation and subsequent proteasomal degradation of IL-17 receptor A (IL-17RA)\(^{54}\). IL-17 signalling and interactions with Th17 cells has been reported to contribute to PCa growth by promoting angiogenesis, tumour cell survival and proliferation, as well as inflammation. This is corroborated by several studies of Pten conditional knockout mice showing that PCa formation and growth is reduced by IL-17 abrogation\(^{60,61}\).

Critically, other data have also shown that Pten-null mice fed a high fat diet (HFD) have increased tumour growth, increased infiltration by MDSCs and increased “M2-like” macrophages. Immunohistochemical staining suggested that stromal immune cells, not tumour cells, secreted IL-6 which in turn activated STAT3 in tumour cells to promote growth. This finding was supported by correlative clinical evidence that higher BMI (defined as BMI > 25 kg/m\(^2\)) associates with increased MDSC counts in PCa biopsies, although there was no association with “M2-like” macrophages\(^{55}\). Interestingly, a clinical study of peri-prostatic fat collected after neoadjuvant ADT reported induction of a pro-inflammatory transcriptional program in peri-prostatic adipocytes. This included upregulated IL-6/JAK/STAT signalling, CXCL10, myeloid cell nuclear differentiation antigen, toll-like receptor 8 (TLR8), interferon-gamma (IFN-γ), and interferon-alpha (IFN-α)\(^{44}\). Some of these cytokines stimulate adaptive immunity; the overall impact of these factors on PCa cell survival needs further study.

Another proposed pro-tumorigenic mechanism is the facilitation of extracapsular extension, a key step in PCa progression, by peri-prostatic adipocytes; evidence for this has been reported using TRAMP-C1P3 PCa cell lines \textit{in vitro} and \textit{in vivo}, with peri-prostatic adipocytes producing an array of chemokines promoting metastases. Notably, CCL7 secreted by adipocytes is reported to activate CCR3 overexpressed by human PCa cells in obese individuals, and result in more aggressive disease. This pro-metastatic impact of this

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CCR3/CCL7 axis is amplified in obese mice; inhibition of the signaling of other adipocyte-derived chemokines including CXCR2 and CXCR4 decreased PCa cell migration. Overall, a key challenge to translating these findings clinically will be deciphering functional redundancies in these chemokine/receptor interactions.

Recent studies provide additional mechanistic insights into how dietary fat can impact PCa progression at a transcriptional level. In a murine prostate cancer model driven by c-MYC (Hi-MYC Mice), a high-fat diet (HFD) enhanced the MYC transcriptional program through metabolic alterations favouring histone H4K20 hypomethylation at promoter regions of MYC regulated genes, which increased proliferation and tumour burden. Interestingly, clinical studies also indicated that saturated fat intake also enhanced a MYC transcriptional signature that associated with prostate cancer progression and death. Interestingly, conditional inactivation of Pml in the transgenic mouse prostate transformed more indolent Pten-null tumours into lethal metastatic disease. Mechanistically, the authors proposed that Pml loss in this model stimulated hyperactivation of an aberrant SREBP pro-metastatic lipogenic program. Importantly, a HFD induced lipid accumulation in prostate tumours and drove metastases. An SREBP transcriptional signature was also reported as associated with poorer prognosis human PCa. Overall, the data indicate that a HFD promotes prostatic carcinogenesis and intra-prostatic inflammation and need to be considered when reflecting on increasing PCa incidence in countries with a changing diet such as China and Japan (Figure 1).

2.3. Prostatic cell senescence may fuel oncogenic inflammation.

Prostatic inflammation can be amplified and perpetuated by cellular senescence triggered by genotoxic stresses due to oncogenic signalling such as AR signaling, DNA damage, loss of tumour suppressors, or prostate cancer therapy (Figure 2); these lead to a state of stable, and sometimes reversible, cell cycle arrest and can safeguard against carcinogenesis. Senescent cells can acquire a senescence-associated secretory phenotype (SASP); this involves the secretion of cytokines, immunomodulators, and proteases. The SASP initially stimulates immune surveillance and reinforces growth arrest, but can drive inflammation,
immunosuppression, epithelial-to-mesenchymal transition, and tumour metastases through
paracrine and autocrine signalling\textsuperscript{71-74}. Efforts are ongoing to develop senolytic
pharmacological strategies that can eradicate such oncogenic senescent cells. The induction
and regulation of specific cellular senescence programs are discussed in Box 1.

2.4. Inflammation can drive prostate carcinogenesis and tumour
growth.

Inflammation can promote prostate carcinogenesis with leucocytes including myeloid cells,
macrophages and lymphocytes increasing in the TME and associating with worse outcome
\textsuperscript{19,75,76}. Interestingly in advanced PCa high neutrophil-to-lymphocyte ratios reflecting an
expanded circulating myeloid compartment correlate with worse OS and decreased
sensitivity to anti-androgens and chemotherapy\textsuperscript{77-79}. We have reported that some myeloid
cells (MDSCs) secrete IL-23 which in a paracrine fashion activates IL-23 receptors on
tumour cells, driving downstream AR target genes through STAT3-ROR\textgreek{g} signalling. MDSCs
also produce free radicals that can cause DNA single and double-strand breaks (DSB) which
can induce cellular senescence (probably through ATM activation) and further fuel
inflammation through the SASP, with this vicious cycle likely resulting in ‘a perfect storm’
or ‘inflammatory storms’ that drive prostate carcinogenesis\textsuperscript{80-82}.

Importantly, AR signalling drives transcriptional programs that alter the three-dimensional
architecture of the genome and causes DNA DSBs at the sites of activated genes including
DNA repair genes in a topoisomerase-II\textbeta-dependent manner\textsuperscript{83-86}. The clustering of DSBs,
along with AR target gene spatial localization and increased AR target gene accessibility,
increases the likelihood of defective DSB repair by error-prone non-homologous end-joining,
resulting in multiple, non-random rearrangements and fusions occurring in concert, a
phenomenon also termed chromoplexy\textsuperscript{83,84,86-88}. This process is accelerated in the presence of
defective DNA repair, for example in BRCA2 or ATM mutation carriers. Recurrent gene
fusions of the androgen-regulated TMPRSS2 gene to the oncogenic ETS family of
transcription factors have been reported in 56% of advanced PCa\textsuperscript{89}; it is postulated that the
chronic transcription of tumour suppressor genes, AR target genes and DNA repair genes

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(DRG), and their resulting altered chromatin state, renders them more susceptible to chronic inflammatory insults thereby fuelling a vicious cycle of further DNA damage and defective repair \(^{80-82}\) (Figure 3). It is, however, important to note that studies offering mechanistic dissection of the link between inflammation and prostate carcinogenesis have been limited to the preclinical context. Studying the mechanistic impact of inflammation in human PCa cells remains a major challenge.

3. DNA repair defects and prostate cancer ‘immunogenicity’.

Despite the central role that inflammation and the SASP play in prostate carcinogenesis, PCa is purported to be an immunologically ‘cold’ tumour with a low response rate to immune checkpoint inhibition \(^{3,90-92}\). Nevertheless, a subset of lethal PCa (20-30%), including those with defective homologous recombination DNA repair genes (HRD; BRCA2, PALB2, RAD51), mismatch repair (MMR) genes, CDK12 bi-allelic alterations, and DNA polymerase epsilon (POLE) mutations may be associated with higher tumour mutation burden (TMB), neoantigens, and an increased likelihood of anti-tumour immunity \(^{89,92-99}\). Deleterious HRD gene alterations are the commonest DNA repair defects in aggressive PCa, and may be early events in carcinogenesis especially in germline mutation carriers; these associate with increased inferred immune infiltrates but may result from the inflammatory process as noted in the previous section, although they may also arguably increase anti-PCa immunogenicity.

Defective mismatch repair (dMMR) occurs in 3-5% of lethal PCa and confers worse prognosis \(^{94,99}\). Both germline and somatic MMR gene mutations are associated with PCa \(^{89,100}\). dMMR mutational signatures correlate with: i) canonical gene mutations, ii) increased mutational load, iii) increased expression of immune checkpoint molecules, and iv) increased and more complex inferred immune infiltrates \(^{94,99}\). A phase II study of 149 patients with dMMR tumours showed approximately 40% of patients with dMMR respond to checkpoint inhibitors (CPI) \(^{101,102}\). Correlation of dMMR status with the expression of immunosuppressive genes and checkpoints, such PD-L1 which is expressed at low frequency in mismatch-repair intact tumours, indicate a potential mechanism of CPI resistance \(^{94,103}\).

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Pathogenic mutations in the DNA replication machinery can create hypermutated immunophenotypes similar to dMMR. Somatic mutations in the \textit{POLE} proofreading domain have been reported in PCa\textsuperscript{93}. \textit{POLE} encodes the catalytic subunit of DNA polymerase \(\varepsilon\), which replicates the leading DNA strand before cell division. POLE proofreading is essential for replication fidelity. \textit{POLE} mutated tumours harbour some of the highest single nucleotide aberrations reported to date\textsuperscript{104,105}. This microsatellite-stable hypermutated phenotype associates with high levels of CD8\(^+\) T cell infiltration, expression of cytotoxic T-cell markers, and effector cytokines - similar to dMMR cancers\textsuperscript{106-109}. Recent report of durable response to CPI in a PCa patient with a \textit{POLE} mutation corroborate similar findings in other histologies\textsuperscript{106,107}.

Somatic bi-allelic alterations of cyclin dependent kinase 12 (\textit{CDK12}) are enriched in lethal PCa (4-5%), generate another immunogenic subset of PCa that is genetically, transcriptionally, and phenotypically distinct from homologous recombination deficient (HRD) and dMMR tumours\textsuperscript{97}. CDK12 deleterious alterations were reported to induce genomic instability through regulation of HR, but also impact other effectors of DDR\textsuperscript{110-114}. Like dMMR and HRD mCRPC, \textit{CDK12} mutant PCa has a significantly higher neoantigen load compared to other mCRPC; however, neoantigens were mostly generated through gene fusions as opposed to indels and single nucleotide variants\textsuperscript{97}. These antigens have a strong affinity for MHC Class I. CDK12 mutant tumours have high immune infiltration, characterised by dendritic cell migration, T cell infiltration and clonal expansion; however, pro-tumour chemokines (CCL18 and CXCL8) are also activated\textsuperscript{97}. Cases of response to CPI is reported in a small clinical series\textsuperscript{97}. Further study in clinical trials are required to evaluate these hypotheses.

Interestingly, these data also indicate that PCa with deleterious ATM mutations may be “immune cold” \textsuperscript{97}; ATM has been reported to be key to activation of senescence and the SASP phenotype with ATM loss possibly decreasing this inflammatory response. Studies are now needed to further evaluate this finding.

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4. Immune evasion
PCa thwarts immune surveillance through multiple mechanisms. The prostate TME harbours a complex network of immunosuppressive cells including myeloid, lymphoid, senescent cells, stromal cells, inflammatory chemokines and cytokines. Several key oncogenic pathways, including MYC, PI3K/AKT and Wnt signalling are also implicated in PCa immune evasion.

4.1. MDSCs, macrophages and prostate cancer
Inflammatory myeloid derived cells, including MDSCs and TAMs, have emerged as key players linking inflammation and immunosuppression to PCa development\textsuperscript{19,75,76,115-118}. MDSCs are enriched in mCRPC biopsies when compared with CSPC and are localised adjacent to epithelial tumour cells in the TME\textsuperscript{19}. MDSCs associate with the development of metastatic PCa, with higher levels conferring worse OS\textsuperscript{115,119,120}. MDSCs are broadly categorized as monocytic MDSC (M-MDSC) and polymorphonuclear MDSC (PMN-MDSC) based on cell surface marker expression and origin\textsuperscript{121}. Both subtype appear to have a role in PCa although their relative contribution is unclear.

MDSCs mediate immunosuppression through direct contact with other immune cells, paracrine and endocrine signalling and the release of reactive oxygen species and reactive nitrogen species\textsuperscript{122}. MDSCs upregulate nitric oxide synthase (NOS), arginase, and indoleamine 2,3-dioxygenase-1 to deplete T-cell nutrient factors (L-arginine, L-cysteine, and tryptophan) and nitrate tyrosine residuals in T cells to induce anergy\textsuperscript{122,123}. In a murine model of prostate-specific deletion of \textit{Pten/p53/smad4}, MDSCs induced tyrosine nitration of lymphocyte-specific protein tyrosine kinase (LCK). LCK is the initiating tyrosine kinase in the T cell activation pathway; its nitration led to T cell anergy evidenced by reduced IL-2 secretion and impaired T cell proliferation\textsuperscript{123}. Another study showed using co-culture experiments of CD33+HLA-DR- cells derived from patients with advanced PCa with autologous T cells showed decreased proliferation and effector function, with decreased IL-2 and IFN-\gamma secretion. Myeloid cells isolated from healthy donors had little impact on the proliferation of their autologous T cells\textsuperscript{119}. Notably, myeloid cells used in this study were
selected using the cell surface marker of early-stage MDSCs, rather than markers such as CD11b, CD14 and CD15, commonly used to identify PMN- and M-MDSCs, subsets upon which much of the existing literature is based. Whilst immaturity and heterogeneity are common features of MDSCs, the specific functional differences between early-MDSCs and more mature subtypes are unclear.

MDSCs influence the TME by releasing cytokines that impact on other myeloid cells, T cells, and tumour cells. Studies of human PCa biopsies showed high MDSC density correlates with increased circulating cytokines IL-6 and IL-8, and higher clinical stage\textsuperscript{119}. Both IL-6 and IL-8 are key pro-inflammatory cytokines that activate multiple oncogenic pathways to induce tumour cell proliferation and metastases\textsuperscript{124,125}. MDSCs release IL-10 to skew macrophage differentiation towards an immunosuppressive pro-mitogenic M2-like phenotype\textsuperscript{75}. The impact of MDSC on IL-23-STAT3-ROR\textgreek{g}-AR signalling also represents an important therapeutic resistance mechanism to AR-directed therapies (Box 2). Future studies need to dissect the exact mechanisms of MDSC recruitment and expansion during PCa development, and how this is perhaps triggered by bacterial infection or other exogenous insults.

Like MDSCs, TAMs can promote inflammation and impact on adaptive immunity, angiogenesis, tumour proliferation and metastases\textsuperscript{76,117,118,126}. TAMs can also have anti-tumour effects\textsuperscript{76,117,118,126-128}. These cells are conceptually categorized into M1-like (classically activated) and M2-like (alternatively activated) phenotypes representing a spectrum of functional states. The M2 subset subverts adaptive immunity by promoting differentiation of T helper 2 cells and Tregs, thus promoting immunosuppression, tumour progression and metastases\textsuperscript{126}. M2 macrophages correlate with PCa aggressiveness and the development of castration resistance\textsuperscript{117,127}. PCa cell and stroma-derived factors, ADT, and obesity can induce TAM recruitment and the M2 phenotype\textsuperscript{76,116,117,129}. Various chemokines and cytokines have been implicated in TAM recruitment (CSF-1, IL-34 (another CSF-1R ligand), IL-6, MCP-1, and SDF-1) and differentiation (IL-10, CSF-1, IL-13, CXCL1, CXCL2, CXCL5)\textsuperscript{76,117,118}. Future work is required to elucidate the mechanisms driving TAM
recruitment and programming in order to determine the exact effects that specific subsets of TAMs have on PCa tumorigenesis and progression.

4.2. Immunosuppressive oncogenic signalling

PI3K/AKT pathway oncogenic alterations associate with adaptive immune-resistance in PCa, contributing to prostate tumorigenesis by playing a role in the initiation and maintenance of prostatic inflammation and immunosuppression. PI3K pathway activation leads to the dysregulation of inflammatory chemokines and chemokine receptors in the prostate epithelium and stroma\textsuperscript{75,130-132}. PTEN loss in PCa cell lines is associated with upregulation of the chemokine CXCL8/IL-8 and its receptors CXCR1 and CXCR2\textsuperscript{131}. Importantly, PCa cell lines stably transfected to express IL-8: i) downregulate AR expression and develop androgen independence; ii) upregulate signalling via PI3K/AKT, mitogen-activated protein kinase, epidermal growth factor receptor (EGFR), Src Family Kinases, and nuclear factor-κB (NFκB); iii) develop angiogenic and metastatic potential\textsuperscript{125}. Deletion of one of the IL-8 receptors, CXCR1, leads to spontaneous apoptosis of androgen unresponsive PCa cells\textsuperscript{133}. IL-8 also facilitates functional cooperation between tumour cells and its stroma by increasing the expression of the chemokine receptors CCR2 and CXCR4 in PCa cells and the secretion of their ligands, CCL2 and CXCL12 by stromal cells. These receptor-ligand interactions promote PCa proliferation and stroma-directed PCa cell migration\textsuperscript{131}. Interestingly, PTEN loss leads to significant upregulation of the \textit{Csf1, Il1b, Il1ra} genes, which induce MDSC recruitment and expansion intratumorally in \textit{Pten} knockout models\textsuperscript{75}. Future studies are required to delineate the impact of AKT blockade on these immunosuppressive functions and to identify potential compensatory mechanisms that lead to therapeutic resistance.

Somatic alterations in Wnt genes occurs in approximately 20\% of lethal PCa\textsuperscript{89,134}. In addition to promoting tumour invasion, metastases, and therapeutic resistance, Wnt pathway signalling likely also plays a role in PCa immune evasion\textsuperscript{41,135-137}. Activation of tumour intrinsic Wnt/β-catenin signalling in murine melanoma models has been shown to upregulate of immune checkpoints, defective dendritic cell recruitment, and T cell exclusion\textsuperscript{138}. In a study of interrogating high-risk prostate cancer biopsies, multi-region sequencing showed

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Wnt pathway activation was associated with a lower CD8+/FOXP3+ ratio, suggesting a potential mechanism for immunosuppression\textsuperscript{135}. The parallel evolution of three different gain-of-function mutations in CTNNB leading to activation of Wnt/β-catenin signalling in a patient with dMMR PCa, with associated high inflammatory infiltrate but low CD8+/FOXP3+ ratio, also supports this hypothesis\textsuperscript{135}. A recent study of the impact of Wnt signalling inhibition on PCa cell lines and lymphocytes derived from patients with biochemically recurrent PCa showed that a tankyrase inhibitor inhibited Wnt/β-catenin signalling by inhibiting β-catenin translocation to the nucleus in PCa cells and CD4+ T cells, but not CD8+ T cells. At doses where cancer cell and immune cell differentiation, proliferation, and viability were not impacted, Wnt/β-catenin signalling inhibition in lymphocytes, but not PCa cells lines, initially accelerated the elimination of PCa cells. However, acquired resistance to immune elimination occurred over time and appeared to be due to adaptive upregulation of Wnt/β-catenin upregulation in PCa cell lines. This was overcome by Wnt/β-catenin signalling inhibition in the PCa cell lines\textsuperscript{139}. Whilst cell line co-culture models do not encapsulate the complexity of anti-tumour immune responses and tankyrase inhibitors can impact other cellular functions\textsuperscript{140}, this study highlights that Wnt/β-catenin signalling can act on both cancer cells and immune cells to facilitate immune evasion.

Further functional dissection of the immunologic and non-immunologic impacts of Wnt signalling in\textit{ in vivo} models are required to validate the aforementioned findings. The differential effect of Wnt/β-catenin signalling on different immune cell subsets also warrant further investigation.

MYC gene upregulation is another genomic event implicated in a subset PCa associating with aggressive disease\textsuperscript{141}. In a study using a murine model of c-Myc-driven PCa (Hi-Myc) PCa, from a precancerous stage, mice showed a higher accumulation of immune cells in their prostate than wild-type mice, with macrophages being the most abundant. The onset of invasive adenocarcinoma was delayed in immunodeficient Hi-Myc mice compared to immunocompetent mice, suggesting immune cells played a role in tumour progression\textsuperscript{142}. In addition to MYC amplification, MYC mRNA and protein is overexpressed in the vast majority of prostatic carcinomas\textsuperscript{141}. Although the mechanisms responsible for MYC
overexpression in many cases is not clear, several oncogenic pathways converge on MYC signalling to co-opt its immune-evasive effects. In a murine model of Pten loss and BrafV600E activation, upregulation of Myc pathway genes was noted, suggesting alternative routes for Myc signalling. Additionally, germline BRCA2 mutations correlate with copy number gains of chromosome 8, which encodes c-MYC. In a series of 20 BRCA2 mutation carriers and 20 non-carriers, copy number gains in the 8q24.21 region were observed in 89% of patients, as compared with 12.5% of non-carrier tumours. MYC-targeted therapy may therefore be relevant to a significant subset of lethal PCa, with effects predicted to affect tumour-intrinsic oncogenic pathways in addition to the immune components of the TME.

5. Weathering the storm

Prostate carcinogenesis and tumour progression are driven by the complex interplay between inflammation, immunity, and tumour genomics. The soluble and cellular components of the PCa TME serve as potential druggable targets. Dissecting these key processes is crucial to the development of novel therapies for patients with lethal PCa, and for the implementation of surveillance, lifestyle, and chemoprevention strategies that address heritable and modifiable risk factors (Figure 4).

5.1. Modulating inflammation and immunity

The well-established link between inflammation and prostate carcinogenesis indicates that therapies preventing prostatic inflammation, whether directed at the host microbiome, dietary or lifestyle factors, or chemical irritation, may play a role in preventing the development and progression of PCa. Prevention strategies may be particularly relevant in those at high risk of developing aggressive PCa, such as those who harbour DNA repair defects (DRD) and especially BRCA carriers.

5.1.1. Modulating the host microbiome

Current evidence suggests that inflammation is likely due to chronic exposure to various organisms rather than a single culprit. Antibiotics, probiotics, and faecal microbial transplantation are all potential methods for augmenting the host microbiome. These studies require a cautious approach since methods such as faecal transplantation carries the risk of...
transplanting potentially harmful donor organisms and antibiotics can also impact ‘favourable’ organisms, or contribute to the unintentional outgrowth of deleterious species. To date, studies have focused on correlating microbial composition with disease\textsuperscript{25,27,28}. A more targeted therapeutic approach may be to address the specific mechanism through which microorganisms promote carcinogenesis. To this end, further functional dissection of the microbial interactions with the host, whether it is through inflammation or the secretion of carcinogenic metabolites or hormones, is critically important.

5.1.2. Modulating diet and obesity

The role of diet and obesity in inducing prostatic inflammation, and on steroid hormone synthesis, and how this contributes to PCa progression can also be addressed through pharmacologic and lifestyle measures\textsuperscript{44,52-57,147,148}. Although there is no consensus on lifestyle interventions for men with advanced PCa, a normal BMI and the prevention of metabolic syndrome are likely to be protective. Further research into how certain foods may interact with the gut microbiome to cause metabolic dysfunction or induce steroid synthesis, or provide a source of exogenous steroid and sex hormones, will shed light on protective dietary interventions\textsuperscript{36,149}. Interventions targeting diet and obesity are likely to have a role not only in the management of advanced disease, but could be considered in the adjuvant and preventative setting, particularly in high-risk individuals.

At a pharmacologic level, abrogation of the endocrine and paracrine functions of adipokines could be achieved using pharmacologic blockade, although given the array of factors implicated, deciphering chemokine/cytokine receptor nodes will be critical to therapeutic development\textsuperscript{44,54,62}. An added complexity is the functional redundancy and compensatory mechanisms shared by chemokines/cytokine receptors; combinatorial strategies may therefore be required. Recent evidence showing that oncogenic signalling interacts with transcriptional regulators of lipid metabolism to promote PCa progression suggests a role for pharmacologic agents, such as fatostatin, which blocks the transcriptional regulator SREBP, in conjunction with lifestyle measures\textsuperscript{150}.

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5.1.3. Targeting inflammatory myeloid cells

Chronic inflammation and immunosuppression triggered by exogenous factors are mediated through inflammatory immune cells in the PCa TME. MDSCs are key players in this process and are thus prime therapeutic targets. Several therapeutic approaches have been demonstrated in preclinical models of PCa: i) depletion of MDSC; ii) interruption of MDSC trafficking; iii) inactivation of MDSC; and iv) blockade of effector functions. MDSC depletion with anti-Gr1 neutralizing antibody sensitized tumours in a novel non-germline mCRPC mouse model to CPI. Treatment of prostate-specific conditional Pten-null mice with a selective CSF-1 receptor antagonist decreased MDSC infiltration and alleviated their immune-suppressive effects on dendritic cells and macrophage maturation. CXCR2 blockade inhibited PMN-MDSC infiltration in prostate-specific conditional Pten-null mice, with prostate cancer allografts (TRAMP-C1 and Myc-CaP), delaying castration-resistant disease. CXCR2 blockade has also been shown to ameliorate the anti-senescence effects of MDSC and synergized with docetaxel and to reduce tumour proliferation in mice with Pten-null PCa. A challenge in chemokine-directed therapy against myeloid inflammatory cells is the significant heterogeneity in the expression of cell surface markers as well as the expression of redundant receptors by healthy myeloid cells, therefore therapies targeted at protumorigenic myeloid cell functions may be better tolerated.

Several preclinical studies have investigated ways in which the pro-mitogenic effects of MDSCs can be targeted. IL-23 has downstream effects on AR target genes; IL-23 blockade has shown preclinical efficacy in murine models of Pten-null PCa resistant to enzalutamide. Strategies to target AR signalling through these novel mechanisms are discussed in Box 2. Inhibition of MDSC effector function can also be achieved by inhibiting JAK2/STAT3 signalling; STAT3 silencing abrogates the immunosuppressive effects of PCa patient-derived MDSCs on effector T-cell function, reducing the expression and activity of Arg1. The combination of CPI and agents which neutralise reactive nitrogen species (which prevented LCK nitration-associated T-cell inhibition) led to a significant anti-tumour immune-response and to tumour response in a mouse model of mCRPC with prostate-specific deletion of Pten.
Given the broad impact of MDSCs, MDSC-directed therapies may be combined with immunotherapy or chemotherapy to overcome treatment resistance.

5.1.4. Targeting prostatic cellular senescence

Senescence and the SASP play an important role in prostate carcinogenesis and progression. The development of therapies targeting senescence mechanisms are complicated by the context-dependent, pleotropic regulation and functions of senescence and the SASP. PCa therapies that induce senescence (e.g. docetaxel, γ-secretase inhibitor, Skp2 inhibitor) may protect against tumorigenesis\(^1\)\(^2\)\(^3\). Additionally, the acute immune-stimulating functions of the SASP may be induced by chemotherapy or ADT\(^6\)\(^7\)\(^8\)\(^9\)\(^10\)\(^11\). However, since the accumulation of senescent cells and the SASP can be tumour-promoting, therapies that promote senescence may need to be coupled with compounds that either enhance their immune-clearance (e.g. STING agonists, CPI), remove these cells from tumours (i.e. senolytics) (e.g. navitoclax), or reprogram the SASP (e.g. JAK inhibitor) to promote immune surveillance\(^7\)\(^1\)\(^5\)\(^7\). Dissection of the regulation of specific functions of senescent cells is critical to the uncoupling of the protective and deleterious effects of the senescence program (Box 1).

5.2. Inducing anti-PCa cell immunity

Despite the advances made by immunotherapy in cancer treatment over the last decade, PCa treatment has not been significantly impacted. Sipuleucel-T, an autologous cellular immunotherapy consisting of autologous peripheral-blood mononuclear cells, including antigen presenting cells, remains the only immunotherapy to confer an OS benefit in mCRPC\(^1\)\(^5\)\(^8\). Due to various factors, this agent is currently not widely administered. The lack of efficacy from CPI in PCa is likely in part due to the overall low mutation rate (2-4.4 single nucleotide alterations per megabase in mCRPC), the lack of PD-L1 expression, and the multiple immune-evasive mechanisms operative in these diseases\(^8\)\(^9\)\(^1\)\(^0\)\(^4\)\(^6\)\(^7\)\(^9\). Two phase III trials have shown showed no OS benefit from CPI targeting cytotoxic T lymphocyte antigen-4 (CTLA-4) in molecularly unselected mCRPC patients. This combination is also associated with significant toxicities\(^9\)\(^0\)\(^1\). A phase II study (KEYNOTE-199) of 258 men with molecularly unselected mCRPC treated with anti-PD-1 monotherapy demonstrated an

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objective response rate of 5% in the subset of patients with measurable disease, with outcomes being similar in PD-L1-positive and -negative patients\textsuperscript{92}. Overall response rate was 11% in patients with BRCA or ATM mutations. Genomic analysis of 50 DRG in responders showed that four of the six responding tumours (nine responders in total) harboured DRD. Exploratory biomarker analyses of two long-term responders revealed dMMR by immunohistochemistry that was below the cut-off for high MSI by next generation sequencing, with associated high TMB and tumour infiltrating lymphocytes, highlighting the need for orthogonal methods in biomarker identification. In another phase II trial studying the impact of anti-CTLA-4 and anti-PD-1 antibodies in men with ARV7-expressing mCRPC, 40% of patients had DRD (three with mutations in BRCA2, two in ATM, one in ERCC4). Objective responses (40%) were only seen in those with DRD\textsuperscript{96}. Collectively, studies of CPI in mCRPC demonstrate that a small subset of patients benefit CPI and identification of predictive biomarkers remains key\textsuperscript{90-92,96,159,160}. However, caution needs to be exercised when interpreting these single-arm studies which are underpowered to draw conclusions about the relationship between the presence of DRD and CPI-responsiveness. Their findings need to be further evaluation in large, randomised studies, with prospective, orthogonal methods of biomarker-selection.

The preliminary demonstration of efficacy with anti-PD-1 therapy – albeit in a very small subset of patients – has led to several ongoing clinical trials exploring anti-PD-1/PD-L1-based combinations. The combination of PARP inhibitors and immunotherapy is supported by evidence of potential synergy. Whilst the exact mechanisms are incompletely understood, it is hypothesised that PARP inhibitors potentiate DNA damage, which in conjunction with the inability to resolve DNA damage in tumours with BRCA1, BRCA2, or BRCAness defects, associate with higher TMB, higher associated neo-antigen load, and increased likelihood of anti-tumour immune response\textsuperscript{161,162}. DNA damage causing release of cytosolic DNA can also activate the eGAS-STING pathway and trigger type-1 interferon-mediated adaptive immune response\textsuperscript{163}. In a phase II study studying of 17 unselected patients with mCRPC who received an anti-PD-L1 antibody and a PARP inhibitor, nine (53%) had a PSA decline of $\geq 50\%$. Germline or somatic aberrations of DNA repair genes (BRCA2, NBN) were more common

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among responders than non-responders. This study showed that an early immune response, characterised by an increase in mature CD83⁺CD141⁺ dendritic cells and increased expression of T cell activation markers, was associated with longer PFS\textsuperscript{164}. It remains unclear whether these responses are due to synergy or the additive benefit of the combination.

Our understanding of the factors required to generate robust anti-tumour immunity is far from complete. Factors currently in consideration include: TMB, tumour genomics, functionality and infiltration of lymphocytes, and immunosuppressive mechanisms operative within the TME. Future studies are required to quantify the presence of aberrations of DRG at a genetic and epigenetic level, to characterize the magnitude and clonality of these genomic events, and how they correlate with markers of tumour immunogenicity. Innate immunity may be harnessed by inducing cytosolic DNA recognition in vulnerable DRD tumours through PARP inhibition or other DNA damaging agents\textsuperscript{163}. At a genetic level, single nucleotide polymorphisms (SNPs) of the components of adaptive and innate immunity, such as the STING pathway, PD-L1 and B7-H3, have been described\textsuperscript{165-167}. The effect of single or multiple cumulative SNPs could contribute to a failure of immune surveillance or to the over-activation of innate immune pathways triggered in response to exogenous stimuli, driving chronic inflammation. Thus, those SNP’s may have prognostic, and potentially predictive, significance. Functional assays dissecting the longitudinal profile and interaction of stromal cells, lymphocytes, and pathogenic myeloid cells will be key in informing therapeutic development.

6. Conclusion

There has been tremendous progress in our understanding of the biological processes underpinning prostate carcinogenesis and progression. Here, we have highlighted the mechanisms through which environmental and heritable risk factors promote prostatic inflammation, a process amplified by inflammatory myeloid cells and senescent cells. Inflammation can drive AR signalling, which in tandem with defective DNA repair, contribute to tumorigenesis. Oncogenic drivers co-opt cellular and soluble components of the PCa TME, by processes including the SASP, to facilitate a highly immunosuppressive TME.
that fosters tumour immune evasion (Figure 4). Emerging data suggest that many of the genetic risk factors that increase prostate cancer risk impact these processes. In light of these findings, it is now time to re-envision the management of PCa, from prevention to systemic therapy, with a goal of leveraging these biological insights into effective and novel therapeutic strategies.
Box 1: Regulation of Cellular Senescence in Prostate Cancer:

Several mechanisms of oncogene-induced senescence have been described in PCa. PTEN loss and TP53 loss are enriched in mCRPC. Mouse models with prostate-specific Pten-loss were protected from the development of invasive tumour growth through the induction of the p19Arf-p53-p21 senescence pathway. Concurrent loss of both Pten and p53 resulted in the development of lethal, invasive tumours. These findings were corroborated by another study, which in addition, showed that Pten-loss induced cellular senescence occurred in the absence of cellular proliferation and DNA damage response.

Pten loss has also been implicated in senescence evasion. Murine models of Pten-null PCa are infiltrated by MDSCs which antagonise cellular senescence through paracrine signalling by secreting interleukin-1 receptor antagonist. Further, Pten-null tumours have also been shown to upregulate Notch signalling. Inhibition of Notch signalling arrested the growth of Pten-null prostate tumours by inducing p27-driven senescence.

Both persistent AR signalling and supraphysiologic doses of androgens have been shown to induce senescence in human PCa cell lines and PCa tissue ex vivo. In a study using PCa cell lines and prostate tumour mouse models (both with inducible AR), persistent AR activation triggered senescence by upregulating p21, and decreasing phosphor-Rb and p63. In another study of PCa cell lines, supraphysiologic androgen levels mediated senescence through the p16-Rb-E2F1 pathway, Src tyrosine kinase, and PI3K pathway. In contrast to Pten-loss induced cellular senescence, AR-induced senescence appears to be p53-independent. These findings highlight the complexity of oncogene-induced senescence, which can be mediated through multiple pathways.

PCa therapies generate genotoxic stress and trigger cellular senescence. Androgen-dependent LNCaP cell lines treated with ADT became morphologically senescent and upregulated their expression of senescence markers. Imputing a direct mechanism, ADT was unable to induce senescence in androgen-independent PCa cell lines. In vitro, ADT has also been reported to induce senescence and the SASP by down-regulating S-phase kinase.

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associated protein 2, which degrades the cyclin dependent kinase inhibitor p27, leading to the upregulation of SASP-associated inflammation\textsuperscript{68}. A study of 69 patients who received neoadjuvant ADT showed a significant increase in the expression of GLB1, the protein product of the $\beta$-galactosidase gene and a well as marker of senescence\textsuperscript{67}.

PCa therapy also induce cellular senescence\textsuperscript{155,156}. Treatment of PCa cell lines with docetaxel, doxorubicin or 5-aza-2’-deoxycytidine led to the upregulation of genes associated with senescence and the SASP, as well as associated morphologic changes\textsuperscript{155}. A recent study of human prostate stromal cells provides mechanistic insight. Chemotherapies drive the activation of the ATM-TRAF6-TAK1 axis, which upregulates the transcription factor Zscan4, to promote the transition from an acute stress associated phenotype to SASP. TAK1 collaterally activates p38 which engages PI3K/Akt/mTOR signalling to support persistent SASP signalling by stromal cells and promote tumour growth. TAK1 suppression reduced the viability of PCa cells\textsuperscript{156}. There are several important implications for understanding therapy-induced senescence. First, mechanistic dissection allows the potential uncoupling of the protective functions of senescence from its deleterious roles. Further, a recent study mapping the fate of embryonic senescent fate \textit{in vivo} showed some cells had the ability to re-enter the cell cycle\textsuperscript{169}. This hypothesis warrants investigation in the oncological setting as ‘reversible’ senescence instead of cell death could represent an important therapeutic resistance mechanism.

\textbf{Box 2: Novel strategies to targeting AR signalling}

AR blockade remains the mainstay of PCa management. Despite the development of next-generation AR signaling inhibitors (abiraterone, enzalutamide, apalutamide, darolutamide), which have significantly improved PCa survival, resistance is inevitable\textsuperscript{170}. Resistance to ADT can be due to restored AR signaling, AR bypass signaling, and complete AR independence. Restored AR signaling is often mediated through AR point mutations, amplification, over-expression, rearrangements, splice variants, increased activity of AR-coactivators, extra-testicular androgen production, or steroid hormone-induced activation of

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mutant AR genes\textsuperscript{171-174}. Therapeutic strategies targeting AR have primarily focused on cell-autonomous mechanisms of AR signaling.

In this review, we describe several cell non-autonomous mechanisms of AR signaling. This includes the paracrine effects of immune cells of the TME driven by inflammation, adipokines, dietary factors, the GI microbiome, as well as senescence mechanisms. Each of these processes can be targeted individually or in combination. For example, blockade of IL-23 mediated AR activation have been shown to reverse endocrine resistance to enzalutamide in murine models\textsuperscript{19}. Inhibition of adipocyte secretion of steroid hormones may also be effective in a subset of obese patients with resistance to ADT. Therapeutic development in this field need to target not only the PCa cells but the broader TME and host context in which these cells exist.

Table 1. Putative factors implicated in the development of lethal prostate cancers

<table>
<thead>
<tr>
<th>Putative factor</th>
<th>Key mechanism</th>
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<tbody>
<tr>
<td>Chronic inflammation</td>
<td>Microbes • Uropathogens • Gut microbes • Uropathogens cause prostatic inflammation, epithelial injury and hyperplasia\textsuperscript{6,7,11,12,25-30}. • Gut microbes have the potential to produce metabolites and androgens that promote PCa development\textsuperscript{35,36}.</td>
</tr>
<tr>
<td>Diet\textsuperscript{10,42,49}.</td>
<td>• Dietary fat • Red meat • Charred meat • High fat diet enhances the oncogenic MYC transcriptional program through epigenetic mechanisms\textsuperscript{63}. • High fat diets may activate the SREBP prometastatic transcriptional regulator of lipid homeostasis in PCa\textsuperscript{56}. • Adipocytes can release growth hormones, sex hormones, steroids, and inflammatory cytokines which promote PCa progression\textsuperscript{44,52,53,55,57}. • Periprostatic fat promotes tumour metastases through paracrine signalling\textsuperscript{62}. • Hyperinsulinemia prevents the degradation of the receptor to the inflammatory cytokine IL-17\textsuperscript{54}.</td>
</tr>
<tr>
<td>Obesity\textsuperscript{8,45,50,51}.</td>
<td></td>
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</tbody>
</table>

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<table>
<thead>
<tr>
<th>Genetic factors</th>
<th>Chemical reflux</th>
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<tbody>
<tr>
<td></td>
<td>Germline mutation of DNA repair gene</td>
<td>• Epithelial injury and hyperplasia(^{5,23})</td>
</tr>
<tr>
<td></td>
<td>Somatic mutation of DNA repair gene</td>
<td>• Germline (e.g. BRCA 1, 2, ATM) or somatic mutations of DNA repair genes lead to ineffective DNA repair(^ {144,145,175}).</td>
</tr>
<tr>
<td></td>
<td>Single nucleotide polymorphisms (SNPs)</td>
<td>• SNPs in promoter and enhancer elements, and transcription factor-binding sites, of genes such as androgen receptor, ERG and FOXA1, as well as DNA repair genes, cell cycle regulators, and oncogenes increase the risk of PCa(^ {176,177}). It is likely that many of the SNPs associated with increased PCa risk alter AR signaling, DNA repair and/or inflammatory responses.</td>
</tr>
</tbody>
</table>

| Androgen receptor signalling | Extra-testicular androgen production | • Adipocytes generate steroid hormone which activate the androgen receptor\(^ {58}\). |
| | Exogenous hormones | • A significant increase in risk of PCa in the French West Indies is linked to increasing plasma concentration of chlordecone, an environmental oestrogen. Chlordecone can have agonistic effects on oestrogen and may activate other receptors involved in steroid homeostasis\(^ {178}\). |
| | Ligand-independent androgen receptor activation | • MDSCs release IL-23 which can cause downstream activation of AR-target genes\(^ {19}\). |

* Prostate carcinogenesis and progression are likely caused by the complex interplay of a multiple heritable and environmental factors. The processes described above do not necessarily occur in a step-wise manner.
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**Prostate Carcinogenesis: Actionable Inflammatory Storms?**
Glossary:

Metastatic castration-resistant prostate cancer (mCRPC)
Prostate cancer which has spread to sites of the body beyond the prostate and continues to progress despite androgen deprivation therapy.

DNA repair genes (DRG)
Genes which encodes the key enzymes involved in the recognition and restoration of the normal base-pair sequence and structure of damaged DNA.

Microbiome
Genetic material of all the microbes – bacteria, fungi, protozoa and viruses – that live on and inside the human body.

Tumour microenvironment (TME)
The environment around a tumour including the surrounding blood vessels, immune cells, stromal cells, signalling molecules and extracellular matrices.

Myeloid-derived suppressor cells (MDSCs)
A heterogenous group of immune cells from the myeloid lineages that expand under pathological conditions such as cancer, chronic infection, and inflammation.

Androgen deprivation therapy (ADT)
Treatments (e.g. luteinising hormone releasing hormone agonists and surgical castration) which significantly reduce the level of testosterone and other antiandrogens in the circulation and is generally the first type of hormone therapy used to treat advanced prostate cancer.

Tumour-associated macrophages (TAMs)

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A class of macrophages found in abundance in some solid tumours and often contributes to immunosuppression in the tumour microenvironment.

**Senescence**
Cells enter a state of cell cycle arrest without undergoing cell death.

**Non-homologous end joining (NHEJ)**
Pathway that repairs DNA double-strand breaks by ligating broken DNA ends without the use of a template.

**Senescence associated secretory phenotype (SASP)**
Secretory phenotype of senescent cells that leads to the expression of a spectrum of soluble factors which promotes inflammation, epithelial-mesenchymal transition, immunosuppression, drive tumour progression, amongst other effects.

**Chromoplexy**
A class of complex and coordinated DNA rearrangements observed in the genome of cancer cells.

**DNA damage response (DDR)**
A network of cellular pathways and machineries that sense, signal and repair DNA lesions to prevent the generation of potentially deleterious mutations which threaten cell viability and potentially lead to neoplasia.

**Mismatch repair (MMR)**
A process that corrects mismatched nucleotides in the otherwise complementary paired DNA strands.

**Tumour mutational burden (TMB)**
The total number of mutations carried by tumour cells
Hormone sensitive prostate cancer (HSPC)
Prostate cancer that is still sensitive to androgen deprivation therapy.

Senolytics
Drugs that specifically target and induce death of senescent cells.

Contributions:
All authors researched data for the manuscript and made substantial contributions to the discussion of the content. J.S.B. and C.G. wrote the manuscript. B.G, A.M.M., K.S.S., R.S.M., J.G., C.G.D., A.A. reviewed and edited the manuscript before submission.

Ethical Declaration:
J.D.B., B. G., and C.G. are employed by The ICR, which has a commercial interest in abiraterone, in the use of PARP inhibitors in DNA repair defective cancers and in IL-23 driving AR signalling in prostate cancer. J.D.B. has served on advisory boards for many companies including Astra Zeneca, Astellas, Bayer, Boehringer Ingelheim, Cellcentric, Daiichi, Genentech/Roche, Genmab, GSK, Janssen, Merck Serono, Merck Sharp & Dohme, Menarini/Silicon Biosystems, Orion, Pfizer, Qiagen, Sanofi Aventis, Sierra Oncology, Taiho. His institution has received funding or other support for his research work from AZ, Astellas, Bayer, Cellcentric, Daiichi, Genentech, Genmab, GSK, Janssen, Merck Serono, MSD, Menarini/Silicon Biosystems, Orion, Pfizer, Sanofi Aventis, Sierra Oncology, Taiho. He has been the CI/PI of many industry sponsored clinical trials. J.G. owns equity and has acted as a consultant for Unity Biotechnology and Geras Bio. J.G. is an inventor in a MRC patent related to senolytic therapies (PCT/GB2018/051437). CGD has served on Advisory boards for AZ Medimmune, BMS, F-Star, Genocea, Janssen, Merck, Pfizer, Pierre Fabre, Roche / Genentech, Sanofi Aventis. His institution holds BMS, AZ Medimmune, Janssen patents, and

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he has interests in Harpoon, Kleo, Shattuck Labs, Tizona, Werewolf. A.M.D.M. has served as a sponsored research recipient from Janssen R&D and Myriad Genetics, and currently serves as a consultant for Cepheid Inc. A.A. is a cofounder and shareholder of Oncosence; he is inventor of the following patents: WO2019142095A1, WO2019142097A1, WO2019180636A1. No relevant conflicts of interest were disclosed by other authors.
Figure 1. The role of diet and obesity in prostate carcinogenesis and progression.
The gut microbiota, diet, and obesity are collectively implicated in prostate cancer progression. High fat diet and sedentary lifestyle lead to obesity. Adipose tissues are endocrine organs which produce pro-inflammatory chemokines, cytokines, metabolites, as well as steroids hormones. Peri-prostatic adipocytes can also induce prostatic inflammation impacting peri-vascular and intra-tumour macrophages as well as MDSCs, and can promote tumour cell migration through their paracrine function. Lethal prostate cancers harbour premetastatic lipogenic transcriptional programs which may serve as another mechanism through which excessive dietary fat promotes tumour progression.
Figure 2. Cellular senescence and the senescence associated phenotype in prostate cancer.

Cellular senescence is a state of stable cell cycle arrest induced by oncogenic signaling, loss of tumour suppressors, prostate cancer therapy, and inflammation. Cellular senescence can safeguard against tumorigenesis. The senescence associated secretory phenotype (SASP) is a transcriptional program involving the secretion of chemokines, cytokines, proteases, and immune-modulators which promote immune clearance and reinforce senescence through paracrine and autocrine functions. Both senescent cells and the SASP, however, can also contribute to tumorigenesis by contributing to immunosuppression and inflammation in the tumour microenvironment, and can facilitate tumour invasion and metastases. There is emerging data in embryonic models that senescent cells can also re-enter the cell cycle.
Figure 3. Androgen receptor signaling, inflammation, and defective DNA repair create the perfect storm for prostate carcinogenesis.

Nuclear androgen receptor (AR) signaling alters the three-dimensional architecture of the genome and recruits the AR-topoisomerase 2-β (TOP2B) complex to promoter regions of AR-target genes, where TOP2B catalyzes double-strand breaks (DSB). Most DSB are correctly repaired. Inflammation generates free radicals that cause DNA DSB. Myeloid-derived suppressor cells release IL-23 which activates cell surface IL-23R, JAK/STAT signaling, RORγt expression and AR signaling. DSB are repaired by high-fidelity homologous recombination (HR) or error-prone non-homologous end joining (NHEJ); AR signaling and DNA repair alterations lead to an imbalance favoring more error-prone NHEJ. The clustering of DSB along with AR target gene spatial localization and increased AR target gene accessibility increases the likelihood of DSB mis-repairs by processes such as chromoplexy resulting in the formation of non-random rearrangements and fusions such as TMPRSS2-ERG. Chronic inflammation exacerbates further DNA damage, and is postulated to be particularly deleterious at transcriptionally active AR target genes and DNA repair genes, thus fueling a vicious cycle of further DNA damage and DNA mis-repairs. Inflammation also results in reduced expression of NKx3.1 which leads to dysregulation of pro-oxidant and antioxidant enzymes creating further oxidative stress.
Figure 4. Overview of prostate carcinogenesis and progression in the context of inflammation and immunosuppression.
Prostate carcinogenesis is initiated by intraprostatic inflammation, potentially triggered by dietary factors, chemical injury, or an altered microbiome (uropathogens or gut microbiota). Intraprostatic myeloid inflammatory cells (e.g. myeloid-derived suppressor cells and tumour-associated macrophages) release cytokines, chemokines, and free radicals which cause DNA double strand breaks and can also directly trigger expression of AR-target genes through interleukin-23 mediated paracrine signaling. This fuels further senescence and the SASP and can amplify the inflammatory response whilst promoting an immunosuppressive milieu. AR signaling and free radicals can mediate further DNA strand breaks. In the setting of chronic inflammation and persistent AR signaling, chronic activation of DNA repair genes (DRG) and tumour suppressor genes expose these genes to DNA damage and generate ‘a perfect inflammatory storm’. This process can be accelerated by germline defects in DNA repair genes. Obesity and metabolic syndrome play a role in prostate cancer progression through the secretion of proinflammatory cytokines, growth hormones, and steroid hormones that exert endocrine and paracrine effects promoting tumour growth and metastases. Advanced prostate cancers also have dysregulated lipogenic programs (e.g. SREBP, transcriptional regulator of lipid metabolism) that also promote tumour growth and metastases –another mechanism through which dietary fat contributes to carcinogenesis. Potential novel therapies targeting these mechanisms are highlighted (yellow).