

Models of neuroendocrine prostate cancer

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Abstract

Prostate cancer remains the second leading cause of cancer death in men in the USA and most western countries. Prostatic acinar adenocarcinoma is the most commonly diagnosed form of prostate cancer. Small-cell neuroendocrine carcinoma is less frequently identified at the time of initial diagnosis, but this highly aggressive form of prostate cancer is increasingly observed in patients who have failed first- and second-line hormone therapy. Thus, developing and exploring models of neuroendocrine prostate cancer (NePC) are of increasing importance. This review examines the relevant xenograft tumor and genetically engineered mouse models of NePC, with the aim of addressing salient features and clinical relevance.

Key Words

- ▶ neuroendocrine prostate cancer
- ▶ castration-resistant prostate cancer
- ▶ xenograft tumors
- ▶ genetically engineered mice

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Introduction

Prostate cancer is the second most common cancer in men worldwide (Ferlay *et al.* 2010). With >90% of prostate cancers initially diagnosed as acinar adenocarcinomas (Fine 2012, Humphrey 2012), neuroendocrine carcinomas of the prostate (also described as small-cell neuroendocrine carcinomas (SCNCs)) are rare at the time of initial diagnosis, with only ~0.5–2% of prostate cancers identified as such (Helpap *et al.* 1999, Stein *et al.* 2008, Wang & Epstein 2008, Humphrey 2012, Tan *et al.* 2014). Unlike acinar adenocarcinomas that, according to the 2004 World Health Organization (WHO) classification, are composed of variably differentiated glandular structures, express prostate-specific antigen (PSA) and the androgen receptor (AR), and are subject to Gleason scoring (Eble *et al.* 2004, Humphrey 2004), neuroendocrine prostate cancers (NePCs) are characterized by sheets of highly atypical cells that do not form glandular structures and are immunopositive for markers of neuroendocrine differentiation, such as chromogranin, synaptophysin, CD56, and/or neuron-specific enolase (NSE) (Helpap &

Kollermann 1999, Helpap *et al.* 1999, Stein *et al.* 2008, Wang & Epstein 2008, Komiya *et al.* 2009, Fine 2012, Humphrey 2012, Beltran *et al.* 2014, Epstein *et al.* 2014). The majority of cells in NePCs are immunonegative for PSA and AR (Helpap & Kollermann 1999, Helpap *et al.* 1999, Eble *et al.* 2004, Stein *et al.* 2008, Wang & Epstein 2008, Fine 2012, Humphrey 2012, Beltran *et al.* 2014, Epstein *et al.* 2014), and these tumors have often lost *Rb* (*EPah4*), *p53* (*Trp53*), and/or *Pten* tumor suppressor activities (Beltran *et al.* 2014, Tan *et al.* 2014). Approximately 50% of NePCs exhibit *ERG* gene rearrangements (Mosquera *et al.* 2013, Beltran *et al.* 2014) and over-expression or amplification of the N-Myc and aurora kinase A genetic loci is common (Beltran *et al.* 2011, 2014, Mosquera *et al.* 2013, Beltran 2014). NePCs are also reported to have a high proliferative index, with more than 50% of tumor cells being immunohistochemically positive for Ki67 (Beltran *et al.* 2014, Epstein *et al.* 2014).

Although the diagnosis of NePC at the time of initial cancer identification is rare, these tumors are more

frequently found in patients who have previously received both first- and second-line androgen ablation therapies (Humphrey 2012, Mosquera *et al.* 2013, Epstein *et al.* 2014). It has been suggested that androgen ablation therapy may be selecting for cells with a neuroendocrine phenotype because these cells are predominantly castration-resistant (Beltran *et al.* 2014, Epstein *et al.* 2014, Tan *et al.* 2014). Given the fact that NePCs are largely negative for AR (Beltran *et al.* 2014), NePCs present a therapeutic challenge (Stein *et al.* 2008, Fine 2012, Humphrey 2012, Beltran *et al.* 2014). Compounding this challenge is the fact that NePCs are also often diagnosed at an advanced stage with visceral metastases present (Stein *et al.* 2008, Humphrey 2012, Epstein *et al.* 2014) and associated with shortened survival times (Stein *et al.* 2008, Humphrey 2012, Marcus *et al.* 2012, Epstein *et al.* 2014). Confounding the diagnosis is the fact that patients with NePCs may have seemingly incongruently low PSA elevations (Beltran *et al.* 2014, Epstein *et al.* 2014), and NePCs can coexist with the acinar adenocarcinomas (Eble *et al.* 2004, Stein *et al.* 2008, Wang & Epstein 2008, Fine 2012, Humphrey 2012, Epstein *et al.* 2014). Therefore, novel therapeutics are needed for this clinically significant and challenging variant of prostate cancer.

Importantly, there is no consensus regarding the cell of origin of NePCs. Multiple lineages have been proposed. One theory is that these tumors arise from 'dedifferentiation' of acinar adenocarcinoma cells, essentially resulting in an 'epithelial-to-neuroendocrine' transition (Helpap & Kollermann 1999, Helpap *et al.* 1999, Stein *et al.* 2008, Beltran *et al.* 2014). It has also been suggested that NePCs arise secondary to neoplastic transformation of a multipotential epithelial cell or stem cell within the prostate (Helpap & Kollermann 1999, Helpap *et al.* 1999, Stein *et al.* 2008). Given the fact that neuroendocrine cells are a normal component of the prostatic epithelium and can be identified immunohistochemically within acinar adenocarcinomas, some speculate that NePCs are the result of transformation of prostate-specific neuroendocrine cells that share a common origin with luminal and basal prostatic epithelial cells (Helpap & Kollermann 1999, Helpap *et al.* 1999, Beltran *et al.* 2014). Finally, a proposal that appears to have fallen out favor is that NePCs originate from non-prostate specific neuroendocrine cells of the diffuse neuroendocrine system (formerly called the amino precursor and decarboxylation (APUD) system) that reside within the prostate (Pearse 1969, Helpap & Kollermann 1999, Helpap *et al.* 1999).

Animal models of prostatic NePC are essential for understanding the biology of NePC and developing more

effective therapies. Multiple mouse models of NePC exist, and herein, we have reviewed the major xenograft and genetically engineered mouse models of NePC. We have described the xenograft models and detailed the lesions that the genetically engineered mice develop, their disease progression, and how genetic manipulation of some of these mice has led to a greater understanding of NePC.

Xenograft models of NePC

There are at least seven well-characterized xenograft models of NePC: LUCAP 49, WISH-PC2, UCRU-PR-2, WM-4A, MDA PCA 144-13, LTL352, and LTL370. A summary of the major characteristics of these seven xenograft tumor models can be found in Table 1.

LUCAP 49

The LUCAP 49 xenograft model was first described by True *et al.* (2002) and was derived from a metastasis of a prostate carcinoma that had received radiation therapy. The prostate tumor was histologically and immunohistochemically consistent with a NePC, although a small component (<5%) of the primary tumor was an acinar carcinoma (True *et al.* 2002). Subcutaneous xenograft tumors were established via serial passage of an omental metastasis in Fox Chase CB.17 severe combined immune deficiency (SCID) mice (True *et al.* 2002). Although the cells were unable to survive passage *in vitro*, at the time of publication of the initial report by True *et al.*, tumors had been successfully serially passaged as xenografts in the CB.17 SCID mice for over 4 years (True *et al.* 2002, Clegg *et al.* 2003). Histologically, the xenograft tumors have the same NePC morphology as the original prostate tumor (Fig. 1A and B). Molecular and pathological features of these tumors are consistent with clinical NePCs with lack of PSA, prostatic acid phosphatase (PAP), and AR expression, immunopositivity for the neuroendocrine markers synaptophysin and NSE and the neural marker CD57, and demonstration of a perinuclear configuration of low molecular weight keratin immunoreactivity characteristic of neuroendocrine cells. These tumors are only focally immunopositive for chromogranin (True *et al.* 2002, Clegg *et al.* 2003) and demonstrate loss of heterozygosity of the short arm of chromosome 8p. The xenograft tumors have a high proliferative index, with >75% of nuclei immunopositive for Ki67. The high proliferative index is also reflected in the short tumor doubling time – ~6.5 days. There are no reports of these tumors metastasizing. However, the tumors are

Table 1 Features of the xenograft models of NePC

Xenograft tumor name	Source	Immunohistochemical characteristics	Able to grow in castrated animals?	Able to metastasize?	Other	Selected references
LUCAP 49	Omental metastasis of a NePC	Immunopositive for: synaptophysin, NSE, and CD57. Focally positive for chromogranin Immunonegative for: AR, PSA, and PAP	Yes	Do not metastasize	High proliferative index. Cells demonstrate loss of heterozygosity of the short arm of chromosome 8p	True <i>et al.</i> (2002)
WISH-PC2	Resected NePC	Immunopositive for: chromogranin, synaptophysin, and NSE Immunonegative for: AR, PSA, PAP, PSCA, PMSA, CD19, CD20, CD22, and MDR1	Yes, but tumor growth rate is increased in the presence of androgens	Occasionally, with metastases identified in the liver, lung, and lymph node. Metastatic potential increased following irradiation	Express a mutated form of p53. Tumors develop after orthotopic injection into the prostate, liver, and bone	Pinthus <i>et al.</i> (2000) and Agemy <i>et al.</i> (2008)
UCRU-PR-2	Biopsy of a NePC	Immunopositive for: NSE, epithelial membrane antigen, carcino embryonic antigen Immunonegative for: AR, ER, PSA, PAP, and keratin	Unknown, but presumed to be able to grow in castrated mice, since tumor cells do not express the AR	Do not metastasize, and tumor cells injected intravenously do not form lung tumors	Tumors secrete POMC derived hormones. Implantation site affects invasiveness	van Haaften-Day <i>et al.</i> (1987), Jelbart, <i>et al.</i> (1988), and Jelbart <i>et al.</i> (1989)
WM-4A	Resected prostate tumor with a mixed phenotype	Immunopositive for: chromogranin Immunonegative for: PSA	Unknown	Metastasize following irradiation	Tumors are sensitive to radiation therapy	Agemy <i>et al.</i> (2008)
MDA PCA 144	Resected prostate tumor with a mixed phenotype	Immunopositive for: synaptophysin, chromogranin, CD56. Focally positive for cytokeratin Immunonegative for: AR, PSA, PAP, and AMACR	Unknown, but presumed to be able to grow in castrated mice, since tumor cells do not express the AR	Do not metastasize	High proliferative index. Cells do not express Rb or cyclin D1	Aparicio <i>et al.</i> (2011) and Tzelepi <i>et al.</i> (2012)
LTL352 and LTL370	Resected urethral metastasis of a NePC (LTL352). Resected penile metastasis of a NePC (LTL370)	Immunopositive for: synaptophysin and chromogranin Immunonegative for: AR and PSA	Yes	Reported to metastasize	Tumors express PTEN and do not express ERG or SPINK1	Lin <i>et al.</i> (2014)

castration-resistant; they are able to grow in castrated male mice, and the growth rate is reportedly unaffected by castration of intact male tumor bearing mice (True *et al.* 2002).

WISH-PC2

The WISH-PC2 xenograft model was developed by Pinthus *et al.* (2000). The line was derived from a transurethraly

resected prostate tumor that had been treated with androgen ablation therapy (goserelin and bicalutamide). Although the patient's tumor was initially diagnosed as an acinar adenocarcinoma, the tumor that was resected and served as the source for the xenograft was histologically consistent with a NePC (Pinthus *et al.* 2000). Xenografts were initially established as subcutaneous tumors in CB.17/Icr Beige or NOD SCID mice and Balb/c nude mice. The xenograft tumors resemble typical NePCs and are immunopositive for chromogranin, synaptophysin, and NSE and express a mutated form of *p53* and the anti-apoptotic protein BCL2. Tumor cells have a high proliferative index, as determined by Ki67 immunostaining and exhibit DNA aneuploidy. The cells lack AR, PSA, PAP, prostate stem cell antigen (PSCA), prostate-specific membrane antigen (PSMA), CD19, CD20, CD22, and multidrug resistance 1 (MDR1) (Pinthus *et al.* 2000). Although these tumors are immunonegative for AR and able to grow in castrated male mice, the tumor growth rate is affected by the presence of androgens, with slightly faster growth rates noted when mice are supplemented with testosterone. The tumor volume doubling time in the absence of androgens, however, remains relatively fast, ranging from 13.5 to 18 days, depending on whether the tumors originated from subcutaneously injected tumor cells or implanted tumor sections (Pinthus *et al.* 2000). The subcutaneous xenograft tumors are able to metastasize to a limited extent, with metastases occasionally identified in the lymph node, lung, or liver (Pinthus *et al.* 2000). However, the metastatic potential of WISH-PC2 xenografts is augmented by irradiation, with metastases identified in the adrenal gland, brown fat, and perirenal tissue (Agemy *et al.* 2008). Tumor cells injected orthotopically into the prostate, liver, and bone are able

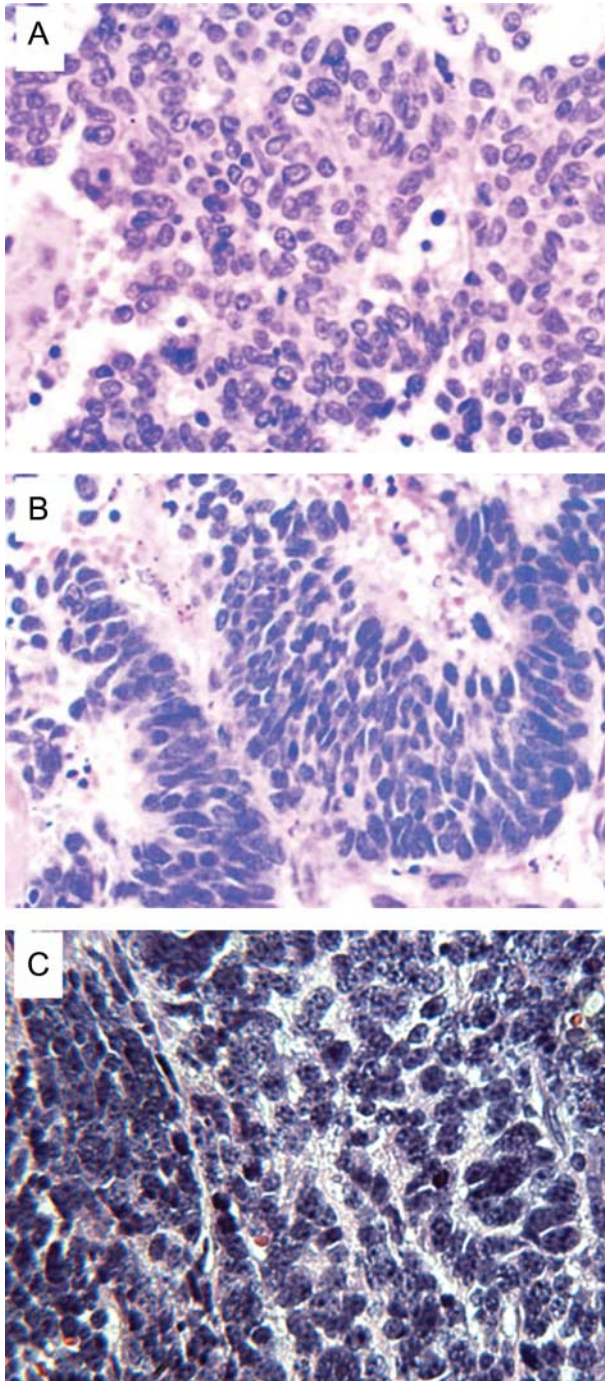


Figure 1

(A) Photomicrograph of the neuroendocrine prostate tumor from which the LuCAP 49 xenograft tumor was derived (H&E, 400× magnification). Reprinted from True LD, Buhler K, Quinn J, Williams E, Nelson PS, Clegg N, Macoska JA, Norwood T, Liu A, Ellis W *et al.* A neuroendocrine/small cell prostate carcinoma xenograft-LuCaP 49. *American Journal of Pathology* 161 705–715, copyright 2002 with permission from Elsevier. (B) Photomicrograph of a LuCAP 49 xenograft tumor. Note that the xenograft tumor and the patient's original prostate tumor have a similar histologic appearance consistent with NePCs (H&E, 400× magnification). Reprinted from True LD, Buhler K, Quinn J, Williams E, Nelson PS, Clegg N, Macoska JA, Norwood T, Liu A, Ellis W *et al.* A neuroendocrine/small cell prostate carcinoma xenograft-LuCaP 49. *American Journal of Pathology* 161 705–715, copyright 2002 with permission from Elsevier. (C) Representative image of a typical TRAMP mouse neuroendocrine prostate tumor exhibiting high nuclear to cytoplasmic ratio, anisocytosis, and anisokaryosis (H&E, 400× magnification).

to grow into discrete tumors, with bone lesions having both an osteolytic and osteoblastic component (Ponthus *et al.* 2000).

UCRU-PR-2

The UCRU-PR-2 xenograft model was first described by van Haaften-Day *et al.* (1987). The patient from whom this xenograft line was derived was initially diagnosed with a prostatic acinar carcinoma that progressed, following androgen-deprivation therapy (bilateral castration), to a NePC. The tissue used to establish the xenograft line was obtained from a biopsy of the NePC. The xenografts were established as subcutaneous tumors in Balb/c nude mice via serial transplantation. Histologically, the xenograft tumors have the same NePC morphology as the primary prostate tumor. The xenograft tumors do not express AR, estrogen receptor (ER), PSA (NPEPPS), PAP (REG3b), or keratin but express NSE, epithelial membrane antigen, and carcinoembryonic antigen (van Haaften-Day *et al.* 1987, Jelbart *et al.* 1989). These tumors also secrete a number of proopiomelanocortin (POMC)-derived hormones *in vivo*, including adrenocorticotrophic hormone (ACTH), somatostatin, and β -endorphin (Jelbart *et al.* 1988). Interestingly, although these xenograft tumors maintain their NePC phenotype regardless of the implantation site, implantation site affects the tumor invasiveness; tumors grafted underneath the renal capsule or within skeletal muscle exhibit local tissue invasion, while tumors inoculated into the subcutaneous fat or peritoneum lack this feature. Tumors do not develop when tissue is implanted under the capsule of the liver or spleen, established xenograft tumors do not metastasize, and *i.v.* injection does not result in lung tumors. The average subcutaneous tumor volume doubling time is ~ 14.7 days (Jelbart *et al.* 1989).

WM-4A

The WM-4A xenograft tumor model was developed by Agemy *et al.* (2008) from a prostate tumor that had been treated with radiation and complete androgen-deprivation therapy. The prostate tumor contained areas consistent with an acinar adenocarcinoma and other areas compatible with a NePC. The patient's tumor was immunopositive for chromogranin, synaptophysin, and CD57, and focally immunoreactive against PSA and PAP. The xenograft model was developed in CB.17/Icr beige SCID male mice. Xenograft tumors express chromogranin but not PSA. Although the xenograft tumors themselves

are sensitive to radiation, irradiation appears to promote metastasis in this model, with irradiated xenograft tumors metastasizing to the adrenal gland, perirenal fat, and brown fat (Agemy *et al.* 2008).

MDA PCA 144

The MDA PCA 144 xenograft tumor model was established by Aparicio *et al.* (2011). The xenograft tumor line was derived from a prostate tumor that had been treated with radiation therapy, androgen-deprivation therapy (leuprolide), carboplatin, docetaxel, cisplatin, and etoposide. The histological appearance of the resected tumor was mixed, containing areas of acinar adenocarcinomas, SCNCs, and large-cell neuroendocrine carcinomas. Areas of SCNCs were immunopositive for chromogranin, synaptophysin, and the neural cell adhesion marker CD56, and immunonegative for AR, PSA, and PAP. To establish the xenograft model, a number of fragments from the resected prostate tumor were implanted into the subcutaneous tissue of male CB.17 SCID mice, and four of the xenografts were consistent with a prostatic SCNC (MDA PCA 144 lines 11, 13, 20, and 23). The cells of these MDA PCA 144 xenografts have the typical histological appearance of a SCNC and resemble a clinical NePC, being immunonegative for the AR, PSA, PAP, and alpha-methylacyl-CoA racemase (AMACR). These xenografts are immunopositive for synaptophysin, chromogranin, and CD56, focally immunopositive for cytokeratin and have a high proliferative index as determined by Ki67 immunohistochemistry and by the prominence of mitotic figures (Aparicio *et al.* 2011). Extensive analysis was done on the MDA PCA 144-13 xenograft, and it was determined that its cells do not express *Rb* or cyclin D1, and they upregulate mitotic genes such as ubiquitin-conjugating enzyme E2C (*Ube2C*). In addition, MDA PCA 144-13 cells demonstrate nuclear *p53* immunostaining (Tzelepi *et al.* 2012).

LTL352 and LTL370

The LTL352 and LTL370 xenografts were established by Lin *et al.* (2014). These two xenografts were derived from biopsies of a urethral metastasis of a NePC (LTL352) and a penile metastasis of a NePC (LTL370). No information was provided by the authors regarding whether these patients had been treated with androgen-deprivation therapy, radiation therapy, and/or chemotherapy before the biopsy. Fragments of tissue were implanted into the subrenal capsule of testosterone-supplemented male NOD SCID (NOD.CB17-Prkdcscid/J) mice (Lin *et al.* 2014).

The xenografts resemble NePCs histologically and exhibit an expression pattern typical of these tumors. Neoplastic cells are immunopositive for synaptophysin and chromogranin, and immunonegative for AR and PSA. The tumors are castration-resistant, being able to grow in mice after androgen-deprivation. These tumors do not express *ERG* or serine protease inhibitor Kazal-type 1 (*SPINK1*), but do express *PTEN*. As with other NePC xenografts, these tumors have a rapid doubling time, with tumors doubling in size in ~10–12 days. These NePC xenografts reportedly metastasize, but the authors do not describe the frequencies or locations of the metastases (Lin *et al.* 2014).

Experimental manipulations

In addition to the described xenograft tumor models that spontaneously exhibit neuroendocrine differentiation, experimental manipulations can induce cells within more traditional xenograft prostate cancer lines to transition to a neuroendocrine phenotype. For example, castration of mice bearing the LTL331 prostate adenocarcinoma subrenal capsule xenograft causes the tumors to transition to a NePC phenotype (LTL331R). While LTL331 xenografts express AR and PSA, LTL331R tumors express synaptophysin, chromogranin, and CD56 and do not express AR or PSA (Lin *et al.* 2014). In addition, androgen-deprivation can increase the number of cells that express the markers of neuroendocrine differentiation within PC-310 (Noordzij *et al.* 1996, Jongsma *et al.* 2000, 2002), PC-295 (Noordzij *et al.* 1996, Jongsma *et al.* 1999), and CWR22 xenograft tumors (Huss *et al.* 2004). Interestingly, ionizing radiation stimulates a population of cells within LNCAP xenograft tumors to express chromogranin (Deng *et al.* 2011). Taken together, these models demonstrate that commonly used therapies (androgen-deprivation and irradiation) can stimulate cells within more 'classical' prostatic carcinomas to develop a neuroendocrine phenotype.

Summary of xenograft models

Herein, we have reviewed seven of the xenograft models of established NePC. These xenografts serve as clinically relevant tumor models for human NePC. Importantly, five of the xenograft tumor lines were derived from patients that had been treated with androgen-deprivation therapy, radiation therapy, and/or chemotherapy (van Haaften-Day *et al.* 1987, Pinthus *et al.* 2000, True *et al.* 2002, Agemy *et al.* 2008, Aparicio *et al.* 2011). The treatment statuses of

the patients from whom LTL352 and LTL370 were derived were not available (Lin *et al.* 2014). Thus, the majority of these xenograft tumors serve as appropriate models for NePC that arise post-treatment. In addition, these tumors model human NePC molecularly, with all of these xenograft tumors demonstrating at least one marker of neuroendocrine differentiation (see Table 1) and lacking expression of AR and/or prostate epithelial-specific markers, such as PSA (van Haaften-Day *et al.* 1987, Jelbart *et al.* 1989, Pinthus *et al.* 2000, True *et al.* 2002, Agemy *et al.* 2008, Aparicio *et al.* 2011, Lin *et al.* 2014). Finally, these xenograft tumors are able to model the rapid growth rate that is characteristic of many NePCs. High proliferative indices as determined by Ki67 immunohistochemistry are features of LUCAP 49 (True *et al.* 2002), WISH-PC2 (Pinthus *et al.* 2000), and MDA PCA 144 (Aparicio *et al.* 2011) xenograft tumors. Similarly, the average tumor doubling time for UCRU-PR-2 is ~2 weeks (Jelbart *et al.* 1989), and LTL352 and LTL370 tumors double in ~10–12 days (Lin *et al.* 2014).

Although these xenograft models are valid *in vivo* models for NePC, they are not without limitations. For example, even though xenograft models allow one to study the behavior of a human prostate tumor *in vivo*, the ability to study metastatic potential is limited (Sausville & Burger 2006). Although WISH-PC2 xenografts demonstrate the ability to metastasize spontaneously, they metastasize rarely and never to bone (Pinthus *et al.* 2000). LTL352 and LTL370 reportedly metastasize, but the locations and frequencies of the metastases were not detailed in the original description of these models (Lin *et al.* 2014). This is an important limitation, given the metastatic nature of NePCs. With the exception of LTL331R, a NePC xenograft that develops after castration of mice bearing LTL331 prostate adenocarcinoma xenograft tumors (Lin *et al.* 2014), another disadvantage is that NePC xenograft tumors only allow for the investigation of the characteristics and behaviors of established post-treatment NePCs, not the molecular mechanisms involved in the development of a NePC. Moreover, although the majority of NePCs observed in the clinic arise in patients that have previously received androgen-deprivation therapy, some NePCs occur in treatment naïve patients. Therefore, new models are needed for 'treatment naïve' *de novo* NePCs, because the currently available xenograft tumors reflect post-treatment NePCs with biologic behaviors and molecular alterations distinct from 'treatment naïve' NePCs. In addition, the effects of the human immune system on tumor growth, invasion, and metastasis cannot be studied because the tumors grow in a mouse microenvironment

(Sausville & Burger 2006). Finally, it is unclear how many of the established xenograft tumor lines are readily available given the extensive handling required to propagate and maintain them. Thus, despite the utility of the established NePC xenograft tumor lines, additional xenograft models of NePC are needed to further explore this clinically challenging variant of prostate cancer.

Genetically engineered mouse models of NePC

A number of genetically engineered mouse models of NePC exist. The salient features of these models can be found in Table 2. All of these models autochthonously develop prostate tumors that histologically resemble human NePC and express at least one marker of neuroendocrine differentiation (most commonly synaptophysin or chromogranin). In most of these models, prostate carcinomas with neuroendocrine phenotypes (i.e. neuroendocrine carcinomas) are induced via the expression of one or both of the simian virus 40 (SV40) early genes (the large and small T antigens) in prostate epithelial cells (Greenberg *et al.* 1995, Gingrich *et al.* 1996, 1999, Perez-Stable *et al.* 1996, 1997, Kasper *et al.* 1998, Masumori *et al.* 2001, Gabril *et al.* 2002, 2005, Reiner *et al.* 2007) or in prostate neuroendocrine cells (Garabedian *et al.* 1998). This results in prostate tumorigenesis because the large T antigen inhibits the activities of the tumor suppressors *p53* and *Rb* (Greenberg *et al.* 1995, Gingrich *et al.* 1996), and the small T antigen interacts with protein phosphatase 2A (Pallas *et al.* 1990, Greenberg *et al.* 1995, Gingrich *et al.* 1996). Conditional knockout of *p53* and *Rb* in prostate epithelial cells has been used as an alternative way of producing neuroendocrine prostate carcinomas in mice (Zhou *et al.* 2006).

Genetically engineered mouse models utilizing the SV40 T antigens

Transgenic adenocarcinoma of the mouse prostate model

The most well-known transgenic mouse model of NePC is the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. In the TRAMP model, the rat probasin promoter (a 426 bp long fragment of the promoter and 28 bp from the 5' UTR) drives the expression the SV40 large and small T antigens in prostatic epithelial cells (Greenberg *et al.* 1995, Gingrich *et al.* 1996, 1999, Irshad & Abate-Shen 2013). The rat probasin promoter is an androgen and zinc-dependent promoter, and transgene

expression should be highest in the dorsal, lateral, and ventral lobes of the prostate (Greenberg *et al.* 1995, Gingrich *et al.* 1996). Prostate lesion development commences at puberty (~6 weeks of age) with the appearance of low-grade prostatic intraepithelial neoplasia (PIN). By ~10–16 weeks of age, the prostates of most TRAMP mice exhibit high-grade PIN that progresses by ~18 weeks to well-differentiated adenocarcinomas. By ~24 weeks of age, most TRAMP mice will have poorly differentiated carcinomas with neuroendocrine features (i.e. neuroendocrine carcinomas, Fig. 1C; Gingrich *et al.* 1999, Kaplan-Lefko *et al.* 2003). These neuroendocrine carcinomas metastasize readily, with metastases identifiable in the adrenal gland, kidney, liver, lung, lymph nodes, and, rarely, the vertebrae with spinal cord compression (Gingrich *et al.* 1996, 1999, Kaplan-Lefko *et al.* 2003).

TRAMP mouse neuroendocrine carcinomas are immunopositive for synaptophysin and the SV40 large T antigen and have a high proliferative index as determined by Ki67. The neuroendocrine carcinomas are largely immunonegative for the epithelial markers cytokeratin 8 and E-cadherin. These carcinomas have variable to no AR expression (Kaplan-Lefko *et al.* 2003). TRAMP mouse tumorigenesis is largely castration-resistant, with ~80% mice castrated at 12 weeks, developing neuroendocrine carcinomas with metastases by 24 weeks of age (Gingrich *et al.* 1997, Kaplan-Lefko *et al.* 2003).

Interestingly, the TRAMP mouse strain affects lesion development and progression. For example, while extensive tumor burdens result in most C57BL/6 TRAMP×FVB F1 mice being killed before 33 weeks of age, C57BL/6 TRAMP mice appear to develop their tumor burden at a slower rate and are frequently able to survive until ~36–40 weeks of age. Neuroendocrine carcinomas in C57BL/6 TRAMP mice can invade the seminal vesicle and urethra, whereas these tumors C57BL/6 TRAMP×FVB F1 mice tend to spare the seminal vesicles (Gingrich *et al.* 1999, Kaplan-Lefko *et al.* 2003). C57BL/6 TRAMP mice may also have a lower incidence of neuroendocrine carcinoma development (Chiaverotti *et al.* 2008).

Similar to human NePC, there is no consensus regarding the cell lineage of TRAMP neuroendocrine carcinomas. Two basic theories exist. The first is that these neuroendocrine carcinomas originate from bipotential stem cells that are capable of expressing both epithelial (E-cadherin) and neuroendocrine (synaptophysin) markers and not from epithelial cells within PIN lesions (Chiaverotti *et al.* 2008). The second theory is that the neuroendocrine carcinomas arise from

Table 2 Features of the genetically engineered mouse models of NePC

Genetically engineered mouse model	Genetic manipulation	Pathologic progression	Castration resistant disease?	Immunohistochemistry	Metastatic potential	Other	Selected references
TRAMP	Rat probasin promoter driving the SV40 large and small T antigens	PIN by 6 weeks. Well-differentiated adenocarcinoma by 18 weeks. Neuroendocrine carcinoma by 24 weeks	Yes	Immunopositive for: synaptophysin Immunonegative for: cytokeratin 8 and E-cadherin. Variable to no expression of the AR	Yes: adrenal gland, kidney, liver, lung, and lymph nodes	Lesion development is affected by mouse background strain. Mice can develop extra-prostatic lesions that interfere with research with research	Greenberg et al. (1995), Gingrich et al. (1999), and Kaplan-Lefko et al. (2003)
12T-10 LPB-Tag	Large probasin promoter (LPB) driving SV40 large T antigen	PIN when mice are 2–5 months old. Carcinoma in mice of 6 months of age and older. Neuroendocrine carcinomas in mice older than 8 months	Not evaluated, presumed based on the fact that the tumors do not express the AR	Immunopositive for: chromogranin A, weakly immunopositive for cytokeratin Immunonegative for: AR	Yes: liver, lung, and lymph node	Allograft tumors metastasize	Masumori et al. (2001)
12T-7f LPB-Tag/ PB-hepsin	Large probasin promoter (LPB) driving SV40 large T antigen/rat probasin promoter driving hepsin overexpression	21-week-old mice demonstrate prostate adenocarcinomas with some areas resembling neuroendocrine carcinomas	Not evaluated	Immunopositive for: synaptophysin	Yes: bone, liver, and lung	Metastatic lesions express the AR	Klezovitch et al. (2004)
FG-Tag	Fetal globin promoter driving the SV40 large and small T antigens	PIN in 16–20-week-old mice. Carcinomas with epithelial and neuroendocrine features in 16–32-week-old mice	Yes	Immunopositive for: chromogranin A, synaptophysin, cytokeratin 8. Consistent low AR expression Immunonegative for: E-cadherin, mouse dorsolateral prostate secretory protein, and connexin 32	Yes: adrenal gland, bone, kidney, lung, and lymph node	Fetal globin promoter is not prostate specific. Mice can develop a number of extra-prostatic lesions	Perez-Stable et al. (1996, 1997) and Reiner et al. (2007)

Table 2 Continued

Genetically engineered mouse model	Genetic manipulation	Pathologic progression	Castration resistant disease?	Immunohistochemistry	Metastatic potential	Other	Selected references
PSP-TGMAP	Prostate secretory protein of 94 amino acids (PSP94) promoter/enhancer region driving SV40 large and small T antigens	Epithelial hyperplasia in 10-week-old mice. PIN in 12–19-week-old mice. Well-differentiated adenocarcinomas in 24–32-week-old mice. Poorly differentiated prostatic carcinomas without apparent glandular architecture have been described in mice as young as 16 weeks of age	Yes	Expresses markers of neuroendocrine differentiation, such as chromogranin A (as determined by cDNA microarray)	Yes: lymph nodes	Three different lines of mice exist. TG183-2 mice have the highest incidence of neuroendocrine carcinoma development. High transgene copy number has been associated with the development of extra-prostatic expression and lesions	Gabril <i>et al.</i> (2002, 2005)
PSP-KIMAP	The SV40 large and small T antigens are knocked-in at the PSP94 promoter/enhancer locus	PIN is present age 6–7 weeks, and well-differentiated adenocarcinomas are apparent by 10–12 weeks of age. Well-differentiated and moderately differentiated adenocarcinomas are the most common cancer types in mice older than 12 weeks. Neuroendocrine carcinomas have been found in mice that are older than 1 year	No	Immunopositive for: chromogranin A	Yes: liver, lungs, and lymph nodes	Tumors with neuroendocrine differentiation are rare in this model	Duan <i>et al.</i> (2005) and Gabril <i>et al.</i> (2005)
CR2-Tag	Cryptidin-2 promoter driving the the SV40 large and small T antigens	PIN in 8–10-week-old mice with microinvasion by 12–16 weeks. Neuroendocrine carcinomas by 24 weeks of age	Yes	Immunopositive for: chromogranin A, synaptophysin Immunonegative for: AR	Yes: bone marrow, liver, lung, and/or lymph nodes	The cryptidin-2 promoter is not prostate specific	Garabedian <i>et al.</i> (1998)

Table 2 Continued

Genetically engineered mouse model	Genetic manipulation	Pathologic progression	Castration resistant disease?	Immunohistochemistry	Metastatic potential	Other	Selected references
p53 ^{PE-/-} ; Rb ^{PE-/-}	Conditionally knocking-out p53 and Rb from the epithelium of all lobes of the mouse prostate	PIN by 8 weeks of age. Poorly differentiated prostate carcinoma with neuroendocrine features by ~32 weeks of age	Yes	Immunopositive for: synaptophysin, cytokeratin 8, AR Immunonegative for: cytokeratin 5	Yes: adrenal gland, liver, lung, and lymph node	Half of p53 ^{PE-/-} ; Rb ^{PE-/-} mice will develop prostate carcinomas with systemic metastases similar to those of the p53 ^{PE-/-} ; Rb ^{PE-/-} mice by ~72 weeks of age	Zhou <i>et al.</i> (2006)

an 'epithelial-to-neuroendocrine' transition (Kaplan-Lefko *et al.* 2003). Given the fact that there is debate regarding the origin of human NePCs, this controversy does not negate the use of TRAMP mice as a model of NePC.

In addition to being used for evaluating the efficacy of novel therapeutics, TRAMP mice have been extensively manipulated in order to investigate prostate cancer progression and metastasis. For example, TRAMP mice that are heterozygous for the *Pten* tumor suppressor (TRAMP/*Pten*^{-/+}) have larger prostate tumors and shorter survival times than TRAMP mice that are WT for *Pten*. These two lines of TRAMP mice have similar rates of visceral metastasis. Together, this indicates an important role for *Pten* in prostate cancer progression but not metastasis (Kwabi-Addo *et al.* 2001). This is clinically relevant, given the frequency of *PTEN* loss in human NePC (Tan *et al.* 2014). TRAMP mice that lack the ubiquitin ligase *Siah2* (TRAMP/*Siah2*^{-/-}) develop fewer neuroendocrine prostate carcinomas and fewer visceral metastases than TRAMP with WT *Siah2*, suggesting a role for *Siah2* in the acquisition of a neuroendocrine phenotype and metastasis (Qi *et al.* 2010).

Despite its utility as a model of NePC, the TRAMP mouse model is not without limitations. For example, TRAMP mice can develop a number of extra-prostatic transgene-associated tumors that complicate research studies and necessitate early removal (Berman-Booty *et al.* 2014). These include epithelial-stromal (phyllodes-like) tumors of the seminal vesicles (Tani *et al.* 2005), renal tubulo-acinar carcinomas (Suttie *et al.* 2005), neuroendocrine tumors of the urethra (Suttie *et al.* 2005), anaplastic midbrain tumors (Berman-Booty *et al.* 2014), and poorly differentiated submandibular salivary gland adenocarcinomas (Berman-Booty *et al.* 2014). Thus, although the TRAMP mouse is one of the best-characterized genetically engineered models of NePC, demonstrating a lesion progression and an immunophenotype similar to human NePC, it is not without shortcomings.

12T-10 LPB-Tag model

The 12T-10 LPB-Tag model is a derivative of the LADY transgenic mouse model. The LADY model is genetically engineered to have a portion of the rat probasin promoter (LPB: a 11 500 bp long fragment of the promoter and 28 bp from the 5' UTR; Yan *et al.* 1997) drive the prostate-specific expression of a mutant SV40, which only produces the large T antigen (Kasper *et al.* 1998, Masumori *et al.* 2001, Irshad & Abate-Shen 2013). Unlike most LADY mice that develop adenocarcinomas that metastasize infrequently

(Kasper *et al.* 1998, Irshad & Abate-Shen 2013), 12T-10 LPB-Tag mice develop neuroendocrine carcinomas originating from the dorsal, lateral, or ventral lobes of the prostate. The initial lesion these mice develop is PIN, which typically appears when the mice are between 2 and 5 months of age. Interestingly, aggregates of cells with an immunohistochemical profile consistent with neuroendocrine cells (immunopositive for chromogranin A) are reported to be found commonly within high-grade PIN lesions in mice 5 months of age and older (Masumori *et al.* 2001). Prostates from mice older than 6 months of age typically have invasive foci and evidence of carcinomas, although some mice develop microinvasion and carcinomas as early as 4 months of age. Invasive foci and early carcinomas can have a mixed phenotype with some areas histologically consistent with adenocarcinomas and other areas typical of neuroendocrine carcinomas. More extensive invasive foci and carcinomas in mice older than 8 months often have a histologic appearance consistent with a neuroendocrine carcinoma (Masumori *et al.* 2001). Like the human NePCs that they are modeling, these tumors are immunopositive for chromogranin A and largely immunonegative for AR, although they are also weakly immunopositive for cytokeratin, (Masumori *et al.* 2001). Tumors most commonly metastasize to the liver, lung, and lymph node, with over 80% of mice 9 months of age and older having metastases. Metastatic lesions have the same histologic and immunohistochemical profile as the primary neuroendocrine prostate tumors (Masumori *et al.* 2001). When neuroendocrine carcinomas from 12T-10 LPB-Tag mice are implanted into the subcutaneous tissue of athymic nude mice, the allograft tumors (NE-10 allograft) maintain the original tumor's neuroendocrine phenotype and metastasize to the liver and lung (Masumori *et al.* 2001, 2004).

Although the 12T-10 LPB-Tag model is the only LPB-Tag mouse line capable of spontaneously developing neuroendocrine prostate carcinomas, prostatic carcinomas with neuroendocrine metastases develop in 12T-7f LPB-Tag mice that overexpress hepsin, a serine protease (Klezovitch *et al.* 2004, Irshad & Abate-Shen 2013). These mice are generated by crossing 12T-7f LPB-Tag mice with mice that overexpress hepsin under the control of the rat probasin promoter (PB-hepsin). By ~21 weeks of age, the resulting 12T-7f LPB-Tag/PB-hepsin mice develop PIN and prostate carcinomas with some areas that histologically resemble neuroendocrine carcinomas. Tumor metastases are found in bone, liver, and lung. Metastases are immunopositive for synaptophysin, thus confirming their neuroendocrine differentiation, and express AR

(Klezovitch *et al.* 2004). Given the fact that 12T-7f LPB-Tag/PB-hepsin neuroendocrine tumors express AR, this model may not be completely clinically relevant.

Fetal globin-T antigen model

Prostate tumors with epithelial and neuroendocrine characteristics have been described in one line of male mice expressing the SV40 T antigens under the control of the fetal globin promoter (fetal globin-T antigen (FG-Tag) model), a global promoter (Perez-Stable *et al.* 1996, 1997). Prostate tumors are identified in 16–32-week-old FG-Tag mice. These tumors likely originate from within PIN lesions, which are found in 16–20-week-old mice. Consistent with the mixed histological appearance, cells within the prostate tumors express epithelial and neuroendocrine markers, namely cytokeratin 8 and chromogranin A. The tumor cells do not express mouse dorsolateral prostate secretory protein or connexin 32 (Perez-Stable *et al.* 1997). Later work with these mice further elucidated the immunohistochemical characteristics, namely, that tumor cells are immunopositive for synaptophysin and immunonegative for E-cadherin. FG-Tag mouse prostate tumors are believed to originate from p63-expressing basal epithelial cells, because the T antigen has been immunohistochemically localized to these cells before PIN and tumor development (Reiner *et al.* 2007).

FG-Tag mouse prostate tumors are able to metastasize to the adrenal gland, bone, kidney, lung, and perirenal lymph node. Mice that are homozygous for the transgene have a higher incidence of tumor development than hemizygous mice. Prostate tumor development in FG-Tag mice appears to be castration-resistant, with ~50% of male mice castrated at age 4–6 weeks, developing prostate tumors by 20–28 weeks of age (Perez-Stable *et al.* 1997). Despite their androgen-independent behavior, tumors consistently express a low level of the AR, with highest AR expression levels in the tumor cells surrounding blood vessels (Reiner *et al.* 2007).

Despite the fact that FG-Tag mice develop prostate tumors, the fetal globin promoter is not prostate specific, therefore mice can develop neoplastic lesions in other organs as well. For example, subcutaneous, pericardial, and periadrenal hibernomas arise in other lines of male FG-Tag mice, and adrenocortical tumors have been found in female FG-Tag mice (Perez-Stable *et al.* 1996). In addition, male mice that develop prostate tumors have also been reported to develop adrenal tumors, hibernomas, and seminomas (Perez-Stable *et al.* 1997). Therefore, although FG-Tag mice develop castration-resistant

prostate tumors that share some features with human NePCs, given the potentially mixed phenotype (immunopositivity for both neuroendocrine and epithelial markers) and development of extra-prostatic lesions, this model is not without its limitations.

PSP94 gene-directed transgenic mouse adenocarcinoma in the prostate model

Poorly differentiated prostate carcinomas with a neuroendocrine phenotype typify late-stage tumors in the *PSP94* gene-directed transgenic mouse adenocarcinoma in the prostate (PSP-TGMAP) model of prostate cancer. In this model, expression of the SV40 T antigens is driven by a 3.84 kb long section of the prostate secretory protein of 94 amino acids (PSP94) promoter/enhancer region, which is reportedly prostate specific (Gabril *et al.* 2002). Three lines (lines TG183-2, TG186-3, and TG186-9) were initially established. Lines TG186 differ from line TG183, in that the transgene for line TG186 includes exons 1 and 2 as well of part of the first intron of the *PSP94* gene following the promoter/enhancer region (Gabril *et al.* 2002). Prostate tumorigenesis begins with prostate epithelial hyperplasia when the mice are ~10 weeks of age, followed by PIN in 12–19-week-old mice. Well-differentiated adenocarcinomas are typically identifiable in 24–32-week-old mice. Poorly differentiated prostatic carcinomas without apparent glandular architecture have been described in TG183-2 mice as young as 16 weeks of age. These tumors have also been identified in TG186-9 mice that are 28 weeks of age and older (Gabril *et al.* 2002). Poorly differentiated carcinomas show elevated expression of genes involved in neuroendocrine differentiation, including chromogranin A. Thus, these tumors have been described as having a neuroendocrine phenotype (Gabril *et al.* 2005). TG183-2 prostate tumors most frequently metastasize to the renal lymph nodes (Gabril *et al.* 2002). TG183-2 prostate tumors also appear to be castration-resistant, with 67% of mice castrated between 20 and 26 weeks of age having prostate carcinomas 1 month post-castration (Gabril *et al.* 2002).

One of the disadvantages of this model is that the rate of lesion development varies between lines. For example, although line TG183-2 mice develop poorly differentiated carcinomas with neuroendocrine features, they develop PIN and well-differentiated adenocarcinomas at a slower rate than line TG186-3 and TG186-9 mice. Therefore, care must be taken when selecting the mouse line for use. Transgene copy number also varies between lines of mice, and having a high transgene copy number has been

associated with the development of extra-prostatic lesions, which can complicate studies and necessitate early removal. For example, line TG186-9 mice have the highest copy number and are most prone to extra-prostatic tumor development (Gabril *et al.* 2002).

Interestingly, when the SV40 T antigens are targeted ('knocked in') to the *Psp94* gene locus, the resulting mice (*Psp94*-knockin mouse adenocarcinoma in the prostate (PSP-KIMAP) mice) display a different phenotype than when the transgene is randomly integrated into the genome, as with PSP-TGMAP mice (Duan *et al.* 2005). For example, PIN is evident in PSP-KIMAP mice by age 6–7 weeks, and well-differentiated adenocarcinomas are apparent by 10–12 weeks of age. Well-differentiated and moderately differentiated adenocarcinomas are the most commonly found cancer types in PSP-KIMAP mice, ranging from 12 to ~52 weeks. Tumor metastases to the liver, lungs, and lymph nodes can be identified in mice older than 1 year of age. Metastases express AR. Unlike TG183-2 tumors, PSP-KIMAP tumors appear to be sensitive to castration (Duan *et al.* 2005). PSP-KIMAP mice rarely develop prostatic neuroendocrine carcinomas (as identified by histological appearance and immunoreactivity against chromogranin A), and, when present, these tumors have only been identified in mice older than 1 year of age (Duan *et al.* 2005, Gabril *et al.* 2005). Studies with PSP-TGMAP and PSP-KIMAP mice seem to suggest that the way that the transgene is integrated into the genome affects lesion development (Duan *et al.* 2005, Gabril *et al.* 2005).

Cryptdin-2 T antigen model

When the mouse cryptdin-2 promoter (6500 bp of the promoter and 34 bp of the 5' UTR) is used to drive the expression of the SV40 T antigens in prostate neuroendocrine cells, prostate tumorigenesis, culminating in neuroendocrine carcinomas, results (Garabedian *et al.* 1998). Lesion development in the cryptdin-2 T antigen (CR2-Tag) model begins with the appearance of PIN in 8–10-week-old mice. By age 12–16 weeks, microinvasion is evident within PIN lesions, and by 24 weeks of age, the majority of CR2-Tag mice will have poorly differentiated, anaplastic prostatic carcinomas, lacking glandular architecture with metastases evident in the bone marrow, liver, lung, and or lymph nodes. Targeting of the SV40 T antigens by the CR2 promoter to prostatic neuroendocrine cells and confirmation of the neuroendocrine carcinoma phenotype are determined by the presence of cells within PIN and microinvasive lesions that express both neuroendocrine

markers (chromogranin A and synaptophysin) and the SV40 T antigens (Garabedian *et al.* 1998). Expression studies have also found that cells from tumor bearing prostates express chromogranin A and B, in addition to a number of other neural and endocrine biomarkers (Hu *et al.* 2002). Prostate tumorigenesis in CR2-Tag mice is castration-resistant, because mice castrated at 4 weeks of age develop similarly sized tumors as their intact littermates. In addition, tumor cells are immunonegative for AR (Garabedian *et al.* 1998).

CR2-Tag mice have been used to elucidate the roles of matrix metalloproteinases (MMPs) in NePC progression. For example, CR2-Tag mice deficient for *Mmp2* (CR2-Tag/*Mmp2*^{-/-} mice) develop smaller prostate tumors, reduced tumor neovascularization, fewer foci of invasion, and fewer lung metastases than CR2-Tag mice with WT *Mmp2*. In contrast, CR2-Tag mice deficient for *Mmp9* (CR2-Tag/*Mmp9*^{-/-} mice) exhibit a more invasive phenotype than CR2-Tag mice with WT *Mmp9*. These studies suggest different roles for *Mmp2* and *Mmp9* in NePC (Littlepage *et al.* 2010).

Summary of models that utilize the SV40 T antigens

A detailed review of the features of the above mouse models clearly demonstrates that utilization of the SV40 T antigens is one of the most robust ways to induce prostate carcinomas with neuroendocrine features as evidenced by the fact that all of these models develop neuroendocrine carcinomas with systemic metastases (Irshad & Abate-Shen 2013). Use of the SV40 T antigens results in rapid tumorigenesis, because neuroendocrine carcinomas are found in many of these models by the time the mice are ~6-month-old (Perez-Stable *et al.* 1997, Garabedian *et al.* 1998, Gingrich *et al.* 1999, Gabril *et al.* 2002). However, the incidence of neuroendocrine tumors varies between models. For example, although the majority of end-stage tumors in most of these models are neuroendocrine carcinomas (Irshad & Abate-Shen 2013), the incidence of poorly differentiated carcinomas with neuroendocrine features in PSP-TGMAP (line TG183-2) may be as low as 25% (Gabril *et al.* 2002), and PSP-KIMAP mice rarely develop neuroendocrine carcinomas (Duan *et al.* 2005, Gabril *et al.* 2005). Importantly, with the exception of PSP-KIMAP (Duan *et al.* 2005) and 12T-7f LPB-Tag/PB-hepsin mice (Klezovitch *et al.* 2004), most tumors that develop secondary to expression of the SV40 T antigens appear to be castration-resistant (Gingrich *et al.* 1997, Perez-Stable *et al.* 1997, Garabedian *et al.* 1998, Masumori *et al.* 2001,

Gabril *et al.* 2002) as determined by continued tumor development in castrated mice or lack of tumor cell AR expression. Castration-resistance is an important and clinically relevant feature of these genetically engineered mouse models.

In addition to the models described previously, there are other mouse models that employ the SV40 T antigen for oncogenesis, but the tumors that develop do not strictly fit the criteria of 'neuroendocrine carcinomas'. For example, when the expression of the SV40 T antigens is controlled by a promoter composed of the 5' flanking region and part of the first exon of the rat prostatic steroid binding protein (C3(1)), poorly differentiated prostate carcinomas can be found in the male mice (C3-(1)-Tag) after 8 months of age. However, despite the poorly differentiated and anaplastic histological appearance of cells within these carcinomas, these tumors have not been immunohistochemically confirmed as neuroendocrine (Maroulakou *et al.* 1994).

Conditional knockout models of NePC

P53 and *Rb* conditional knockout

Simultaneously, conditionally knocking out *p53* and *Rb* (*p53*^{PE-/-}; *Rb*^{PE-/-}) from the epithelium of all lobes of the mouse prostate results in the development of prostate carcinomas with neuroendocrine differentiation. Loss of *p53* and *Rb* is limited to the prostate secondary to the prostate-specific expression of Cre recombinase under the control of *Arr2pb* promoter, a modified rat probasin promoter (Zhou *et al.* 2006). The average *p53*^{PE-/-}; *Rb*^{PE-/-} mouse will develop PIN at ~8 weeks of age followed by a poorly differentiated prostatic carcinoma with neuroendocrine features by ~32 weeks of age (range 24–50 weeks). These tumors metastasize readily to the adrenal gland, liver, lung, and lymph node. Interestingly, cells within the primary tumors and metastases vary with regard to their reactivity against synaptophysin, cytokeratin 8, and AR, with up to 80% of cells immunopositive for each of these biomarkers and ~50–90% of the cells immunopositive for all three biomarkers. Tumor cells are immunonegative for cytokeratin 5 (Zhou *et al.* 2006).

Although prostate tumors from *p53*^{PE-/-}; *Rb*^{PE-/-} mice express AR, tumorigenesis appears to be castration-resistant. Mice castrated at ~8 weeks of age develop prostate tumors with a similar histological appearance and frequency as their intact counterparts by ~22 weeks of age. In addition, castration of 22-week-old mice with

prostate tumors does not decrease tumor cell proliferation, although AR expression may be reduced (Zhou *et al.* 2006).

Interestingly, half of mice that lack prostate-specific expression of both alleles of *p53* and one allele of *Rb* ($p53^{PE-/-}$; $Rb^{PE+/-}$ mice) will develop prostate carcinomas with systemic metastases similar to those of the $p53^{PE-/-}$; $Rb^{PE-/-}$ mice by ~72 weeks of age. Many of the tumors from these mice will have lost expression of the remaining WT allele of *Rb*. This is in contrast to mice with prostate-specific inactivation of either *p53* ($p53^{PE-/-}$ mice) or *Rb* ($Rb^{PE-/-}$ mice) and mice that lack prostate-specific expression of *Rb* but maintain one *p53* allele ($p53^{PE+/-}$; $Rb^{PE-/-}$ mice). These mice develop PIN that does not progress to carcinoma (Zhou *et al.* 2006). This suggests that both *p53* and *Rb* must be lost for neuroendocrine prostate tumor development and that homozygous loss of *p53* may facilitate loss of the second allele of *Rb*.

Summary of genetically engineered mouse models of NePC

Herein we have described a number of currently available genetically engineered mouse models of NePC. Interestingly, since all of the genetically engineered models discussed herein exhibit functional loss of *p53* and *Rb*, these models illustrate the apparent importance of *p53* and *Rb* loss in the development of a neuroendocrine phenotype. However, it is not clear whether loss of *p53* and *Rb* is 'necessary and sufficient' for neuroendocrine carcinoma development. For example, although work with the $p53^{PE-/-}$; $Rb^{PE-/-}$ mice (Zhou *et al.* 2006) suggests that loss of *Rb* in the background of complete *p53* loss is 'necessary and sufficient' to induce prostatic neuroendocrine carcinomas since both $p53^{PE-/-}$; $Rb^{PE-/-}$ and $p53^{PE-/-}$; $Rb^{PE+/-}$ mice develop NePCs, while $p53^{PE+/-}$; $Rb^{PE-/-}$, $p53^{PE-/-}$, and $Rb^{PE-/-}$ mice do not (Zhou *et al.* 2006), studies with TgAPT₁₂₁ mice indicate otherwise (Hill *et al.* 2005). TgAPT₁₂₁ mice express a mutant SV40 large T antigen that only abrogates the activity of RB family members (*Rb*, *p107*, and *p130*) and spares *p53* (Hill *et al.* 2005). These mice develop PIN and well-differentiated adenocarcinomas. When further manipulated, they lose *p53*, but the resulting TgAPT₁₂₁; $p53^{-/-}$ mice do not develop neuroendocrine prostate tumors. This implies that the loss of function of other tumor suppressors and/or gain of function of other oncogenes is required for NePC development (Hill *et al.* 2005). Further research is needed to determine the exact molecular alterations necessary for NePC development and growth.

One of the advantages of using genetically engineered mouse models to study NePC is that the tumors autochthonously develop within the prostate and, as the lesions typically progress through a number of stages (including pre-neoplastic) before culminating in NePC, the molecular mechanisms involved in most stages of prostatic NePC development can be studied. Another advantage of these genetically engineered mouse models is that most of these models produce castration-resistant disease with solid organ and or lymph node metastases (Gingrich *et al.* 1997, Perez-Stable *et al.* 1997, Garabedian *et al.* 1998, Masumori *et al.* 2001, Gabril *et al.* 2002, Zhou *et al.* 2006) – an important feature of human NePC. In addition, the mice have intact immune systems (Becher & Holland 2006), allowing for studies into the effect of the immune system on cancer development and regression.

A major disadvantage of all of the genetically engineered mouse models is that simultaneous loss of the *p53* and *Rb* activity, either through the use of the SV40 T antigens (Greenberg *et al.* 1995, Perez-Stable *et al.* 1997, Garabedian *et al.* 1998, Masumori *et al.* 2001, Gabril *et al.* 2002, Klezovitch *et al.* 2004, Duan *et al.* 2005), or secondary to conditionally knocking-out both of these genes from the prostatic epithelium (Zhou *et al.* 2006), is the genetic alteration that results in neuroendocrine tumor development. Although loss of both *p53* and *Rb* is a common finding in human NePCs (Tan *et al.* 2014), it is unlikely that these two major tumor suppressors are lost simultaneously or as the initial event leading to tumorigenesis. Therefore, models that lose both *p53* and *Rb* functionality as the initiating event may not accurately model the molecular events that occur during the development of human NePC. The $p53^{PE-/-}$; $Rb^{PE+/-}$ mouse model is the one that most closely resembles nonsimultaneous loss of *p53* and *Rb* (Zhou *et al.* 2006), although the timing of the loss of the second allele of *Rb* during tumorigenesis is unknown. In addition to the nonclinically relevant simultaneous loss of *p53* and *Rb* activity induced by the SV40 T antigens, the clinical applicability of mouse models that use these viral oncogenes is further challenged by the fact that the SV40 T antigens interact with a number of cellular targets in addition to *p53* and *Rb*, and these interactions may be irrelevant to human prostate cancer. Specifically, the large T antigen inhibits *p107* and *p130*, two other members of the RB family of proteins, and binds the chaperone Hsc70 and the transcriptional co-activators CBP, p300, and p400, while the small T antigen interacts with protein phosphatase 2A (Ali & DeCaprio 2001, Ahuja *et al.* 2005, Pipas 2009). In addition, all of these genetically engineered mice

develop neuroendocrine carcinomas spontaneously, that is without therapeutic interventions such as androgen deprivation. Therefore, instead of modeling the more common 'post-treatment' NePC, these mice model primary (treatment naïve) NePCs.

Finally, prostate lesion development in some of these models, specifically the TRAMP (Berman-Booty *et al.* 2014), FG-Tag (Perez-Stable *et al.* 1996), and PSP-TGMAP (Gabril *et al.* 2002) models, can be confounded by the development of extra-prostatic lesions and tumors.

Conclusions and future directions

Herein, we reviewed the major xenograft and genetically engineered mouse models of NePC. These models underscore the aggressive nature of NePCs. However, current mouse models of NePC only partially mimic the salient features of clinical disease. Thus, careful consideration of the biological behaviors and characteristics of the available models are needed in order to ensure that the model of choice is appropriate for the research question posed. For example, it may be inappropriate to use one of the xenograft models to study how NePC develops in the background of PIN or to use TRAMP mice to try to model castration-sensitive disease. In addition, the prostate tumorigenesis of the established genetically engineered mouse models may not reflect that of 'post-treatment' NePCs, because these mice develop neuroendocrine carcinomas without therapeutic intervention (such as androgen-deprivation) and castration does not significantly alter their phenotype.

Xenografts that are derived from *de novo* 'treatment naïve' NePC as well as more xenografts that reliably metastasize are clearly needed. Conversely, genetically engineered models that develop neuroendocrine carcinomas after androgen ablation therapy are needed in order to model the more common form of NePC seen in the clinic. Genetically engineered mice with truly sequential loss of function of multiple tumor suppressors (such as *p53* and *Rb*) or gain of function of multiple oncogenes (through the use of inducible promoters) would also allow for more accurate modeling of human NePC. Given the increasing prevalence of NePC in the clinical setting, development of new models that address the limitations of the current models is of increasing urgency.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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