

# Patterns of indolence in prostate cancer (Review)

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**Abstract.** Although prostate cancer is a major cause of cancer-related mortality worldwide, most patients will have a relatively indolent clinical course. Contrary to most other types of cancer, even the diagnosis of locally advanced or metastatic disease is not always lethal. The present review aimed to summarize what is known regarding the underlying mechanisms related to the indolent course of subsets of prostate cancer, at various stages. The data suggested that no specific gene alteration by itself was responsible for carcinogenesis or disease aggressiveness. However, pathway analysis identified genetic aberrations in multiple critical pathways that tend to accumulate over the course of the disease. The progression from indolence into aggressive disease is associated with a complex interplay in which genetic and epigenetic factors are involved. The effect of the immune tumor microenvironment is also very important. Emerging evidence has suggested that the upregulation of pathways related to cellular aging and senescence can identify patients with indolent disease. In addition, a number of tumors enter a long-lasting quiescent state. Further research will

determine whether halting tumor evolution is a feasible option, and whether the life of patients can be markedly prolonged by inducing tumor senescence or long-term dormancy.

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*Abbreviations:* ADT, androgen deprivation therapy; PCa, prostate cancer; RP, radical prostatectomy; PSA, prostate specific antigen; DNA, deoxyribonucleic acid; ERG, ETS-related gene; ETV1, ETS variant transcription factor 1; ETV4, ETS variant transcription factor 4; FLII, friend leukemia integration 1 transcription factor; SPOP, speckle-type POZ protein; FOXA1, forkhead box A1; IDH1, isocitrate dehydrogenase 1; CNA, copy number alterations; MEN1, menin 1; MAP4K2, mitogen-activated protein kinase kinase kinase 2; SF1, splicing factor 1; PPP2R5B, protein phosphatase 2 regulatory subunit B $\beta$ ; TMPPRS2, transmembrane serine protease 2; PTEN, phosphatase and tensin homolog; TP53, tumor protein p53; FOXP1, forkhead box P1; RYBP, RING1 And YY1 binding protein; SHQ1, H/ACA ribonucleoprotein assembly factor; AR, androgen receptor; NCOA2, nuclear receptor coactivator 2; NCOR2, nuclear receptor corepressor 2; PI3K, phosphoinositide 3-kinase; RAS, rat sarcoma virus; RAF, rapidly accelerated fibrosarcoma; NEPC, neuroendocrine prostate cancer; RB, retinoblastoma; DNMT1, DNA methyltransferase family member 1; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; HDAC, histone deacetylases; TIME, tumor immune microenvironment; DDR,

DNA damage response and repair; CD8, cluster of differentiation 8; PD-L1, programmed death-ligand 1; Foxp3, forkhead box P3; TGF $\beta$ , transforming growth factor- $\beta$ ; MHC, major histocompatibility complex; TILs, tumor infiltrating lymphocytes; CAFs, cancer-associated fibroblasts; COL5A2/6, collagen, type V,  $\alpha$ 2/6; NRG1, neuregulin 1; HER3, receptor tyrosine-protein kinase erbB-3; CDKN1A, cyclin dependent kinase inhibitor 1A; FGFR1, fibroblast growth factor receptor 1; PMP22, peripheral myelin protein 22; CDX2, caudal type homeobox 2; HOXB13, homeobox B13; EGFR, epidermal growth factor receptor; HER2/neu, receptor tyrosine-protein kinase erbB-2; DAD1, defender against cell death 1; BCL2, B-cell lymphoma 2; VEGF, vascular endothelial growth factor; CXCR4, C-X-C motif chemokine receptor 4; CXCL12, C-X-C motif chemokine ligand 12; BMP, bone morphogenetic protein; GDF10, growth differentiation factor 10; Wnt5a, Wnt family member 5A; GAS6, growth arrest-specific 6; LIF, leukemia inhibitory factor; DKK3, Dickkopf WNT signaling pathway inhibitor 3; MIA, melanoma-derived growth regulatory protein; NGAL, neutrophil gelatinase-associated lipocalin; p38MAPK, p38 mitogen-activated protein kinase; SOX2, SRY-related HMG-box; NANOG, Nanog homeobox; NR2F1, nuclear receptor subfamily 2 group F member 1; TYRO3, TYRO3 protein tyrosine kinase; MERTK, MER proto oncogene tyrosine kinase

*Key words:* indolence, epigenetic, genetic, prostate, cancer

## 1. Introduction

The diagnosis of prostate cancer (PCa) is usually not a death sentence. Overall, the relative survival rate in five years is almost 100 percent (1). Even when the disease progresses, it usually has a relatively slow clinical course. Prostate cancer is usually diagnosed in elderly men who often have several comorbidities and reduced expected lifespan. This suggests that if PCa patients were able to survive 15 or 20 years with the disease, prostate cancer-specific mortality would greatly decrease.

Androgen deprivation therapy (ADT) is the mainstay of therapy in early disseminated prostate cancer. Despite the initial response to treatment, the disease eventually relapses into an androgen independent state. Several attempts have been made to therapeutically target the mechanisms of resistance to ADT (2-4). However, the responses to these novel treatments are generally short lived (5). New candidate treatment regimens or combinations that counter the effects of resistance mechanisms are not always successful (6,7). Similar to all biological systems, cancer cells have molecular redundancies and it is rare that a viable combination of therapeutic compounds can kill every single cancer cell while sparing normal cells. In addition, tumors within individual patients are highly heterogeneous, hence there is a marked probability that at least a few tumor clones will survive a certain treatment (8). Most research in prostate cancer is following 'down the rabbit hole' of discovering and subsequently targeting the emerging resistance mechanisms in order to eliminate every single cancer cell (3-7). Not much research has been directed towards discovering novel ways to slow the progression rate of the disease.

## 2. Indolence in localized disease

Early stage localized PCa can be successfully treated with radical prostatectomy (RP) and/or radiation therapy (9). The 5-year biochemical disease-free survival in patients with Grade Group 1 through 5 disease after RP is 96, 88, 63, 48 and 26% respectively (10). Among patients with T0-2 clinical stage disease, only 11% eventually die from prostate cancer (11). However, the effects of RP and radiotherapy are not so dramatic. These high survival rates mostly result from the indolent clinical course of the tumors. In early localized disease, excellent survival rates can be achieved even without intervention. In a 2015 meta-analysis of active surveillance studies, only 8 out of 7,627 patients eventually died from prostate cancer, with a median follow up of 3.5 years (range of 1.5-7.5 years) (12). In a prospective randomized trial, radical prostatectomy did not increase survival compared to watchful waiting in patients above 65 years old with early-stage prostate cancer (13). Longer follow up confirms the relatively indolent course of most localized prostate cancers. The 15-year metastasis-free survival in patients with Gleason 6 or less and PSA between 10-20 ng/ml is 94%. In Gleason 3+4 and PSA <20 ng/ml is 84%, while in Gleason 4+3 and PSA <20 ng/ml is 63% (13).

## 3. Indolence in high-risk disease and biochemical recurrence

Contrary to most other cancers, in prostate cancer, even the diagnosis of locally advanced or metastatic disease is not

always a death sentence. A study conducted in 1997 found that patients with locally advanced disease had a corrected 15-year survival rate of 57% (11). Even 6% of patients discovered with initial metastatic disease did not die from prostate cancer after 15 years (11). Several attempts have been made to develop a genetic signature to inform us regarding which patients will have a reduced survival. This has led to the development of several experimental molecular assays, such as Decipher, and others (14-16). After RP, patients with low, intermediate or high risk Decipher scores have 10-year cumulative metastasis rates of 5.5, 15 and 26.7% respectively (17). This means that patients with the most aggressive genetic signatures will potentially be 73.3% metastasis-free at 10 years based on this method (17). Patients with biochemical-only recurrence also have a mostly indolent disease course. Post-RP biochemical relapse has a 37% likelihood of metastatic disease in 5 years (18). Median time to clinical metastases after PSA elevation is approximately 8 years (18). Even for patients who develop biochemical relapse <1.2 years after RP, the ten-year cancer-specific mortality rate is about 10 percent (19). Similarly, biochemical relapse after prostate radiotherapy yields high survival rates (20). Post-RP salvage radiotherapy results in 10-year prostate cancer specific survival rates of 86 percent (21).

## 4. Indolence in advanced disease

Even in the recurrent or metastatic setting, a subset of patients can achieve remarkable and durable responses to ADT and novel antiandrogen therapies (22). Among men with biochemical recurrence who are placed on immediate ADT, the 5-year overall survival rate is 91.2% (22). Among patients who develop distant metastatic disease, the 5-year prostate cancer-specific mortality is 57% (23). In the phase 3 AFFIRM study, there was a group of long-term responders to enzalutamide after treatment with docetaxel, who had a median survival of 7.9 years (24). In the STAMPEDE trial, almost half of the patients with metastatic disease who received ADT plus abiraterone acetate in the hormone-naive setting, were free from disease progression after 4.5 years (25). While subsets of prostate cancers can have a remarkably indolent course and show good response to therapy, other subsets can be particularly aggressive and refractory to treatments (26,27). Hence, the fundamental question that arises is what is the underlying cause of these differences? Is it an inherent property of the tumors, dictated by their genetic and epigenetic signature? Is it a matter of the tumor microenvironment, including the immune microenvironment? In this review, we will attempt to summarize what is already known regarding the underlying molecular mechanisms related to the indolent course of subsets of prostate cancers. Our hope is to provide evidence that the mechanisms that drive the aggressive variants are reversible.

## 5. Genetic determinants of indolence and aggressiveness in prostate cancer

Several distinct genomic subsets of PCa exist. Unsupervised clustering of molecular profiling (which include gene mutations, fusions, copy number alterations, gene expression levels and DNA methylation) indicate that 74% of all tumors can be assigned in one out of seven classes based on oncogenic drivers:

fusions that involve 1) ERG, 2) ETV1, 3) ETV4, 4) FLI1, or mutations in 5) SPOP, 6) FOXA1, 7) IDH1 (28). The relative distribution of these subgroups is similar in tumors derived from both primary and metastatic sites. This molecular taxonomy cannot accurately predict whether the tumors will be aggressive or indolent. The tumor mutational burden in prostate cancer is relatively low (28). Overall, increased number of copy number alterations (CNA) is associated with worse prognosis (29,30). One of the most frequent events in the prostate cancer genome with prognostic significance is a loss in the short arm of chromosome 8 (31,32). Loci frequently lost (>40%) include 8p21.2 and 8p23.2 (31). The latter is associated with advanced disease and is most commonly found in progressors vs. non progressors (50 vs. 31%) (31). The most frequent gains (>50%) include 11p15.4, 2p25.1, 13q34, and 11q13.1. The latter is associated with biochemical recurrence independent of tumor stage and grade. Genes that overlap with this region include MEN1, MAP4K2, SF1, PPP2R5B and others (31). Fusions of androgen-regulated promoters with members of the ETS family of transcription factors are also very common. About 53% of prostate cancers have ETS-family fusions (33-36). TMPRSS2-ERG fusion analysis for CNAs reveals three important regions of copy-number loss: Two regions spanning PTEN and TP53 and the third spanning the region at 3p14, which likely contains FOXP1, RYBP and SHQ1 genes (30).

Whole exome sequencing reveals that there is only a small number of recurrent genes with alterations among various subtypes (28). In primary tumors, the most frequently altered gene is PTEN (17%), followed by TP53 (8%) (28). This suggests that no specific gene alteration by itself is solely responsible for carcinogenesis or disease aggressiveness. However, when distinct pathways as a whole are analyzed, the hypothesis changes. Taylor *et al* reported androgen receptor (AR) pathway gene aberrations in 56 percent of primaries and 100% of metastases (30). While AR gene amplifications and mutations were almost exclusively found in metastatic disease, it appeared that aberrations in NCOA2 and NCOR2 genes were important in primary tumors (30). PI3K signaling pathway was affected in 42% of primary tumors and 100% of metastases. The RB signaling pathway was affected in 34% of primary sites and 74% of metastases. The RAS/RAF signaling pathway was affected in 43% of primary sites and 90% of metastases (30). These findings support the notion that genetic factors are associated with the development and progression of prostate cancer. It is unlikely that clinically significant prostate cancer is a result of a single altered gene. The frequencies of altered critical pathways in primary tumors (many of which are indolent) suggest that clinically significant prostate cancers are unlikely to also result from a single altered molecular pathway. On the contrary, the evidence points towards combinations of genetic aberrations, which result in several altered molecular pathways. Accumulation of critical genomic aberrations over time and divergent clonal evolution are also hallmarks of the disease progression towards a more aggressive state (37) (Table I). Identifying and targeting key aberrant genes or pathways simultaneously may theoretically 'force' the disease to regress into a more indolent state. However, it is unknown whether the aggressive state is actually reversible once it occurs.

## 6. Epigenetic determinants of prognosis in prostate cancer

The low mutation rate in PCa suggests that other factors might also determine the clinical course of the disease. It is now well established that epigenetic modifications play an important role in prostate cancer (38-41) (Table I). Epigenetics is the study of heritable changes in gene expression, without the presence of changes in the DNA sequence itself (42). While cells can alter their epigenome as a response to various conditions, it is known that epigenetic abnormalities frequently accumulate in cancer (43,44). Epigenetic changes can predispose genes to mutations, while genes that modify the epigenome are frequently mutated (45-47). DNA methylation has been implicated in the lineage plasticity of PCa (48). Several hypermethylated cell cycle genes and growth suppressor genes have been linked to worse prognosis (43). Aberrant DNA hypomethylation has also been observed more frequently in late stages of PCa (49). Epigenetic reprogramming is associated with loss of luminal epithelial identity and the transition from a typical prostate adenocarcinoma towards an aggressive neuroendocrine PCa (NEPC) (37,50,51). Neuroendocrine PCa cell lines possess a unique chromatin accessibility profile, distinct from prostate adenocarcinoma (52). Inactivation of TP53 and/or RB1 leads to upregulation of DNA methyltransferase family member 1 (DNMT1) (53,54). DNA methylation is linked to the activity of EZH2, which serves as a recruitment platform for DNMTs (55). EZH2 is a central regulator of neuroendocrine differentiation and the transition from an androgen receptor (AR)-dependent disease towards an aggressive state that is independent of AR signaling and indifferent to the effects of antiandrogens (51,56-58). The activity of AR can also be directly regulated by epigenetic modifiers, such as histone deacetylases (HDAC) (59). Post-transcriptional mechanisms, such as mRNA splicing or regulation by miRNA also play a role in the progression of prostate cancer (60-62). EZH2 can act both as a transcriptional activator or repressor, depending on post-translational modifications of EZH2 (63-65).

## 7. The role of tumor microenvironment

Prostate tumors with 'bad' morphologic or genetic features can still run an indolent clinical course. This suggests that the cellular and secreted factors in the tumor immune microenvironment (TIME) might play a role in the balance between tumor clearance and tumor progression, as well as the response to treatment. However, PCa in general has an immunologically 'cold' TIME (65). Overall mutation rates, as well as DDR gene defects in PCa are low, especially in the early disease setting (66). Hence, neoantigen expression is diminished compared to many other cancers. This results in a non-inflamed TIME, where tumor cells proliferate freely and evade immune-mediated elimination. Although the presence of cytotoxic and helper T-lymphocytes within tumor margins have been associated with favorable prognosis across several cancer types, PCa exhibits unique features (66-69). Studies suggest that high density of stromal CD8+ tumor infiltrating lymphocytes (TILs), and high PD-L1 expression are not associated with better outcomes in PCa (66,70-72). Some studies indicate that they might even be detrimental (73). Prostate cancer-infiltrating TILs are frequently dysfunctional (71,74)

Table I. Factors that maintain tumor indolence and mechanisms mediating a switch into aggressive disease.

Indolence factor	First author, year	Escape mechanism/aggressiveness induction	(Refs.)
Low mutation rate	Taylor <i>et al</i> , 2010	Additional genetic aberrations	(30)
	Bonollo <i>et al</i> , 2020	CAF effects	(88)
	Aggarwal <i>et al</i> , 2018	Epigenetic modifications	(50)
Slow proliferation	Taylor <i>et al</i> , 2010	Additional genetic aberrations	(30)
	Bonollo <i>et al</i> , 2020	CAF effects	(88)
	Sugiura <i>et al</i> , 2021	Cell cycle gene hypermethylation	(43)
	Sejda <i>et al</i> , 2020	Neurotrophic signaling	(98)
Androgen dependence	Taylor <i>et al</i> , 2010;	Additional genetic aberrations	(30,37)
	Beltran <i>et al</i> , 2016		
	Blom <i>et al</i> , 2019	TME factors	(89)
	Ngollo <i>et al</i> , 2014;	Epigenetic adaptation	(40,59)
Nutrient scarcity/hypoxia	Fu <i>et al</i> , 2006		
	West <i>et al</i> , 2001	VEGF upregulation	(129)
	Ngollo <i>et al</i> , 2014;	Epigenetic adaptation	(40,48)
Immunosurveillance	Ge <i>et al</i> , 2020		
	Taylor <i>et al</i> , 2010;	Additional genetic aberrations	(30,37)
	Beltran <i>et al</i> , 2016		
	Bonollo <i>et al</i> , 2020	Crosstalk with CAFs	(88)
	Ness <i>et al</i> , 2014	Dysfunctional TILs	(71)
Fibroblast/stromal-induced inhibition of tumor growth	Nardone <i>et al</i> , 2016	High regulatory Foxp3+	(75)
	Mariathasan <i>et al</i> , 2018	High M2 macrophages	(77)
	Heninger <i>et al</i> , 2016	MHC Class 1 silencing	(78)
	Blom <i>et al</i> , 2019	CAFs activity/epigenetic changes in CAFs	(89)
Senescence phenotype	Bonollo <i>et al</i> , 2020	Increased stromal stiffness	(88)
	Sejda <i>et al</i> , 2020	Perineural invasion	(98)
	March <i>et al</i> , 2021	Neurotrophic growth factors	(99)
	Ewald <i>et al</i> , 2010	Treatment resistance/therapy escape	(110)
Low visceral tropism	Wang <i>et al</i> , 2020	Secretome sends tumorigenic signals to neighboring cells	(108)
	Beltran <i>et al</i> , 2016	Additional mutations/CNA in critical genes	(37)
	Davies <i>et al</i> , 2020	Neuroendocrine differentiation	(51)
Dormancy induction	Yegnasubramanian <i>et al</i> , 2008	Epigenetic adaptation	(49)
	Recasens <i>et al</i> , 2019	Additional genetic aberrations	(148)
	Decker <i>et al</i> , 2017	Beta-adrenergic signaling	(149)
	Cackowski <i>et al</i> , 2017	TYRO3, MERTK activity	(145)

There are several factors that contribute to an indolent phenotype in subsets of prostate cancers. They include inherent properties of a tumor (such as slow proliferation rate, low visceral tropism), effects of treatment, immunosurveillance, TME-related effects, induction of dormancy/quiescence/senescence phenotype. However, as genetic and epigenetic alterations continue to accumulate, combined with the TME-endothelial compartment crosstalk, many tumors eventually escape dormancy and switch to aggressive disease.

(Table I). However, high proportions of Foxp3+ regulatory TILs are associated with worse progression-free and overall survival in prostate cancer (75). In addition, high levels of M2 macrophages are pro-tumorigenic, suggesting that TGF $\beta$  might play a role in immune exclusion in prostate cancer (76,77). Epigenetic silencing of MHC Class I expression is common in advanced prostate tumors (78,79). Several signaling pathways, including the INF axis can also affect the expression of MHC Class I and the subsequent activation and expansion of CD8+ TILs within the invasive margins of

a tumor (80). PTEN loss and other DDR defects also impact the TIME by modulating the activation of cellular INF pathways (66,81-83). It is known that the PTEN axis can confer sensitivity to T-cell-based immunotherapies (84). The development of bone metastases promotes the interaction between the tumor cells and the bone microenvironment. This further decreases the immunogenicity of the lesions (85). However, a few prostate cancers are immunologically ‘hot’ tumors and show durable responses when treated with immunotherapeutic agents (85-87). This suggests that the TIME has the potential

to affect the clinical course of a patient who develops PCa, given the right circumstances.

Cancer associated fibroblasts (CAFs) constitute the most abundant cell population in the TME. They have been shown to play a major role in prostate cancer progression (88). During tumorigenesis, stromal fibroblasts crosstalk and likely coevolve with the epithelial compartment and become CAFs. Experiments revealed that CAFs from aggressive disease are sufficient to drive the progression of prostate cancer cells with low tumorigenic potential (88). They can also contribute to castration-resistance (89). On the other hand, normal fibroblasts can slow the proliferation rate of aggressive prostate cancer models (90). The amount of tumor-associated stroma diminishes as prostate cancer becomes more aggressive (89). Although it is hard to prove causality, TME features that characterize aggressive disease include a high proportion of CAFs, low proportion of smooth muscles, high vimentin expression, asporin expression, increased manifestation of matrix metalloproteinases, increased expression of COL5A2, and decreased expression of COL4A6 (89,91-93). The increased deposition of various collagen types, such as I or III, contributes to matrix stiffness, which leads to increased tumor invasiveness and metastatic potential (88). It was also suggested that CAF-derived neuregulin 1 (NRG1) induces antiandrogen resistance, via a NRG1-HER3 axis (94). CAFs don't have the genetic alterations of the epithelial compartment. On the contrary, CAFs from aggressive prostate cancers have discrete methylation differences compared to CAFs from moderate risk disease (95). For example, epigenetic regulation of Ras activity in prostatic CAFs, was found to regulate the metabolic and neuroendocrine activity in prostate cancer that fails ADT (96). Stromal AR expression also diminishes early in prostate tumorigenesis and continues to gradually decrease as the disease evolves into a more aggressive phenotype. It has been suggested that stromal AR inhibits the growth of malignant epithelial cells (97). Neural tissue is also an active TME element in prostate cancer. Perineural invasion is a known adverse prognostic factor (98). Several neural transmission receptors are present in cancer cells (98). Moreover, it has been recently shown that the abundance of neurotrophic growth factors in the patient's urine may perform better than PSA to separate aggressive prostate tumors from indolent ones (99).

## 8. A senescence phenotype is associated with indolence

There are currently no reliable molecular signatures to identify prostate tumors destined to run an indolent clinical course. Emerging evidence suggests that the upregulation of pathways related to cellular aging and senescence can distinguish between indolent and aggressive disease (100) (Table I). The 'indolence signature' includes inactivation of Nkx3.1, and increased expression of CDKN1A (p21), FGFR1 and PMP22 genes (100) (Fig. 1). CDKN1A is a cell regulatory gene associated with senescence (101). FGFR1 is known to play a critical role in prostate development and prostate tumorigenesis (102). This suggests a potentially complex activity of FGFR1 in prostate cancer. FGF signaling plays an important role in stem cell renewal, cellular aging and senescence (103). Although PMP22 is a gene highly expressed in neurons, it has also been associated with cellular proliferation regulation

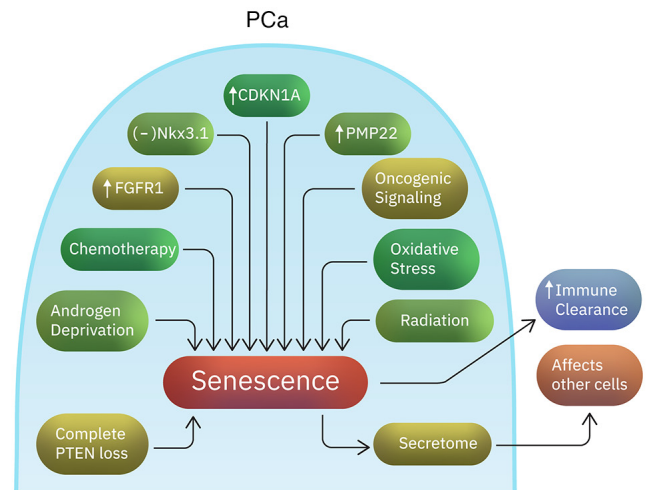


Figure 1. Factors associated with a 'senescence phenotype' in prostate cancer. Studies suggest that the upregulation of aging- and senescence-related pathways is able to distinguish between indolent and aggressive prostate tumors. The senescent signature includes Nkx3.1 inactivation and increased expression of CDKN1A, FGFR1 and PMP22 genes. Moreover, factors such as oxidative stress, oncogenic signaling, radiation, androgen deprivation or chemotherapy might induce senescence. While senescent cells can trigger immune clearance, their secretome can provide pro-tumorigenic signals to neighboring non-senescent cells.

in other tissues and growth arrest in fibroblasts (104,105). Increased senescence has also been associated with reduced PSA recurrence rates (106). Senescent cells not only undergo cell cycle arrest, but they also trigger an immune response that can help the clearance of neoplastic cells (107). However, they are metabolically active and their secretome can impact the surrounding non-senescent cancer cells in ways that promote cancer progression and metastasis (108,109). Senescence can also be caused by long term oncogenic signaling or DNA damage and increased oxidative stress as a result of anticancer agents or radiation (109,110). In addition, complete PTEN loss triggers a p53-dependent cellular senescence response (The overwhelming majority of patients have PTEN loss heterozygosity, which results in tumor initiation and progression) (111-113). Moreover, androgen deprivation frequently induces senescence in prostate tumor cells (114,115). In conclusion, the induction of senescent molecular signatures might contribute to the indolent clinical course in some patients with PCa, before and after treatment, especially antiandrogen therapy.

## 9. What makes high risk disease?

Data from histology and gene expression analysis can provide useful prognostic information. Luminal B tumors carry the worst outcome (69% overall survival at 10-years), followed by basal and luminal A tumors (10-year overall survival at 80% and 82% respectively) (116). It is well known that the amount of Gleason 4 disease in the primary tumor is strongly associated with clinical outcomes and disease aggressiveness (117,118). High grade localized tumors are marked by epigenetic loss of heterogeneity and common trans-regulatory signatures. They exhibit enrichment for FOXA1, CDX2 and HOXB13 transcription factor binding sites (119). A few studies

compared the differential gene expression between Gleason grade 3 and Gleason grade 4 lesions (120,121). The genes exclusively expressed in Gleason 4 tumors are those that are upregulated in neuronal, embryonic and hematopoietic stem cells. Overexpression of EGFR and HER2/neu are almost exclusively confined to Gleason 4 and above cancers (120,121). These genes are associated with independent tumor cell proliferation and enhanced cell motility (117). Gleason 4 and 5 lesions have lesser frequencies of cyclin D2 methylation, which results in cyclin D2 activation and CDKN1B sequestration. This subsequently results in cell cycle entry. CDKN1B immunostaining progressively diminishes with increasing Gleason score (122-124). Gleason score is also strongly associated with expression levels of the anti-apoptotic genes DAD1 and BCL2 (125,126). Moreover, indolent cancers are more capable of subverting a brake in replication. High Gleason grade lesions also show decreased androgen signaling, suggesting dedifferentiation. The downregulation of androgen responsive genes in high grade tumors results in increased cell proliferation (127,128). In addition, poor prognosis tumors are related to increased VEGF production and microvessel density, as well as irregularity in vessel diameter (129-131). Microvessel pericyte density score is also associated with disease aggressiveness (132). Higher grade lesions overexpress elements that are permissive for tumor migration. For example, the chemokine receptor CXCR4, which is overexpressed in Gleason 4 lesions, poses a key role in the development of lymph nodes and bone metastases (133-135). Interestingly, its ligand CXCL12 is secreted in high concentrations by lymph nodes and bone marrow stroma (117).

## 10. The role of tumor dormancy

In PCa, metastatic disease may occur years after RP. This suggests that in some cases cancer cells undergo a long-lasting quiescent state (136). Quiescent cells are reversibly suspended in the G0 phase, but they retain the ability to re-enter the cell cycle and initiate symptomatic metastatic disease (137) (Table I; Fig. 2). Tumor dormancy might be due to intrinsic factors or due to conditions provided to the tumor cells by the surrounding microenvironment (138,139). The bone is a major site of PCa recurrence, suggesting that the bone microenvironment promotes a state of dormancy (136). *In vitro* studies have shown that two members of the TGF $\beta$ /BMP family, GDF10 and TGF $\beta$ 2 (which are secreted from differentiated osteoblasts), induce quiescence in PCa cells (136,138). Several other osteoblast secreted factors such as Wnt5a (maintains hematopoietic stem cells in dormant state), GAS6, LIF, thrombospondin, DKK3, BMP1, vadorin, neogenin, MIA and NGAL, have also been suggested to promote dormancy in PCa (136,140-142). For example, vadorin, neogenin and DKK3 induce dormancy via activation of the p38MAPK signaling pathway (136). Besides osteoblasts, other bone marrow stromal cells secrete dormancy-inducing factors (136). The transcription factors SOX2, NANOG and the orphan receptor NR2F1 are important for the maintenance of a quiescent phenotype through epigenetic regulation (143-147). Studies suggest that dormant cells at the metastatic sites continue to acquire genomic changes (148). The receptor tyrosine kinases TYRO3 and MERTK (TAM family) were shown to promote dormancy

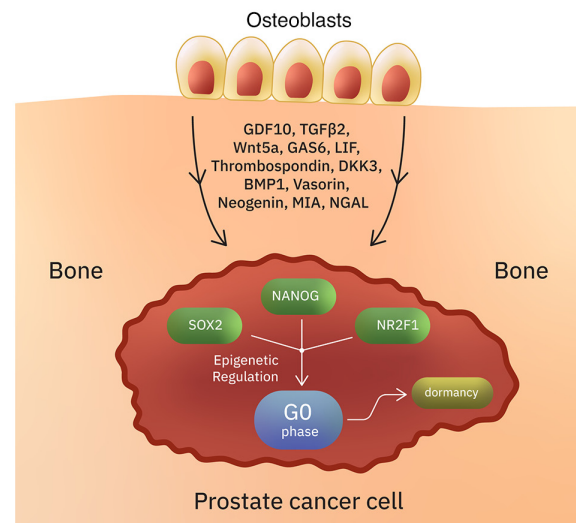


Figure 2. Induction of dormancy/quiescence in prostate cancer cells. Intrinsic factors and conditions provided by the TME can result in the reversible suspension of prostate cancer cells in G0 phase. In some cases, this dormant state can last for several years. Several osteoblast-secreted factors (such as NGAL, MIA, GDF10, TGF $\beta$ 2, and others) have been suggested to induce quiescence in prostate cancer. The transcription factors SOX2, NANOG and NR2F1 are important for the maintenance of a quiescent phenotype through epigenetic regulation.

escape (145). Norepinephrine was also hypothesized to stimulate dormancy escape in PCa through the beta-2 adrenergic signaling (149).

## 11. Conclusions

The majority of prostate cancers follow an indolent clinical course. It is unclear whether there is one or several types of indolence in PCa. Several factors have been linked to disease aggressiveness. However, it is still largely unknown which of these factors actually have a causative role. High quality tumor analyses suggest that complex genetic aberrations in multiple critical pathways are associated with a worse phenotype. The escape from indolence into an aggressive disease is associated with a complex interplay in which genetic and epigenetic factors are likely involved. The effect of the immediate immune tumor microenvironment is also very important. Future studies will show whether halting the step-wise tumor evolution is a feasible option. Further research will also determine whether we can meaningfully prolong the life of PCa patients by inducing senescence or long-term tumor dormancy.

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MS conceptualized this review, reviewed the literature and drafted and critically reviewed the final manuscript. LJF reviewed the literature, and drafted and critically reviewed the final version of the manuscript. SR reviewed the literature, and drafted and critically reviewed the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

Not applicable.

### Patient consent for publication

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### Competing interests

All authors declare that they have no competing interests.

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