Non-human prostate cancer cell lines

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INTRODUCTION

Prostate cancer (PCa) is a multistep disease. It progresses from prostatic intra-epithelial neoplasia (PIN) to locally invasive adenocarcinoma, which further leads to hormone-refractory metastatic carcinoma.[1–4] Therefore, an understanding of the molecular changes that initiate and/or promote prostate cancer progression is essential to design any therapeutic strategy for this disease. Though different animal models have contributed significantly in this regard, none have been able to mimic all the steps of prostate cancer progression. Animal models have other limitations including ethical concerns and high management costs. Therefore, to understand prostate cancer pathogenesis in an effective manner, other avenues must be adopted. One such avenue is the use of cell culture models to study the process of cancer development and progression in an in vitro environment. Furthermore, cell line models also have importance in developing novel therapeutic agents. Importantly, cell culture provides a system that can be easily manipulated experimentally, and enables evaluation of response to a wide range of stimuli on a single cell.

 Despite the high incidence of prostate cancer in men, there are only a few cell lines of human prostate carcinoma available. The cell lines LNCaP, PC-3 and DU-145 cells, and some clonal derivatives of these cell lines are still the most commonly used cells for the majority of...
published research. Information obtained from these cell lines and their sub-lines, while valuable, has limitations and has led to a continued gap in our information regarding molecular basis of prostate cancer pathogenesis. In prostate cancer research, the development of many new animal cell line models has been of great use to answer questions unique to PCa. These cell lines are either established from primary tumor tissues or clonal derivatives of already established cell lines. Aside from humans, dogs and a few strains of rats develop spontaneous prostate cancer. Use of carcinogens also induces prostate cancer in rats. Moreover, some genetically engineered mouse models also develop spontaneous prostate cancer. In recent years, there has been a growing effort towards isolating and establishing animal cell lines from these models. Indeed, many of these animal prostate cancer cell line models have been successfully used to mimic different events of prostate cancer progression and metastasis.

Immense efforts have been made to catalogue the entire array of human PCa cell lines earlier. However, less attention has been paid in discussing the information about cell lines obtained from non-human species (mostly from rodents and canines). Therefore, we have made an effort to review all the available information regarding different animals PCa cells’ method of origin, characterization and use here.

Molecular markers for rodent and canine prostate epithelial cells

Prostate cancer is a multistep process. Therefore, a thorough understanding of the parental lineage of different established cell lines is crucial before their use in different functional studies. In human beings, rodents and dogs, the normal prostate epithelium is characterized by the presence of three distinct types of cells: luminal, basal and neuroendocrine (Fig. 1). The luminal cells are often considered terminally differentiated; and secretory and basal cells are thought to be poorly differentiated. Identification of different markers has been successfully used to distinguish different prostate cell lines as per their lineage of origin. Prostate epithelial cells of different lineages express specific intermediate filament (IF) proteins, cytokeratins (CKs). The luminal cells express low molecular weight cytokeratins CK8 and CK18 as the typical marker. They also produce different secretory proteins such as prostate specific antigen (PSA) in men, and arginine esterase in dogs. Luminal cells also express androgen receptor. Basal cells of humans and mice prostate epithelium express high molecular weight cytokeratins CK5 and CK14. However, in dogs CK5 expression is abundant and CK14 expression is limited and scattered.

Different investigators have also identified other basal cell specific markers such as Ki67, p63, CD44, ETS-2, glutathione S-transferase P and Maspin. These cells rarely express androgen receptors (AR). In normal prostate, Neuroendocrine (NE) cells express both luminal and basal cytokeratins (CK 5, 14, 8 and 18), and the neuroendocrine marker, chromogranin A. However, in cancer, the NE cells express chromogranin A and only luminal cytokeratins. The NE cells do not express androgen receptor and PSA. In addition to the aforementioned three cell types, another intermediate or transition cell type is found in prostate epithelium, which expresses both the basal and luminal CK cell markers, but does not express AR and PSA. Experimental evidence has suggested that prostate stem cell antigen (PSCA) and CD44 are two potential markers for intermediate prostate epithelial cells.

Canine prostate cancer cell lines

Dogs are the only large mammals other than humans, which commonly suffer from spontaneous prostate cancer. Prostate cancer in dogs has many important similarities to prostate cancer in humans. Therefore, canine prostate cancer is a relevant model to understand prostate cancer pathogenesis. Cell lines derived from spontaneous canine prostate cancer have been successfully used for in vitro and in vivo studies. Prostate cancer in dogs is highly invasive and metastasizes to distant organs. Particularly, the ability of many canine prostate cancer cells to metastasize to the bone has created a lot of interest leading to different researches in this field. However, unlike human prostate cancer cells, the canine prostate cancer cells are androgen independent.

Figure 1. A generalized diagram showing specific markers for different type of prostate cells.
from the very early stages of the cancer. Therefore, most of the established canine prostate cancer cell lines are believed to recapitulate only the late stage (androgen independent) of human prostate cancer progression.

**Leo**

Thomas J Rosol’s group from the Ohio State University has established and characterized two important canine prostate cancer cell lines, Ace-1 and Leo. The Leo cell line was generated from a primary spontaneous tumor obtained from a five-year-old castrated male mixed breed dog. The cells were tested positive for CK8 and CK18, thus indicating their secretory epithelial origin. These cells also express ductal cell marker CK7, which suggests that they have differentiated towards both secretory and ductal types. Leo cells also express arginine esterase.

Genomic analysis of Leo cells showed chromosomal imbalances such as hyperdiploidy and bi-armed chromosomes. In standard cell culture, these cells grow in an anchorage-dependent manner and form a monolayer sheet with a tightly packed cobblestone growth pattern. The cells have a doubling time of approximately 38 hours. Subcutaneous injection of ten million Leo cells into nude mice generated tumors of an average size of 0.8 cm in 6 weeks. Tumors were multilobular and the microscopic appearance of subcutaneous xenograft cells was similar to the primary tumor. Furthermore, the authors have also checked the metastatic ability of these cells by left ventricular intracardiac injections. Intra cardiac injection of Leo cells tagged with YFP-Luc resulted in metastasis to different organs. Out of 25 male nude mice, 23 mice were detected with metastasis. After 28 days of injection, histopathological analysis of the different organs obtained from these mice showed the presence of metastasis in brain, spinal cord, adrenal gland, long bones and scapula, and the incidence of brain metastasis was very common (76% mice). The bone metastasis that was detected in athymic (mice deficient in T-cell immunity) mice was predominantly osteolytic type. This cell line is particularly valuable because it repeatedly develops brain metastasis in vivo; therefore, it will have significant contribution in brain metastasis study.

**Ace-1**

Ace-1 cell line was established from Ace-1 xenografts in nude mice. Originally, the authors obtained prostate carcinoma tissue from an 8-year-old male castrated Labrador Retriever, and processed and injected them subcutaneously into nude mice. The Ace-1 xenograft was then serially passed through implantation into new nude mice. The cell line was developed from 3–5 passages of the Ace-1 xenografts. For this, tumors were minced, collagenase-digested, and a single-cell suspension was prepared by using a 70 μM filter. To suppress stromal cell growth, the cells were grown in either DMEM/F12 media or Keratinocyte serum-free media. The Ace-1 cells were noted to express CK 8/14, but not CK 5/14. These cells also express the mesenchymal marker vimentin. The authors felt that the expression of vimentin in these cells might be due to epithelial to mesenchymal transition (EMT). These cells are negative for AR. Ace-1 cells are locally invasive and metastasize to the bone in nude mice. The bone lesions were of a mixed osteoblastic and osteolytic reaction similar to bone metastasis in human patients with prostate cancer. Therefore, this cell line is a potential tool for studies related to bone metastasis.

**DPC-1**

This cell line was established from a spontaneous canine prostate adenocarcinoma. The primary prostate cancer tissue was obtained from an 11-year-old male Doberman Pinscher. After mechanical dissociation and mincing, tumor fragments were plated in supplemented RPMI-1640 medium. In culture, the cells have rapid growth kinetics with an average doubling time of 27 hr; however they failed to produce colonies in soft agar suggesting their inability to grow in an anchorage independent manner. The cells have epithelial morphology in culture, and expressed epithelial-specific CK19. An inconsistent expression of CK14 and vimentin has also been reported. No androgen receptor expression was detected. The karyotype of DPC-1 cells was hypodiploid with a chromosome number ranging from 67 to 70 (78 is normal). DPC-1 cells are tumorigenic in both athymic mice and immunosuppressed dogs. Subcutaneous injection of these cells in nude mice showed a 100% tumor uptake, and no gross metastasis was detected by the authors. Histologically, the tumor cells were highly undifferentiated prostate carcinoma. Orthotopic allograft in immunosuppressed dog produced a poorly differentiated primary tumor. In this system, metastasis to adjacent lymph nodes and lung was detected.

**CPA-1**

This cell line was established from a spontaneous canine prostate tumor acquired from a castrated 10-year-old Doberman dog. Small fragments of the original tumor tissues were washed with PBS and trypsinised for 45 min. The mixture of tissue fragments and single cells was filtered and the filtrate was centrifuged. Further, the cell pellets were resuspended in DMEM medium supplemented with 5% FBS. Initially, the tumor cells obtained from the primary tumor were grown in semisolid agar. Individual
colonies grown in the agar were aspirated and grown in separate wells of a 24-well culture plate. Out of all the isolated clones, the rapidly amplifying clones were further sub-cultured. CPA-1 cell line was generated by this method. In culture, CPA-1 cells have an epithelial growth pattern. At low-density, these cells form a monolayer; however, high density culture forms multi layering of cells. In xenografts obtained from CPA-1, cells have a similar morphology that is found in primary tumor implants. Androgen and estrogen receptors were undetectable in the homogenates of the primary tumor tissue and cell cultures. Moreover, the growth of these cells was insensitive to androgen or estrogen in vitro, and was similar in both sexes of mice in vivo. These cells are tumorigenic in athymic mice, and tumors were of well differentiated type.

CT-1258

This cell line was established from a spontaneous prostate tumor obtained from a 10-year-old male intact Briard dog. The tumor was removed by surgical method, minced, collagenase-treated, and dissociated cells were cultured in medium 199. The cells are tumorigenic in NOD/Scid mice. The authors have reported 89% of tumor incidence when these cells were injected subcutaneously into male and female nude mice. Histopathological analysis showed no metastasis in any of these animals. Histologically, the subcutaneous tumors showed a highly heterogeneous population of cells with different genetic abnormalities. Immunohistochemistry showed a strong Ki67 staining (Ki 67 index 41%) indicating a high proliferative nature of these tumor cells. CT-1258 cells also have the ability to develop tumors when injected intraperitoneally, and in this case 86% of the animals developed local tumor growth at the injection site. In this system the authors did not observe any metastasis. Importantly, the experimental tumors induced in mice (both subcutaneous and intraperitoneally) have a similar growth pattern and histological appearance as the original tumor from which the cell line was established. Cytogenetic analysis of the original tumor, the cell line and experimentally induced tumors showed the presence of hyperdiploid karyotypes. Furthermore, centromeric fusion between chromosomes 1 and 5 and chromosomes 4 and 5 were also detected. There was also presence of a large biarmed marker (mat). These findings support the thought that human and dog prostate cancer have the same molecular events in the generation and/or progression of cancer.

Mouse prostate cancer cell lines

Unlike dog and a few strains of rat, normal mice do not develop spontaneous prostate cancer. However, advancements in science have made it possible to generate genetically engineered mouse models (GEMs) that generate prostate cancer. Due to the spontaneous (without carcinogen) and autochthonous development of prostate cancer in an intact immunity set up, these models have been instrumental in understanding the genetic basis of prostate cancer initiation and progression. Furthermore, different cell lines established from these models have been helpful in different prostate cancer studies. Use of many of the cell lines in xenograft model has also generated potential tools in the prostate cancer therapeutic studies.

TRAMP-C1/C2/C3

These cell lines were established from a 32 week old heterogeneous prostate tumor obtained from a transgenic adenocarcinoma mouse prostate (TRAMP) model. TRAMP model is a transgenic mouse on a C57BL/6 background, which carries a construct that expresses SV40 large T antigen in prostate epithelium. After differential trypsinization to get rid of stromal cell contamination, the cells were maintained in supplemented DMEM-HG media. These cell lines were positive for cytokeratin, E-cadherin and androgen receptor. Immunohistochemical analysis also suggested the absence of mutated p53 in these cells. As reported by Watson AP et al., TRAMP-C1 cells particularly express CK14, CK8, Psca, and Sca-1 markers. Out of these markers, Sca-1 is considered to be a marker for cells with stem cell properties. Therefore, TRAMP cells might have originated from intermediate or stem cell like cells. In soft agar assay, TRAMP-C1 cell had very few colonies after 10 days of growth, while TRAMP-C2 did not form any colonies. Ironically, the three cells lines do not express SV40 T antigen.

The TRAMP-C1 and TRAMP-C2 cells are tumorigenic in syngeneic C57BL/6 mice, and tumor uptake was 100% in both cells. However, TRAMP-C3 which grew very well in vitro, failed to form any tumor in syngeneic C57BL/6 mice. Histological analysis of TRAMP-C1 and TRAMP-C2 xenograft tumors revealed poorly differentiated and disorganized sheets of cells. By using serial prostatic implantation of TRAMP-C1 cells, Somers KD et al. have established a metastatic tumor cell line; TRAMP-C1P3. Orthotopic implantation of TRAMP-C1P3 cells in C57BL/6 mice leads to predominant lymph node metastasis.

Prostate neuroendocrine cancer (PNEC) cell lines

Prostate neuroendocrine cancer (PNEC) cell lines were generated from 20 to 24-week-old FVB/N transgenic...
mice (CR2-Tag) primary tumors and metastases. In these mice, simian virus 40 large T antigen (Tag) is expressed in a subset of prostate NE cells. When cells obtained from different organs were cultured in supplemented neural progenitor medium (NPPM), all of them grew with a similar morphology. PNEC30, PNEC25 and PNEC28 cells were derived from primary tumor, liver metastasis and lymph node metastasis, respectively. All these cells grow in suspension as aggregates on a non coated surface; however, they attach to poly (L-lysine) and human laminin-coated surfaces and grow as monolayer. The average doubling time of these cells is 50 h, and they produce neurite-like process in culture. A subline named PNEC30-3 grows as a monolayer on polystyrene surfaces. In suspension, the aggregation formation of these cells resembles the neurospheres of cultured neural stem cells. Subcutaneous injection of PNEC30 cells in BALBc mice produced tumor in all the recipients and the incidence was independent of sex. Furthermore, on tail vein injection, liver tumors were identified in the injected mice. GeneChiP analysis indicated that PNEC cells express steady features ex vivo and in vivo. Cell lines harvested at different passages (30 or 34), and xenografts share a high degree of similarity with the primary CR2-Tag prostate tumors. PNECs cells express mAsh1, a basic helix-loop helix (bHLH) transcription factor in a cell cycle dependent manner. mAsh1 transcription factor is known to be essential for NE cell differentiation. Based on their phenotypes, PNEC cells are a valuable tool to address questions associated with NE cells proliferation, differentiation and tumorigenesis.

**E and cE series (E2, E4, cE1, and cE2)**

All these four cell lines were established from tumor tissues obtained from different conditionally *Pten* deleted mice (cPten-/-L). Out of several clonal derived cells, PTEN-P2 and PTEN-P8 cell lines were two independent spontaneously immortalized lines. Genotyping of PTEN-P2 and PTEN-P8 cells revealed that both the cells are genetically heterozygous for Pten deletion. Therefore, to generate isogenic lines with homozygous deletion of Pten allele, the authors infected PTEN-P2 and PTEN-P8 cells with Cre-retrovirus. The PTEN-CaP2 and PTEN-CaP8 isogenic lines were generated after 2 weeks of puromycin drug selection. PCR-based genotyping and westernblot analysis confirmed the homozygous deletion of Pten in these cell lines, and this was further corroborated by the detection of increased P-AKT level in these cells. Pten deletion also increased the level of AR receptors, supporting a previous finding by these authors that PTEN negatively regulates AR expression. Q-RT-PCR analysis showed that these cells express both basal (CK14) and luminal (CK8/CK18) cytokeratins. However, they do not express the neuroendocrine markers. Further detection of PSCA expression (an intermediate cell marker) in these cells suggested that these cells have probably originated from a transiently amplified or intermediate cell type. The cytogenetic analysis of all the cell lines showed several common chromosomal abnormalities indicating their common origin. Importantly, PTEN-P8 and PTEN-CaP8 showed the loss of Y chromosome, a frequently found cytogenetic change that is observed in human sporadic prostate tumors.
Additionally, in the soft agar assay (anchorage independent growth) PTEN-CaP2 and PTEN-CaP8 cells formed more and bigger colonies compared to PTEN-P2 and PTEN-P8 cells. Furthermore, compare to isogenic heterozygous pairs, Pten null cells have a decreased level of p27 and E-cadherin expression. After subcutaneous injection, Pten null cells were able to generate visible tumors; however, in a similar condition the heterozygous cells did not form any tumor. Histological analysis showed maintenance of AR and CK8 expression in all the xenografts, and no mesenchymal marker (SMA) and no neuroendocrine makers were detected in any of the xenografts.

**Myc-CaP**

This cell line was established from a 16-month-old Hi-Myc transgenic mouse. The cells were maintained in a growth factor enriched/supplemented DMEM media. Myc-CaP cells express c-Myc as equivalent to the level seen in the original prostate carcinoma tissue. These cells also overexpress AR, which might be due to genomic amplification of AR as detected by FISH analysis. However, the authors were unable to detect any mutation in AR after sequencing of all the exons. Myc-CaP cells express CK14, CK18, Psca and Sca-1. This expression profile of different lineage markers suggests that these cells might originate from an intermediate or stem cell type. Additionally, soft agar assay in the presence or absence of androgen revealed that these cells are androgen dependent for their growth and/or survival.

Myc-CaP cells are tumorigenic when injected in the mammary pad of syngenic male mice. In this study, the authors have reported a 100% tumor uptake. Histologically, the xenografts uniformly composed of undifferentiated cells. To check the effect of androgen on the tumorigenicity of these cells, the authors castrated the tumor bearing mice and measured the tumor volume at multiple time points. Interestingly, though Myc-CaP xenografts regressed initially, later on they grew in an androgen independent manner. As this cell line has been derived from a primary prostate tumor that has never been exposed to hormone deprivation, it is believed to be a prospective reagent for understanding the development of hormone refractory prostate cancer.

**177-2 and 178-2 series cell lines**

These cell lines were established from primary and metastatic tumors of mice that carry prostate tumor through a mouse prostate reconstitution (MPR) model. Firstly, urogenital sinus epithelial cells (UGE) of different p53 genotype were grafted under the renal capsule of adult male syngeneic 129 mice (p53 +/ +). These mice were then provided with a special diet without or with synthetic retinoids N-(4-hydroxyphenyl) retinamide (4-HPR). After several days, when the animals were with obvious distress, the primary and metastatic tumors were harvested and evaluated. 178-2PA cell lines were obtained from the primary tumors of the animals on control diet, and 178-2 BMA, BMB and BMG cell lines were obtained from the bone metastatic tissue of the animal on control diet. Likewise, 177-2 PB/PE cell line was derived from primary tumor of animals with 4-HPR diet and 177-2 BMA cell line was derived from bone metastasis. 178-2 BMA cells also have the capacity to form primary and metastatic (lung) tumors in syngenic orthotopic mouse model. The orthotopic model of this cells line has been extensively used for many prostate cancer therapeutic studies.

**Rat prostate cancer cell lines**

Like humans and dogs, some strains of rats also develop spontaneous prostate cancer. Therefore, spontaneous prostate cancer in rats has created an opportunity to isolate and establish different cell lines that represent different stages of prostate cancer progression. Moreover, carcinogen-induced prostate cancer model is a well established model and has been used to address many prostate cancer associated questions. Investigators have also shown interest to generate different cell lines from these carcinogen-induced prostate cancer tumors.

**Dunning cell lines (G, AT1-3, MAT-LyLu, and MAT-Lu)**

This series of cell lines were derived from Dunning rat prostate cancer sublines (in vivo tumors), which have different biological characteristics. The original tumor from which all the in vivo sublines were established was R3327 tumor (discovered by W.F. Dunning) in a 22-month-old inbred Copenhagen male rat. Further by serial in vivo passages of the R3327 tumor, various tumor sublines (G, AT1-3, MAT-LyLu and MAT-Lu) were generated. And these tumor lines have difference in their histology, androgen sensitivity, growth rate and metastatic ability. To establish the in vitro cell lines, after collagen digestion, filtration and plating, the confluent primary culture cells were continuously passaged. During the culture of these cell lines, glucocorticoid hormone supplementation was observed to be critically required for the attachment of growing cells to the plastic surface. Particularly, AT3 and MAT-LyLu cell lines were...
more susceptible to detachment in absence of glucocorticoid in the culture medium.

Different analysis confirmed that all the Dunning in vitro cell lines have retained the major properties of their relevant parental tumor sublines. For example, G cell line has a slower growth rate and AT1-3, MAT-Ly-Lu and MAT-Lu have a faster growth rate in vitro. To characterize these cell lines in vivo, when these lines were injected subcutaneously back into castrated and intact Copenhagen male rats, all the aforementioned cell lines produced 100% tumor uptake. Like its parental tumor subline, G cells growth was androgen sensitive (grew better in intact than castrated rats) and was low metastatic. All other cell lines like their parental tumor line (AT1-3, MAT-Ly-Lu, and MAT-Lu) grew in an androgen insensitive manner (growth rate was same in both intact and castrated rat). AT-3, MAT-Lu and MAT-Ly-Lu cell lines were highly metastatic. AT3 and MAT-Ly-Lu cell lines metastasized to lymph node and lung. As well as MAT-Lu cell line metastasized to lung. However, AT-1 and AT-2 have very low metastatic ability.

**MDR lines (AT3B-1, AT3B-2, MLLB-1, and MLLB-2)**

MDR lines were developed by culturing the parental AT-3 and MAT-Ly-Lu (MLL) cell lines in increasing concentration of doxorubicin. These cell lines were maintained in 1 μM doxorubicin for more than one year. In contrast to parental cell lines, the MDR lines were more resistant to vinblastine treatment. Like their parental cell line (AT3), AT3B-1 and AT3B-2 cells developed tumor when subcutaneously injected in Copenhagen rats. Interestingly, xenografts produced by these two MDR lines were less responsive to doxorubicin treatment than AT3-xenografts in vivo. Overexpression of P-glycoprotein has been thought to contribute to the MDR phenotypes of these cells. These cell lines are very good model to study chemotherapy resistance in prostate cancer.

**NRP152 and NRP154 cell lines**

In the year 1994, Danielpour A et al. had generated these two cell lines from the dorso-lateral prostate of two Lobund/Wistar rats (rat number 152 and 154) treated with N-methyl-N-nitrosourea and testosterone propionate. To develop NRP152 cell line, dorso-lateral prostate from carcinogen and hormone treated animals was excised, minced, and the fragments were plated on tissue culture flasks. After cells attached to the plates, the cells were treated with trypsin-EDTA to remove fibroblasts from the cultured cells. After serial trypsinization and passages, one clone that remained attached to the culture plate and continued to grow was developed into NRP152 cell line. With some modifications, NRP-154 cell line was also generated in a similar fashion like NRP152 cell lines. After harvesting prostate tissue, prostate tissues were digested with collagenase, passed through a filter, and sequentially washed with WAJC 404 with and without FBS. Further, cells were cultured in a special serum free media (SFM) that inhibits stroma cells growth. After some days, the medium was replaced with serum containing media. Finally one clone that survived and continued to grow was established as NRP-154 cell line.

In culture, both the cell lines have different morphology. At a low density NRP-152 cell monolayers are cuboidal, but sporadically aligned in parallel curved bundles at a high density. Compare to NRP-152 cells, NRP-154 cells are larger and flatter. The NRP-152 cells have many properties similar to normal prostatic epithelial cells. They express functional androgen receptor and prostatic acid phosphatase. On the other hand, NRP-154 cells are malignant type; and the level of AR m-RNA is undetectable in these cells. For optimal growth, in addition to 10% FBS NRP-152 cells need additional growth factors and hormones; in contrast, only 10% FBS is sufficient for NRP-154 cells growth. Addition of TGF-β to the culture media inhibits growth of both the cell lines. However, retinoic acid inhibits growth of only NRP-152 cells, and has no overall effect on growth pattern of NRP-154 cells. Under optimal growth conditions, both the cell lines have a doubling time about 17 h. NRP-152 cells were unable to form tumors in athymic mice (in the presence and absence of matrigel). However, NRP-1524 cells are tumorigenic in athymic mice. Though k-ras mutation is a common finding in MNU-induced prostate cancer, neither of these cell lines has this mutation.

**CONCLUSION AND FUTURE DIRECTIONS**

Advancement in cell line models has been instrumental in understanding the pathogenesis of prostate cancer. However, certain issues associated with cell culture have always generated doubts on many cell line based data. Amongst them, misidentification and cross-contamination of different cell lines are the biggest hurdle in cell-culture based studies. Therefore, investigators working with different cell lines should take personal moral responsibility to make sure that no cell lines are infected with any microorganisms like mycoplasma, and the genetic identity of different cell lines are routinely
checked. During multiple passages, additional genetic changes may accumulate in different cancer cells, which might have an impact on the morphology and phenotypes of those cells. Therefore, detection of any kind of such alterations should be well documented. It is also important to check the characteristic features of tumors that are obtained from different cell lines, and should be compared to the original tumor phenotypes. During tumorigenesis study, more information about the host animals (species, strain, age, and sex) will be very instrumental in using these systems in a more clinically relevant manner.

Like human cancer cell lines, most of the aforementioned animal cancer cells are derived from already transformed malignant cells. Therefore, these cells are not suitable to understand the early genetic events that initiate or promote prostate cancer. Generation of more cell lines from different new GEMs that develop spontaneous tumor is essential to understand the mechanism of prostate cancer initiation and promotion. Cell lines with known genetic alterations will be also helpful in screening the efficacy of drugs with a particular genetic background. Later, information obtained from these models can be translated to personalized human cancer therapy. Due to the origin of spontaneous prostate cancer in dogs, and its biological similarity with human prostate cancer, canine prostate cancer in vivo and in vitro models have a significant importance in the prostate cancer research. Importantly, preliminary evaluation of the canine genome advocates a more resemblance between dog and human than human and rodents in term of nucleotide divergence and rearrangements. Moreover, comparative genomic studies have shown significant homology between different cancer-related genes like MET, mTOR, KIT and IGFR1R both in dog and humans. Therefore, generation and characterization of more canine prostate cancer cell lines will be helpful to the whole prostate cancer research community.

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