Genetically Engineered Mouse Models of Prostate Cancer

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Abstract

Objectives: Mouse models of prostate cancer are used to test the contribution of individual genes to the transformation process, evaluate the collaboration between multiple genetic lesions observed in a single tumour, and perform preclinical intervention studies in prostate cancer research.

Methods: Mouse models for human prostate cancer are generated through genetic engineering of the mouse germline, introducing lesions that reflect those observed in human prostate cancer specimens. The optimal mouse model accurately reflects the pathogenesis of the disease including the sporadic nature of the initiating insult, the identity of the genetic lesions accumulated throughout the transformation process, the hormone dependency of the malignant cells, the incidence and tissue specificity of metastatic lesions, and the responses to therapeutic intervention.

Results: Although this ultimate goal has not yet been reached, the currently existing mouse models for prostate cancer have yielded important insights. These mostly relate to the contribution of individual genes and the mechanism of oncogene collaboration in the early stages of the disease. Modelling metastatic and hormone-refractory prostate cancer, however, remains a major challenge.

Conclusions: Mouse models have made an invaluable contribution in identifying the genetic lesions involved in high-grade prostatic intraepithelial neoplasia lesions and locally invasive prostate cancer. Most mouse models are less accurate in modelling the progression to metastatic disease. Moreover, most mouse models for prostate cancer do not facilitate analysis of hormone-refractory prostate cancer, although this would constitute the most valuable contribution to preclinical testing of novel therapeutic intervention strategies for the human disease.

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1. Introduction

1.1. The pathophysiology of prostate cancer

Prostate cancer is the most common malignancy in men and represents the second-leading cause of cancer-related male deaths in the Western world [1]. The development of prostate cancer advances through a series of distinct stages ranging from atypical hyperplasia, a commonly found early lesion, to metastatic disease present in bone, lung, and liver in advanced stages of the disease. Benign prostatic hyperplasia (BPH) is characterised by a major proliferation of stromal cells. In contrast, prostatic intraepithelial neoplasia (PIN) is characterised by foci of dysplastic ductal and acinar cells and high-grade PIN (HGPIN) is thought to comprise the first stage of invasive prostate adenocarcinoma, which usually occurs in the transition zone of the prostate. The formation of PIN lesions is a common event and is detected in up to one third of all men over age 45 yr [2]. Still, most of these lesions do not progress to clinically detectable tumours. HGPIN lesions are thought to progress to invasive adenocarcinoma, locally confined and locally advanced tumours, and eventually to metastatic prostate cancer [3]. Prostate cancer biopsies usually contain multiple independent sites of cancerous and HGPIN lesions. Interestingly, independent lesions found in a single specimen are often genetically heterogeneous, supporting the concept that prostate cancer originates as a multifocal disease [4]. On radical prostatectomy a biochemical relapse, detected by a rise in prostate-specific antigen (PSA) levels, is observed in about 30% of the cases [5]. Although metastatic prostate cancer can initially be treated by androgen-ablation therapy, the disease invariably relapses as a highly aggressive androgen-independent prostate cancer, which cannot be cured with current treatment modalities [6].

The heterogeneity of prostate cancer has precluded unambiguous identification of the critical genetic lesions contributing to the disease. Several genomic lesions are frequently present in prostate cancer samples, including chromosomal losses at 8p, 10q, 13q, and 17p, as well as gain of 8q and chromosomes 7 and X [3,7]. Loss of 8p21 is considered an early event because it is found with a similar, high frequency in PIN lesions and advanced cancer. The homeobox gene NKX3.1 maps to the critical 8p21 genomic region [8]. Loss of 10q23 is found in 30% of primary prostate cancers and up to 65% of metastatic tumour samples, implicating it as a later event than loss of 8p21 [3,9,10]. The tumour suppressor PTEN, one of the most frequently deleted genes in a variety of human malignancies, maps to the minimally deleted 10q23 chromosomal region [11,12]. The amplified genomic regions contain genes such as MYC (8q) and AR (X chromosome), as well as numerous other genes. Although these kinds of associative studies lend some support to the notion that gain or loss of the individual candidate genes might induce or contribute to the process of malignant transformation of prostate epithelial cells, formal proof of such a hypothesis requires further experimental validation. Since the 1980s, mouse genetic engineering, allowing a wide variety of genetic modifications, has taken centre stage in these kinds of mechanistic studies. In fact, the pioneering work on mouse knockout technology has been awarded the 2007 Nobel Prize in Physiology or Medicine [13]. To date, genetic modelling of the mouse has come to provide an invaluable resource for the detailed characterisation of the molecular events driving the cellular transformation process in prostate and many other cancers.

1.2. Mouse models for prostate cancer

The study of prostate cancer in humans is limited by the ability to perform mechanistic studies with regard to the contribution of individual genetic lesions to the transformation process of prostate epithelial cells. Moreover, the evaluation of novel therapeutic strategies in patients requires large cohort studies, which are difficult to control for disease stage, genetic heterogeneity, diet, age, and other risk factors for prostate cancer [14]. In the mouse model, experimental control over both environmental factors and the genetic lesions applied to the prostate epithelial cells allows for a far more detailed analysis of the mechanisms underlying prostate cancer development as well as the preclinical evaluation of novel therapeutic strategies, thereby facilitating the design of targeted approaches for further clinical studies.

Initially, the mouse was used as a xenograft model for in vivo analyses of human prostate cell lines. However, this approach largely neglects the complexity of cancer as a disease with intricate interactions between the transformed cells and the surrounding resident cells in normal tissue, the stromal cells, the endothelial cells, and the immune cells that all play an critical role in the pathogenesis of the disease [15]. Especially with regard to the therapeutic evaluation of the antineoplastic activity of (novel) compounds, xenograft studies frequently fail as a preclinical approach [16]. Therefore, these xenograft models are no longer referred to or considered to be true mouse cancer models, but
Instead are considered to represent an intermediate step between cell culture and an animal model and relevant only for the evaluation of certain cell autonomous features of clonal cell lines [15]. In contrast, mouse models for prostate cancer are characterised by the occurrence of endogenous prostate tumours that evolve from normal cells. Here, we will focus on transgenic mouse models of prostate cancer, in which relevant genetic lesions have been introduced into the mouse germline to drive the transformation process of the prostate epithelial cells.

The first transgenic mouse prostate cancer models displaying autochthonous tumours depended on prostate-specific overexpression of the SV40 small T and large T antigens (such as the TRAMP model) or SV40 large T antigen only (such as the LADY model) [17]. These models have the drawback of using a very strong transforming agent (SV40 large T inactivates both the p53 and the Rb tumour suppressor proteins), inducing an oncogenic process that does not accurately recapitulate the pathogenesis of the human disease. Moreover, these models have several other shortcomings, such as the strong neuroendocrine component seen in advanced stages of the disease in the TRAMP model, which preclude their use as preclinical models for the evaluation of therapeutic treatments. We will therefore not discuss these SV40 large T-driven models in this update, but focus instead on mouse cancer models using defined genetic lesions as observed in human prostate cancer specimens. We will discuss the current approach towards modelling prostate cancer in the mouse, present some of the data derived from these models with relevance to the pathogenesis or treatment of prostate cancer, and discuss the limitations of these mouse models.

2. Methods and results

In general, cancer is thought to develop through a series of genetic and epigenetic changes driving the transformation of normal cells into malignant cancer cells [18]. In sporadic cancer, the initiating lesion affects a single cell in an otherwise normal tissue environment [19]. The mouse model for human prostate cancer needs to reflect the pathogenesis of the human disease accurately, including the sporadic nature of the initiating insult, the identity of the genetic lesions accumulated throughout the transformation process, the hormone dependency of the malignant cells, the incidence and tissue specificity of metastatic lesions, and the responses to therapeutic intervention.

To date, mouse models for prostate cancer have been used to study (1) the contribution of single genes to the transformation process, (2) the collaboration between multiple genetic lesions, and (3) the preclinical evaluation of therapeutic intervention strategies.

2.1. The contribution of single genes to prostate cancer

Genetic manipulation of the mouse germline has facilitated the generation of mutant mouse strains carrying engineered genomic lesions (Fig. 1). These lesions can result in the loss of a single gene from the germline (knockout mouse strains; Fig. 1B) or the insertion of multiple additional copies of a gene into the germline (transgenic mouse strains; Fig. 1C). In transgenic mice, the expression of the transgene is usually driven by a cell- or tissue-specific promoter sequence, resulting in expression of the transgene during a certain developmental stage or in a specific cell type. In addition, single genes can be replaced by specific mutant alleles to generate allelic series of a certain gene, or by unrelated sequences to generate single-copy transgenic strains (knock-in mouse strains). Finally, to allow experimental control over the loss of gene expression or induction of transgene expression, conditionally mutant mice have been generated using the Cre recombinase [19]. In conditional knockout mice, the gene of interest is placed in between loxP sequences, but remains present and active in the germline (Fig. 1D). The conditional allele can then be removed by Cre-mediated recombination, mediating the fusion of the two loxP sequences with excision of the intervening sequences. By applying a Cre transgene, which acts in a tissue-specific fashion (such as specifically in the prostate epithelium), it is possible to induce loss of the tumour suppressor in a specific tissue or during a specific developmental stage [19]. Conditional transgenic mice carry a transgene, which will only be expressed on Cre-mediated removal of a conditional STOP sequence, which abrogates the translation of the transgene from its mRNA [19]. All of these approaches have been used to study the contribution of single genes to prostate cancer in the mouse. In this approach, over-expression of relevant oncogenes can be modelled in transgenic mouse strains, whilst loss of a tumour suppressor gene can be modelled by generating a (conditional) knockout strain (Fig. 1).

2.2. Oncogenes

As stated above, several genes (including MYC, NKX3.1, and AR) have been found to be frequently amplified in human prostate cancer and have been proposed to contribute to the transformation process. The causal relationship between over-expression of a given oncogene and the induction or progression of a cancerous lesion can only be formally addressed using genetically engineered mouse models. To study the specific contribution of an individual oncogene to prostate cancer, the gene of interest is over-expressed in prostate epithelial cells using a transgenic cassette that drives tissue-specific expression in the mouse (Fig. 1A). To direct transgene expression to the mouse prostate, regulatory sequences derived from the rat minimal probasin promoter containing two androgen-responsive elements are commonly used [17]. This transgenic cassette has been modified by the inclusion of two additional androgen-responsive elements to
Fig. 1 – Genetic engineering of the mouse germline. Most genes present in the mouse genome will contain several exons, indicated as open rectangles, spaced by the noncoding intervening sequences known as introns. Gene expression is controlled by regulatory sequences positioned upstream of the transcription initiation site (both enhancer and promoter sequences), in the intronic sequences or downstream of the last exon (depicted schematically as the black arrow). (B) Single gene knockouts are generated by replacing most of the coding sequences, or a critical part of the coding sequence, by unrelated sequences. Expression from the endogenous locus will result in a nonfunctional protein. The knockout allele is often referred to as a null allele. (C) Multicopy transgenics are generated by genomic insertion of multiple copies of a transgene, usually the cDNA sequence of the gene of interest (indicated as the fused open rectangles) placed under transcriptional regulation of a generic or cell-type specific promoter sequence (indicated by the grey arrow). Transgene integration occurs at a random position in the genome, and copy numbers vary between individual founder mice, allowing for the selection of high- and low-copy transgenic strains. (D) Conditional knockout strains are generated by the introduction of loxP sequences (indicated as solid triangles) at opposite ends of the gene of interest. Expression (usually transgenic) of the Cre recombinase will result in fusion of the two loxP sites, with excision of the intervening sequences, in this case most of the coding sequence of the gene. Hence, a conditional knockout allele allows the normal developmental expression pattern of the gene up to the moment when Cre recombinase is introduced into the (somatic) cells. Depending on the expression pattern of the Cre transgene, this approach allows the generation of tissue-specific knockouts. (E) Conditional transgenic strains are generated by the introduction of a conditional STOP cassette upstream of the coding sequences of the transgene of interest. The STOP cassette abrogates translation from the mRNA and precludes expression of the transgene. Introduction of Cre recombinase results in removal of the STOP cassette and thereby induces expression of the transgene. The expression pattern of the Cre recombinase determines the tissue-specific activation of the transgene.

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1 The drawback of this conventional approach is that most, if not all, of the prostate epithelial cells will express high levels of the transgene, which does not accurately reflect the pathogenesis of human cancer, where the lesion will occur in a single cell within a normal tissue environment. Hence, these models often do not accurately reflect the pathophysiology of the human disease. Despite this shortcoming, this approach has been successfully used to experimentally confirm the critical role of several oncogenes in driving the transformation of prostate epithelial cells [17].

2.3. Tumour suppressor genes

In addition to gene amplification, loss of certain genomic regions is also frequently observed in human prostate cancer specimens, which can contribute to loss of expression of critical tumour suppressor genes. In addition to genomic deletion, epigenetic silencing also contributes to loss of tumour suppressor activity during transformation. In mouse models, loss of a tumour suppressor gene can be mimicked by deletion of the gene of interest from the germline (Fig. 1B). In case of p27Kip1, for instance, it was shown that p27-deficient mice displayed mild hyperplasia of the prostate epithelium, but no mPIN lesions or more advanced stages of the disease [24]. Also, in NKX3.1-deficient mice, hyperplasia and atypical dysplasia were found in the prostate gland, but again, more advanced stages of the disease were not observed [25]. Finally, p53-deficient mice, which are predisposed to developing various types of tumours, do not develop any lesions in the prostate [26], indicating that loss of p53 does not predispose to prostate cancer.

Unfortunately, homozygous deletion of critical tumour suppressor genes from the germline often results in early embryonic lethality, precluding the analysis of the role of the tumour suppressor gene to the transformation process. To circumvent these limitations, gene loss can be achieved by prostate-specific deletion of a conditional allele (Fig. 1D). Using
this approach to generate mutant mice with a prostate-specific deletion of the PTEN tumour suppressor, the critical role of PTEN loss in inducing prostate cancer was shown [27].

2.4. The collaboration between multiple genetic lesions

The availability of genetically engineered mouse models allows for the direct evaluation of the functional interactions between individual genetic lesions in driving the transformation process. This is especially relevant for prostate cancer, because biopsies usually present with multiple independent sites of cancerous and HGPIN lesions [4], precluding the straightforward identification of the critical genetic lesions driving the transformation process. Independent lesions found in a single specimen are often genetically heterogeneous, supporting the concept that prostate cancer originates as a multifocal disease. Hence, multiple genetic lesions found in a single specimen do not necessarily collaborate in the transformation process, but might merely represent independent cancerous lesions present within the specimen. In compound mutant mouse models, the interaction between discrete genetic lesions can be evaluated experimentally. The power of the mouse model to disentangle the complex interactions between individual genetic lesions can be nicely illustrated by the studies on the roles of the tumour suppressors PTEN and p53 in prostate cancer.

The tumour suppressors p53 and PTEN are mutated or not expressed in a wide range of human malignancies. Functionally, the two proteins act in distinct cellular pathways. The p53 pathway integrates a variety of cellular stress signals and responds by inducing cell cycle arrest, cellular senescence, or apoptosis [28]. Using mouse models, it has been shown that loss of p53 as a single genetic lesion does not induce any prostate pathology [26], which could be interpreted to disqualify p53 as a relevant tumour suppressor in prostate cancer. However, the role of the p53 pathway in prostate cancer became apparent only after concomitant loss of PTEN.

PTEN counteracts phosphatidylinositol 3'-kinase (PI3-K) signalling, by reverting the second messenger phosphoinositol-3,4,5-trisphosphate (PIP3; produced by PI3-K activity), precluding the second messenger phosphoinositol pathways relevant to cellular survival and proliferation [29]. Independent lesions found in a single specimen are often genetically heterogeneous, supporting the concept that prostate cancer originates as a multifocal disease. Hence, multiple genetic lesions found in a single specimen do not necessarily collaborate in the transformation process, but might merely represent independent cancerous lesions present within the specimen. In compound mutant mouse models, the interaction between discrete genetic lesions can be evaluated experimentally. The power of the mouse model to disentangle the complex interactions between individual genetic lesions can be nicely illustrated by the studies on the roles of the tumour suppressors PTEN and p53 in prostate cancer.

To analyse in detail the effect of Pten gene dosage on the transformation of the prostate epithelium in mice, a so-called Pten hypomorphic allele was generated. The hypomorphic allele produces lower levels of the wild-type PTEN protein than the germline allele. This allows the analysis of the effects of a wide range of PTEN dosages on cellular transformation in the prostate. Mice heterozygous for one null allele and one hypomorphic Pten allele (resulting in lower PTEN levels than observed in mice heterozygous for one null and one wild-type Pten allele), developed massive mPIN and invasive prostate cancer with intermediate latency and at full penetrance [37]. Still, residual PTEN protein expression was retained in the lesions of these mice [37], indicating that remnant PTEN activity might, in fact, be beneficial for tumour growth. In support of this, recent studies in mouse models show that full loss of Pten leads to oncogene-induced senescence in a p19Arf-dependent fashion [38], counteracting the outgrowth of malignant tumours in PTEN-deficient prostate. Apparently, loss of heterozygosity (LOH) of the wild-type allele in Pten heterozygous mice likely requires inactivating lesions in the p19Arf/p53/p21 pathway. Indeed, concomitant loss of Pten and p53 causes aggressive tumour growth in the mouse prostate and a lack of induction of oncogene-induced senescence [38]. Because p53-deficient mice do not develop any prostate pathology [26], these data indicate that PTEN loss precedes p53 loss in prostate cancer, although p53 loss is required for LOH of PTEN, which facilitates hormone-refractory disease.

In addition to the analysis of the functional interactions between two genetic lesions present within the transformed cells, the mouse model of prostate cancer has also been used to characterise the contribution of genetic lesions in the stromal compartment of the tumour to the progression of the disease. During the transformation process the interactions between the cancerous cells and the surrounding tissue constituents (non-transformed tissue cells, stromal cells and immune cells) govern critical processes such as angiogenesis and metastasis. The progression of cancerous lesions to a fully malignant phenotype is therefore also dependent on the interactions with the tumour microenvironment. Interestingly, in mouse models of prostate cancer it has recently been observed that the stromal compartment of the tumour can acquire specific genetic lesions independently from the cancer cells, underscoring the critical role of the stromal cells to tumour progression and the strong selective pressure on the stromal cells during this process [39].

2.5. The preclinical evaluation of therapeutic intervention strategies

Whereas mouse models have been useful in dissecting the specific contribution of individual genes to the transformation process, and in charting the functional interactions between multiple genetic lesions commonly found in a single tumour, the availability of genetically engineered mouse strains closely mimicking the genetic make-up of human prostate tumours holds the promise of not only modelling the pathogenesis of human prostate cancer but also of evaluating the efficiency of various therapeutic treatment regimens in tumours with a specific genetic fingerprint. As such, validated mouse models of human prostate cancer could facilitate
preclinical testing to optimise treatment regimens for very specific tumour types.

The value of mouse models of prostate cancer for preclinical testing can be further enhanced by the availability of noninvasive imaging techniques, allowing the longitudinal monitoring of the responses of individual tumours to a certain therapeutic treatment regimen. Several approaches to small animal imaging are available, such as magnetic resonance imaging, computed tomography, positron emission tomography, and bioluminescence imaging [40]. Imaging of the mouse prostatic lobes is technically challenging, given their small size, the complex anatomy, and the proximity of the bladder, where specific imaging probes can accumulate. Hence, most progress in imaging prostate cancer in the mouse has been made using bioluminescence imaging techniques. In this approach, a bioluminescent reporter transgene is expressed in a prostate-specific manner [41] or as a conditional reporter, activated only after Cre-mediated recombination and thereby specific for cells carrying an engineered genetic mutation requiring Cre activity [42]. The latter approach has facilitated the detection of recurrent tumour growth after castration of mice with prostate-specific deletion of PTEN [43].

Unfortunately, most currently available mouse models have not been used extensively to study advanced prostate cancer (see below), precluding the application of mouse models for the design of novel treatment regimens for metastatic or hormone-refractory disease, which by and large pose the major clinical problems associated with prostate cancer. Notwithstanding these limitations, mouse models carrying a genetic lesion that is sufficient to induce cellular transformation, the formation of mPIN lesions and locally invasive carcinoma are useful to explore therapeutic intervention aimed at disarming prostate cancer cells carrying that specific genetic lesion. In fact, cancer cells are thought to remain, at least in part, dependent on the activities induced by their primary lesion, a phenomenon called oncogene addiction [44]. Therefore, identifying a potentially effective therapeutic approach acting on cancer cells carrying a very specific genetic lesion might prove to be a fruitful approach for targeting well-characterised subsets of prostate cancer. This approach has proven its validity in treating chronic myeloid leukaemia with a specific inhibitor (imatinib mesylate) of the tyrosine kinase ABL, where complete regression of advanced tumours is observed, and recurrent disease invariably carries mutations in the BCR-ABL oncogene rendering the kinase insensitive to the drug [45].

With respect to prostate cancer, however, this approach might be less straightforward given the multifocal nature of the disease and the presence of genetically heterogeneous independent lesions found in a single specimen [4]. The clinical efficacy of a therapeutic approach targeting an individual pathway can therefore be expected to be limited. Nevertheless, specific approaches aimed at disarming the frequently activated PI3K/Akt pathway (see above) have been evaluated in mouse models for Akt-driven prostate cancer, and given the central role for the PTEN tumour suppressor as an early lesion in a large fraction of prostate cancers, these data are relevant to the treatment of human disease. As discussed above, the PTEN tumour suppressor negatively regulates the PI3-K pathway, thereby limiting the activation of Akt and other downstream pathways. This genetic lesion has been modelled by prostate-specific deletion of PTEN, resulting in invasive and metastatic disease [27], and by prostate-specific over-expression of an activated form of the downstream Akt kinase [23], inducing mPIN lesions but not invasive carcinoma. The latter model has been used to evaluate the response of these lesions to inhibition of mammalian target of rapamycin (mTOR), which is placed even further downstream in this signalling cascade and the levels of which are elevated in human tumours with loss of PTEN or over-expression of Akt [46]. This treatment regimen induced complete regression of the mPIN lesions and seemed to largely affect HIF-1α–driven transcriptional programme in the transformed epithelial cells [47]. These data are supportive of further studies in the mouse model on the inhibition of the PI3-K–activated pathways, especially given the assumed role for PTEN loss in setting the stage for progression to hormone-refractory disease [30,31]. The increased severity of disease in the mouse models driven by loss of PTEN as compared to the activated Akt-driven model argues for the use of the former in such studies and the evaluation of alternative, more upstream kinases as a target for intervention.

3. Discussion

3.1. Limitations of the transgenic mouse model

The mouse model of prostate cancer offers a wide range of experimental possibilities and has been invaluable in dissecting the critical pathways governing the early stages of prostate cancer. Nevertheless, transgenic mouse models of prostate cancer faces several shortcomings. The mouse and human prostate are anatomically dissimilar, complicating the interpretation of data derived from a mouse model. In addition, mice do not spontaneously develop prostate cancer, and it appears that the engineered genetic lesion requires a certain degree of robustness to allow the model to mimic most of the characteristics of the human disease. Finally, numerous mouse models depend on androgen-driven transcriptional cassettes to establish prostate-specific over-expression of the transgenes.

3.2. Anatomic differences between the prostate of man and mouse

Historically, the key anatomic differences between the murine and the human prostate have led to concerns about the validity of the mouse model [48]. The human prostate is a single lobular structure surrounding the urethra at the base of the bladder. In contrast, the mouse prostate consists of three
distinct lobes, which are arranged circumferentially at the basis of the bladder [48,49]. Both mouse and human prostate are composed of similar epithelial cell types (columnar secretory epithelial cells, basal cells, and neuroendocrine cells), although the ratios are different. Also, the mouse prostate has a very modest stromal component. In the mouse, the dorsal and lateral lobes of the prostate share a ductal system and are thought to be most similar to the human peripheral zone of the prostate, where most carcinomas arise. In contrast, the mouse anterior prostate is considered to be analogous to the human central zone, which is rarely a site for neoplastic transformation [49]. In human prostate cancer, the contribution of neuroendocrine cells to advanced stages of the disease is limited, even though they can be present in significant numbers in prostate adenocarcinoma. Hence, mouse models that display preferential transformation of neuroendocrine cells (such as the SV40 T antigen-driven models) or in which tumour outgrowth preferentially occurs in the anterior prostate (such as in some Pten-deficient models) are less informative for the pathogenesis of human disease. Consequently, efforts have been made to standardise the pathologic grading of mouse prostate cancer lesions [50], and expert pathologic evaluation of the lesions found in newly generated models with reference to this Bar Harbor classification system is a prerequisite for the accurate interpretation of the observed phenotype.

### 3.3. Limitations to inducing advanced prostate cancer in the mouse

The mouse does not spontaneously develop benign or malignant prostate pathology. This is in contrast to the human situation, where in elderly men benign proliferations such as nodular hyperplasia have a nearly 100% prevalence and prostate cancer can be found at high frequencies during autopsy. In fact, prostate cancer is a very chronic disease. PIN lesions can already be found in the prostates of men in their twenties, whereas clinically detectable prostate cancer is usually not observed before the sixth decade of life. This chronic nature of the progression of the disease cannot be accurately modelled in the mouse, due to its limited lifespan and the logistical restraints of experimental research. These drawbacks are not a major issue for the study of the early stages of the disease, such as atypical hyperplasia, PIN lesions, and progression to microinvasive carcinoma, and mouse models have confirmed the critical roles of MYC over-expression and loss of PTEN and NKX3.1 in these early stages. However, most of these models do not progress beyond the stage of invasive carcinoma, which has severely hampered the study of advanced disease [17]. In fact, very few mouse models of prostate cancer display metastatic disease to the relevant target organs (bone, liver, and lung). The clinical problems associated with prostate cancer, however, are largely restricted to advanced disease. Whereas locally invasive prostate cancer can be efficiently treated by a surgical approach, metastatic disease present at the time of surgery is largely responsible for the remission of disease, and subsequent androgen-ablation therapy offers only temporary relief. As such, mouse models of prostate cancer will only significantly contribute to the design of novel therapeutic treatment regimens when they accurately model metastatic disease, facilitating the study of androgen-ablation therapy.

This brings us to a final shortcoming in a number of the currently available mouse models. To direct the expression of a transgene to the prostatic secretory epithelium, numerous models have used regulatory sequences driving prostate-specific gene transcription. Unfortunately, these regulatory sequences usually contain several androgen-responsive elements, rendering the expression levels of the transgene androgen dependent. Consequently, transgene expression levels drop dramatically on castration (to mimic androgen-ablation therapy), thereby inducing massive death of the cancer cells and remission of the tumour. These direct effects preclude the use of these transgenic mouse models to study the responses of the transformed cells to androgen ablation, and the mechanisms contributing to the outgrowth of hormone-refractory prostate cancer. In mouse models using prostate-specific deletion of conditional tumour suppressor genes (Fig. 1B) or prostate-specific activation of conditional oncogenes (expressed from generic promoters; Fig. 1C) using Cre-lox technology, these problems can be avoided because only the Cre transgene will depend on androgen-driven transcription.

### 4. Future directions

Mouse models for prostate cancer have made major contributions to the characterisation of the individual roles of distinct genetic lesions in the transformation process. However, most of these data bear relevance only to the early stages of the disease including invasive but not metastatic lesions. The clinical practice, however, could benefit considerably from more detailed knowledge concerning the
mechanisms of metastatic disease and the inevitable hormone-refractory stages. Therefore, modeling advanced prostate cancer using genetically engineered mice will require models allowing both the combination of multiple individual genetic lesions (for advanced stages of disease) and non-invasive imaging techniques to efficiently monitor metastatic spread of the primary tumour. Moreover, the pathways under scrutiny should include those uniquely involved in advanced prostate cancer, such as over-expression of the polycomb group proteins BMI1 and EZH2.

An alternative approach to study advanced prostate cancer could be the orthotopic transplantation of cells carrying the genetic lesions required for the early stages, in combination with an in vitro manipulation of these cells to introduce additional genetic lesions required for metastatic disease. Such orthotopic transplantation models, however, are also faced with certain technical complications, such as the size and the complex anatomy of the target organ and the requirement for the correct stromal compartment to facilitate advanced stages of the disease.

The main goals for mouse models of prostate cancer remain the validation of the specific contributions of individual genetic lesions observed in human disease and the preclinical testing of novel approaches to therapeutic intervention. In both instances, the main challenge lies in the accurate modelling of advances stages of the disease, specifically, metastatic lesions in lung, bone, and liver and hormone-refractory disease.

5. Conclusions

The mouse model has allowed the critical testing of the causal relationship between gene loss or gene over-expression and the induction of prostate cancer. Especially for the early stages of the disease, the contribution of individual lesions, such as loss of PTEN or p53 and gain of MYC has been well characterised using these mouse cancer models. Moreover, the collaboration between some of these genetic lesions and their mechanistic basis of action has been dissected into great detail. However, the progress in modelling metastatic disease as well as hormone-refractory prostate cancer is limited. Current models can be used to evaluate therapeutic approaches directed against the activities conferred on the cancer cells by the single experimentally induced genetic lesion. However, to further preclinical research where it really counts—in the area of metastatic disease and hormone-refractory prostate cancer—a further investment in generating mouse models for advanced prostate cancer will be required.

Conflicts of interest

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References


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1. Mouse models for prostate cancer allow experimental control over:
   A. The genetic lesions leading to prostate cancer.
   B. The environmental factors contributing to prostate cancer.
   C. Both the genetic lesions and environmental factors in prostate cancer.
   D. The progression to hormone-refractory prostate cancer.

2. Mouse models of prostate cancer over-expressing oncogenes in a prostate-specific fashion:
   A. Progress to locally invasive cancer.
   B. Progress to metastatic disease.
   C. Accurately model sporadic prostate cancer.
   D. Have oncogene expression in most prostate epithelial cells.

3. The part of the mouse prostate most closely resembling the peripheral zone of the human prostate is:
   A. The dorsal and lateral lobes.
   B. The ventral lobe.
   C. The lateral lobe.
   D. The lateral and ventral lobes.

4. Noninvasive imaging of mouse prostate tumours has been used to detect recurrent disease after androgen ablation. The imaging technique allowing such analyses is:
   A. Magnetic resonance imaging (MRI).
   B. Single-photon emission computed tomography (SPECT).
   C. Bioluminescence imaging (BLI).
   D. Positron emission tomography (PET).

5. Progression of prostate cancer to androgen-independent disease can be modelled in genetically engineered mice. The mouse model allowing such analyses is:
   A. A conditional knock-out for PTEN in combination with prostate-specific Cre transgene.
   B. A prostate-specific MYC transgene in combination with prostate-specific Cre transgene.
   C. A conditional knock-out for p53 in combination with a prostate-specific MYC transgene.
   D. A conditional knock-out for p53 in combination with a prostate-specific Cre transgene.

6. In mouse models of prostate cancer, loss of p53:
   A. Induces invasive prostate cancer in the absence of other genetic lesions.
   B. Does not induce any prostate pathology, irrespective of other genetic lesions.
   C. Induces hormone-refractory disease only after full loss of PTEN.
   D. Prevents oncogene-induced senescence induced by full loss of PTEN.