A Spectrum of Prostate Cancer Development in Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) Model

Krisna Murti
Anatomical Pathology Department, Medical Faculty, University of Sriwijaya, Indonesia

Abstract

Animal models that closely mimic the clinical disease can be exploited to facilitate translational research are needed. The Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) model is uniquely suited to elucidate how a spectrum of prostate cancer development raised. Prostate cancer growths from premalignant stage, high grade prostatic intraepithelial neoplasia then progressed into highly dedifferentiated tumours that primarily metastasised to lymph nodes and lungs have similar pattern to that seen in humans.

Key words: animal model, prostate cancer, TRAMP, metastasis, high-grade prostatic intraepithelial neoplasia.

Abstrak

Hewan model yang sangat mirip dengan penyakit klinik yang dapat dieksploitasi dalam memfasilitasi penelitian sangat dibutuhkan. The Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) model merupakan suatu model yang sangat cocok dalam menjabarkan suatu spektrum perkembangan keganasan prostat dari stadium pre-ganas, high grade prostatic intraepithelial neoplasia sampai terjadinya tumor prostat yang metastase ke kelenjar limfe dan paru-paru dengan pola yang sama seperti yang terjadi pada manusia.

Kata kata kunci: hewan model, kanker prostat, TRAMP, metastase, high grade prostatic intraepithelial neoplasia.

Prostate Cancer is the most common malignant disease in Western communities and has caused the second cancer related deaths in man after lung cancer. In the USA this cancer caused 27.000 death in 2007 [1]. In addition, the number of men having risk for prostate cancer development is increasing rapidly as shown by demographic shift in population [2]. Prostate cancer (PCa) is an androgen dependent disease that can be treated by androgen ablation therapy, and clinical trials are under way to prevent PCa through the reduction of androgen receptor [3].

Extensive and adequate research is obviously necessary to identify and analyse innovative modalities for prevention, intervention and regression of prostate cancer as well as to study the potential relationship between molecular mechanism and clinical progression. However, it is difficult to obtain human prostate cancer tissues, particularly, at metastatic or very late stages of disease. Therefore, validation and establishment of a model system that is suitable and analytical are in essential to accelerate the velocity of translational research and into improving outcomes for patients with castrate resistant. Animal models are ideal to fulfil these criteria. The use of transgenic mouse models provides benefits as observers can manipulate its genome to analyse molecular events related to the development of prostate cancer as well as milieu and genetic mutation can be controlled [4].

The transgenic adenocarcinoma of the mouse prostate (TRAMP) model developed on C57BL/6 inbred strain by Professor Norman Greenberg. This model was generated by using the prostate-specific rat probasin promoter to drive expression of the simian virus 40 large tumour antigen-coding region that acts as an oncoprotein through interactions with the retinoblastoma (Rb) and p53, tumour suppressor gene products. The rat probasin gene encodes an androgen and zinc-regulated protein specific to the epithelium of prostate dorsolateral and ventral lobes. Cis-acting androgen–response regions within the 5' flanking region have been identified and the ability of the prostate-specific rat probasin gene promoter to target heterologous genes particularly in the prostate of transgenic mice was established. In rats the gene promoter would be expected to act in the same manner since it was isolated from this species [5,6,7].

A full spectrum of prostate cancer development was induced; from premalignant stage, high grade prostatic intraepithelial neoplasia (HGPIN) then progressed into highly dedifferentiated tumours that primarily metastasised to lymph nodes and lungs and these

cancers have similar patterns of the natural history that were observed in human prostate carcinoma [5,7]. Another study by Han et al. (2001), demonstrated that the TRAMP model enabled to examine contributions of AR mutations, and the regulation of the androgen signalling axis, in the development of castrate resistance prostate cancer [8]. Other studies have extensively investigated prostate cancer progression in the TRAMP model and summarized that TRAMP model is suitable for the analysis of histopathobiology and molecular events of progression of prostate cancer [4-10].

In comparison of human and mouse prostate glands, some similarities as well as the differences can be observed. The similarities can support the utilities of mouse models to reveal the underlying molecular aspects that occurred in the development and progression of prostate cancer. Nevertheless the differences between these two species have influence into particular aspects on the analysis and the application of mouse models to certain clinicopathological issue in human prostate cancer [4,5].

Normal Human Prostate

Normal human prostate is a single glandular organ divided into zones that are not clearly demarcated (Figure 1A). A normal human prostate gland is assembled of the periurethral transition zone (TZ), the peripheral zone (PZ), and the central zone (CZ). The PZ is the major site for the incidence of prostatic intraepithelial neoplasia (PIN) and prostate cancer [2,11]. Normal prostate gland has a lobular formation and is surrounded by abundance stroma consists of contracting spindle cells and collagen (Figure 2A). The stroma expands further than glands' outer boundary. In comparison with rodent, the stroma in human is a much more plentiful. Benign prostate glands/acini are comprised of two cell layers; a basal cell layer and a secretory cell layer. The basal cells are the progenitor cells for the secretory cells; they are most probably the true "stem cell". These

cells have not always been clearly seen by light microscopy, but can be identified by immunostaining for high molecular weight cytokeratin (HMWCK). Between the two layers there are a minor population of cells with neuroendocrine (NE) differentiation, ultrastructurally characterized by dense core secretory granules. NE cells can be identified by immunostaining for NE markers, such as chromogranin and Serotonin [11,12].

Normal Mouse Prostate

The mouse prostate is divided into discreting lobes, the anterior prostate (AP) or coagulating gland, the ventral prostate (VP), dorsal (DP) and lateral (LP) prostate, which the DP and LP are grouped together as the dorso-lateral prostate (DLP) (Figure 1B).

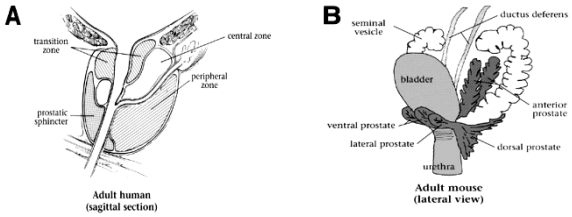

Figure 1. Comparison of human and mouse prostate anatomy. A, adult human prostate is divided into zones consisted of the periurethral transition zones (TZ), the peripheral zone (PZ), and the central zone (CZ). B, mouse prostate is categorized into antero-posterior prostate (AP), ventral (VP), dorsal and lateral lobes, which are grouped together as DLP [19].

The individual glands in each lobe are surrounded by very thin stroma, consisted of a few layers of spindle cells among collagen fibers (Figure 2F, G, H, I) [15].

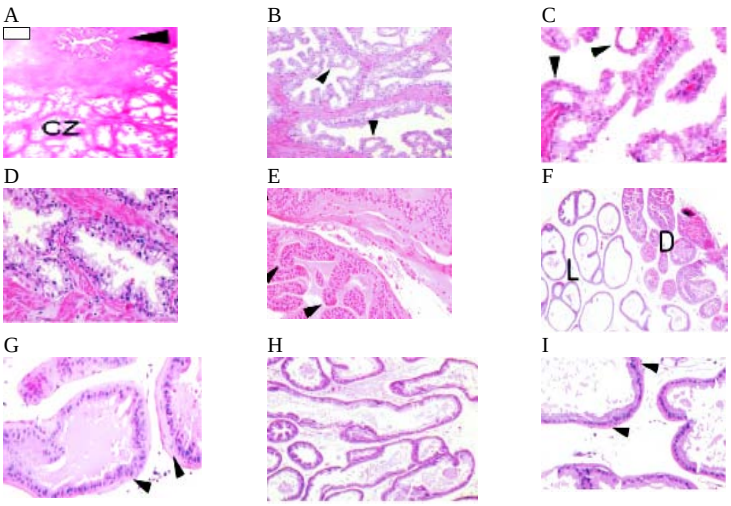

Figure 2. Comparison of human and mouse prostate histology. A-C, low, intermediate, and high magnification of normal CZ human glands. D, high magnification of human normal benign PZ glands that have a tufted or undulated luminal border and the sizes are larger than usual glands in prostate cancer. E-I, mouse prostate glands, with very thin stroma surrounds each gland in each lobe [15].

The glands of each lobe have cell populations that are homologous to the human prostate, involving luminal secretory cells, a basal cell layer, and NE cells [12]. Similar to normal human prostate glands, basal cell layer in mouse prostate is not observable by routine light microscopy. Moreover, the results of ultrastructural studies supported this data as they found the lack of a continuous basal cell layer in normal mouse prostate glands. These cells cannot be identified by antibodies to HMWCK (66kDa and 57KDa), but they can be identified by immunostaining of a rabbit polyclonal antibody to mouse cytokeratin 5 (CK5) and antibodies to CK14 [13,14]. In addition, nerve bundles, which are laid within the prostate stroma in the postero-lateral of the human gland are not identified within mouse stroma. However, in DLP sections thick nerve bundles and ganglia are often seen in the peri-stromal loose connective tissue. Neuroendocrine (NE) cells which are only few (< 1 %) can be observed by immunostaining with chromogranin and synaptophysin [6].

Prostate Pathology

Several disorders have been reported in genetic engineered mouse (GEM) models from development disorders until malignancy, however, in the present study the disorders are restricted to hyperplasia, PIN, well differentiated (WD), Moderate differentiated (MD) and poorly differentiated (PD) adenocarcinoma, and NE tumours [15].

Non-malignancy

Non-neoplastic proliferation in prostate such as hyperplasia also occurs in TRAMP model as well as in human, which is recognized as benign prostatic hyperplasia (BPH) (Figure 3A, B). Shappell et al. (2004) defined hyperplasia as “a non-neoplastic

increase in epithelial (glandular) tissue compared with age-matched wild-type control mice" or "increase epithelial cells within normal appearing gland spaces". The same authors also identified that in GEM, proliferation patterns such as tufting, micropapillary and cribriform are usually found. Hyperplasia in GEM should be defined as focal, in which only a few glands are involved, and diffuse, in which more glands involved. Slight atypia can be present in hyperplasia (Figure 3 C, D) [15].

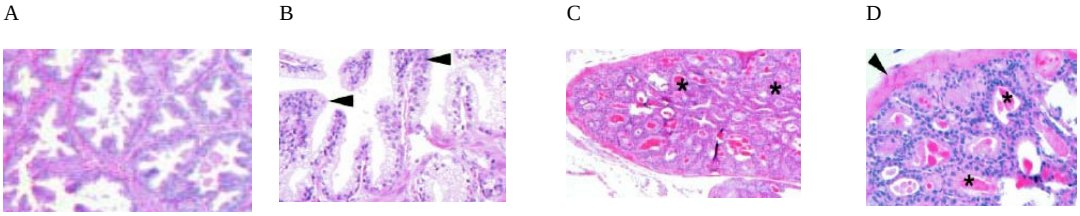

Figure 3. Comparison of human and mouse hyperplasia. A and B, human benign prostate hyperplasia, showing the increase of gland numbers. C and D, low and high power magnification showing hyperplasia in mouse prostate glands with epithelial stratification due to proliferation with cribriform pattern (*) and stroma surround the hyperplastic glands (arrowhead) [15].

Several studies observed, premalignant lesions, PIN in TRAMP model which is called mouse PIN (mPIN) described as "proliferation of atypical epithelial cells within pre-existing glands" are characterized by nuclear stratification, epithelial tufting, micropapillary projections, cribriform structures, elongated nuclei, increased mitoses and apoptosis and the nuclei showed nuclear atypia. This spontaneous lesion can be observed in all lobes by 8 weeks of age. In non-transgenic mice, PIN is observed in 40-60% mice and was observed mostly in VP. In human, PIN is classified into low and high grade (LGPIN and HGPIN). HGPIN is associated with invasive prostate cancer with the glands in this stage shows nuclear stratification, enlargement and distinctly atypical epithelial cells with nuclear enlargement and prominently enlarged nucleoli (macronucleoli) as the human HGPIN characteristic feature (Figure 4A, B). The same authors, also classified mPIN into with documented and without documented progression to invasive carcinoma as follow:

Classification of Prostate Disorders of Genetic Engineered Mouse (GEM) models

Non-neoplastic Disorders

1. Hyperplasia

Neoplastic Disorders

1. Prostatic intraepithelial neoplasia/neoplastic proliferation of premalignant potential
   1.1. With documented progression to invasive carcinoma
   1.2. Without documented progression to invasive carcinoma
2. Carcinoma (invasive)
   2.1. Microinvasive carcinoma
   2.2. Invasive carcinoma
   2.2.1. Adenocarcinoma
      2.2.1.1. Well differentiated
      2.2.1.2. Moderately differentiated
      2.2.1.3. Poorly differentiated
   2.2.2. Neuroendocrine carcinoma
      2.2.2.1. Small cell carcinoma
   2.3.1. Squamous Cell carcinoma
   2.3.2. Spindle cell/Sarcomatoid carcinoma
   2.3.3. Undifferentiated carcinoma
   2.3.4. Mixed carcinoma (specify component: Adenosquamous carcinoma)

Furthermore, mPIN with documented progression is naturally comparable to human HGPIN, while PIN without documented is tentatively categorized at the beginning of identifying an mPIN lesion in a new tissue, hence, the classification should be converted if the invasion is identified afterwards. mPIN should be recognized either focality or/and progression, in which, the lesion should begin focally, rather than homogenous throughout the prostate and shows progression either increased of glands involvement or increased nuclear atypia or both (Figure 4 C, D) [10,15].

Malignancy and Neuroendocrine Differentiation

In human, adenocarcinoma is the majority type of prostate malignancies and histologically it is classified into WD, MD and PD based on gland appearances. WD adenocarcinoma shows well defined glands either small or medium sized lined with a single layer of uniform cuboidal or low columnar epithelium with clear cytoplasm, and

nuclei basically located (Figure 4E). In PD adenocarcinoma, gland formation is difficult to observe, because the merge of glands creating more solid appearance, consequently cells grow in cords, nest or sheets patterns (Figure 4G). MD is the mixture of WD and PD appearances, where gland formation can be found side by side with solid sheets (Figure 4F) [16]. In TRAMP model the stages of cancer can be identified from premalignant lesions, PIN, WD, MD, and PD adenocarcinoma, and NE carcinoma can be observed [15].

Well differenciated PCa is characterized by increased quantity of small glands (Figure 4A) [15]. There is often an associated desmoplastic response or stromal thickening. The cells have round nuclei with fewer hyperchromatic nuclei than in PIN lesions. Increased mitoses and apoptosis are apparent and may be associated with inflammation. MD is characterized by nearly anaplastic sheets of cells that may contain remaining of glandular architecture or the many glands fused and the others preserve individual outlines but are closely packed with surrounding glands (Figure 4B), however, this stage is not frequently observed [16]. PD is characterized by anaplastic sheets of cells containing pleomorphic (many and different shapes) cells with irregular nuclei, very little cytoplasm surrounding the nuclei. There are often normal glands trapped within sheets of cells. These lesions are often highly vascularized, hemorrhagic, and in the larger lesions can be accompanied by necrotic (Figure 4C) [15].

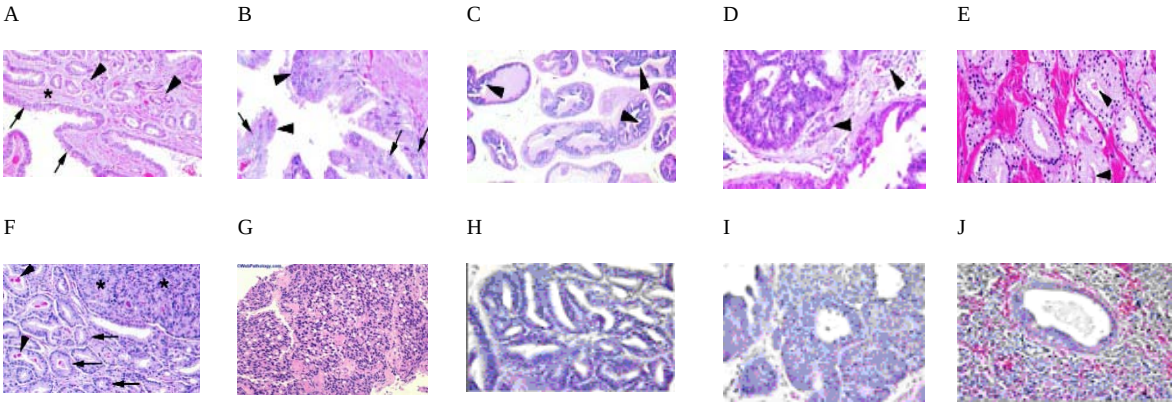

Figure 4. Comparison of prostatic intraepithelial neoplasia (PIN) and adenocarcinoma in human and TRAMP model [15,16,20].

Prostate cancer of human often demonstrates focal NE differentiation cells as, but these cells are not neoplastic and the neoplastic type is found only 5% of all prostatic neoplasms [10]. This type of prostate cancer can also be found in several transgenic mouse models including TRAMP as observed by some studies [4,5]. They identified the transformation into an NE phenotype in PD tumours in untreated and castrated mice, which indicates that NE differentiation is associated with advanced prostate cancer.

The description of this tumour as a NE carcinoma is based on its histological and cytological characteristics that form solid and cribriform pattern with rosette-like formation (Figure 5C) [15,17]. Some authors proposed the spectrum of NE differentiation in prostate cancer from NE with large eosinophilic granules to carcinoid-like pattern to small cell carcinoma [16,18]. The focal NE differentiation can be observed with the spread of individual cells or cell nests in conventional prostatic adenocarcinoma. NE appearance can also be observed as the entirely spread of small tumour cells indicated by nuclear hyperchromasia, nuclear molding, small punctate nucleoli, and vigorous mitotic activity, and this is called the aggressive small cell carcinoma or carcinoid/carcinoid-like tumour (Figure 5A, B). These features should be verified by immunostaining (Figure 5B) for the markers such as chromogranin and synaptophysin [6].

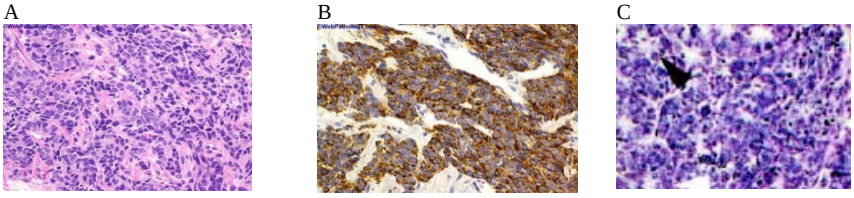

Figure 5. Comparison of NE carcinoma in human and mouse. A, small cell carcinoma of prostate cancer entirely consists of NE cell type. B, Prostate small cell carcinoma shows strong positivity with Chromogranin A immunocytochemistry. C, H&E staining prostate carcinoma with NE differentiation in TRAMP model showing solid sheet area with rosette formation (arrowhead) [15,16].

CONCLUSION

The TRAMP mouse model provides a consistent resource of primary and metastatic tumours representative of those seen in clinical prostate cancer for histopathobiological and molecular analyses to elucidate and further characterise the molecular events involved in the development, progression and metastasis of prostate cancer. The use of TRAMP tissues at different stages of disease progression and treatment response will facilitate molecular and cellular analyses.

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Author:

Krisna Murti, MD., M.Biotech., SpPA : Anatomical Pathology Department, Medical Faculty, University of Sriwijaya, Indonesia.
Current Address: Department of Molecular Pathology, Institute of Pathology, University of Wuerzburg, Germany

Synopsis

In the TRAMP model, a full spectrum prostate cancer development and growths can be observed from premalignant stage, high grade prostatic intraepithelial neoplasia then progressed into highly dedifferentiated tumours that primarily metastasised to lymph nodes and lungs have similar pattern to that seen in humans