9

Importance of Stromal Stem Cells in Prostate Carcinogenesis Process

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1. Introduction

Prostate cancer is a significant health concern for men throughout the world, responsible for the highest rate of morbidity after lung cancer, and its etiology still remains unclear (Siegel et al, 2007). Death from prostate cancer occurs largely in patients with the aggressive androgen-insensitive metastatic disease. Conventional therapies for prostate cancer, especially in its androgen-insensitive form, may result in the survival of small population of resistant cancer stem cells with tumor-initiating potential that are believed to be responsible for cancer relapse. Prostate stem cells may represent a major target for mutations leading to cancer as their longevity assures continued presence during the long latency between exposure and cancer development (Pierce & Wallace, 1971; Reya et al, 2001). The existence of stem cells in the prostate is probably best illustrated by animal studies investigating the effect of androgen on the prostate. Castration leads to rapid involution of the prostate, but once androgen levels are restored; the gland completely regenerates due to, possibly, existence of a long-lived prostate stem cell population (Isaacs etal, 1987). It is generally believed that cancer relapse in patients may be due to this small population of cancer stem cells within the tumor mass which are resistant to conventional therapies.

To date, prostate cancer stem cell researchers are facing many obscurities: 1) the amount of knowledge about prostate stem cells is limited due in part, to the small amounts of primary tumor samples available for investigation; 2) complexity in distinguishing between normal and malignant prostate cells based on surface markers alone; 3) problems due to confirmational analysis of data resulted from cell line experiments with those obtained from primary tumor counterparts; 4) although some investigators are strong supporter of xenograft propagation of human tumors, but the mouse stromal environment is very different from the human prostate stromal niche; and 5) exploitation of the prostate orthotropic xenograft, are also difficult to establish, and there are high rates of mortality. However, the combinatorial use of primary samples, xenografts and cell lines will likely provide the tools for the most rigorous prostate cancer scientists who are studying the complexity of cross-talking between prostatic epithelial cells and stromal stem cells (Marian & Shay, 2009).

This chapter briefly describes what is currently known about this emerging field of prostate cancer- stromal stem cell biology, which is bringing new knowledge to a global disease and may hopefully reveal new ideas and targets to assist in early detection, prognosis, and monitoring of prostate cancer.

2. Anatomy of the prostate

The normal human prostate gland is an organ consisting of a glandular part and a stromal part which can also be divided further on the basis of zones and lobes. The outermost part is called peripheral zone (PZ) and it consists of 70% part of the normal prostate gland in an adult man. It is in the peripheral part that most of prostate cancers occur. The central zone (CZ) is nearly 25% of the normal prostate gland. The central zone surrounds the ejaculatory ducts and the prostate cancers in this region are more serious and in many cases they may even affect the seminal vesicles. The third zone or the transition zone accounts for 5% of prostate volume and this region is responsible for the prostate enlargement problems. The last zone known as anterior fibro-muscular zone or stroma doesn't contain any glandular parts but consists of a variety of cells including fibroblasts, nerves, infiltrating lymphocytes, macrophages, endothelial cells, and smooth muscle cells (Figure 1).

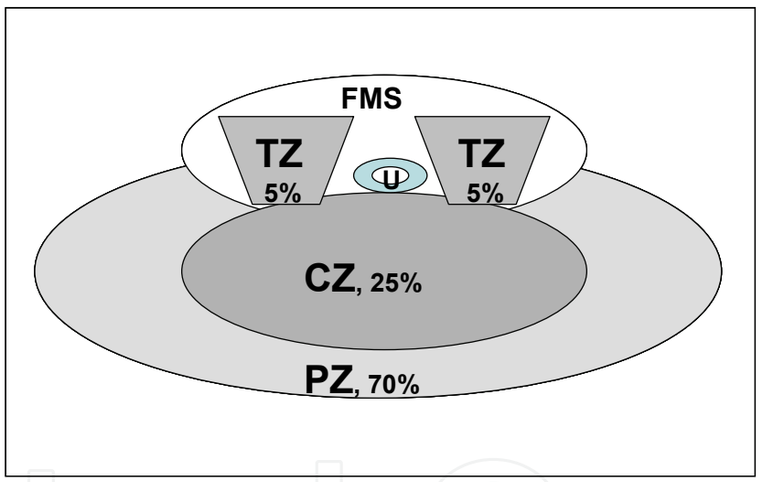


Fig. 1. **Prostate zones.** PZ, peripheral zone; CZ, central zone; TZ, transitional zone; U, urethra

3. Cellular characteristics of the prostate

Glandular part comprised of three anatomically distinct epithelial cell populations that can be distinguished by their morphological characteristics, functional significance, and relevance for carcinogenesis (Abate-Shen & Shen, 2000). **Prostatic proliferative basal cells** form a layer along the basement membrane of each prostatic duct, and **luminal secretory cells** form a layer above the basal cells. The basal cell express K5/14, CD44 (Liu etal, 1997), and BCL-2 markers (McDonnell et al, 1992). The luminal cells express prostate specific antigen (PSA), prostate acid phosphotase (PAP), androgen receptor (AR), and keratins K8/18 markers (Liu etal, 1997). More recently, an **intermediate phenotype** expressing a mixture of basal and luminal markers, with either co-expression of K5 and K18 in the

absence of K14 or of K5 together with PSA, have been described (Verhagen et al, 1992; Bankhoff et al, 1994; Xue et al, 1998). **Neuroendocrine cells** are minor population scattered throughout the basal layer and are identified by the expression of neuroendocrine markers such as synaptophysin and chromogranin A. The prostate also contains several types of **stromal cells** including fibroblasts, myofibroblasts, and smooth muscle cells (Figure 2).

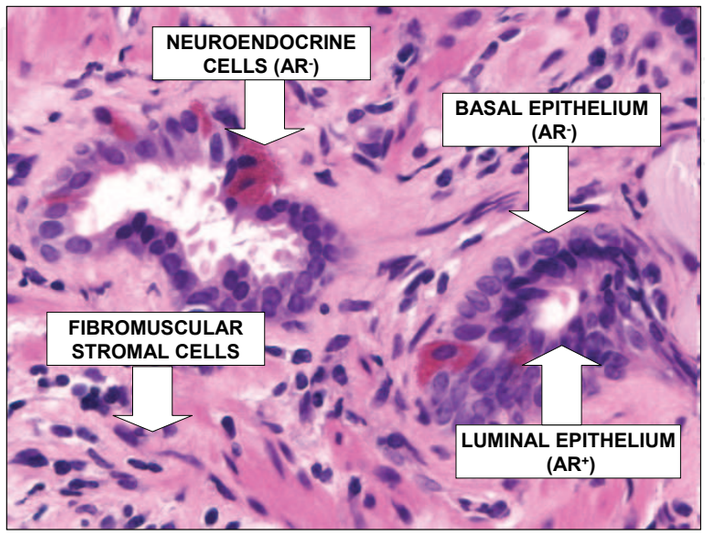


Fig. 2. Hematoxylin-Eosin staining of human prostate tissue, showing both glandular and stromal parts.

A classical androgen cycling experiments suggested that the prostate epithelium contain a stem cell population (English et al, 1987). When rodents are deprived of androgens, the prostate atrophies due to the apoptosis of terminally differentiated luminal cells that are dependent on androgen for growth and proliferation (English et al, 1987). When androgen is replaced, the prostate regenerates and resumes normal secretory function. It was shown that this experiment could be repeated for many sequential cycles and that a stem cell population must exist within the prostate (Isaacs, 1985). These findings have led to the traditionally held hypothesis that prostate stem cells (PSCs) reside within the basal layer of the gland (English et al, 1987). This was supported by findings that mice null for the basal cell marker *p63* were born without the prostate (Mills et al, 1999; Mills et al, 2002; Yang et al, 1999; Signoretti et al, 2000). It was also found that human basal cells express BCL-2, an anti-apoptotic protein that is commonly expressed by tissue stem cells (Verhagen,A.P., etal. 1992). Moreover, it was reported that BCL-2 lies downstream of parathyroid hormone-related peptide (PTHrP), an anti-apoptotic and osteoclastogenic growth factor, in a pathway that controls cellular proliferation and differentiation (Amling, M. et al. 1997).

4. Prostate epithelium differentiation model

The traditional model for prostate epithelial differentiation proposes that the epithelium is composed of multiple stem cell units (Isaacs & Coffey, 1989; Bonkhoff et al, 1994; Bankhoff & Remberger, 1996; Qiu et al, 1998; van Leenders et al, 2000; Hudson et al, 2000) where the prostate stem cells (PSCs) that has unlimited self-renewal capacity but only rarely proliferates residing in the basal cell layer. When PSCs proliferate, they provide progeny

that differentiate into transit-amplifying cells (TACs). The TACs subsequently differentiate into either the luminal secretory cells or basal cells which can be easily distinguished by light microscopy (Litvinov,I.V., et al, 2006; Bonkhoff et al, 1994; Bankhoff & Remberger, 1996) (Figure 3). Neuroendocrine cells are not distinguishable under the light microscope but can be identified by electron microscopy or immunohistochemical staining with antibodies against neuroendocrine markers. Number of neuroendocrine cells are higher in the transition zone and peripheral zone than in the central zone, suggesting that they may be involved in disease processes associated with these areas, such as nodular prostatic hyperplasia and prostate cancer (Santamaria et al, 2002). This model is supported by the existence of TACs that express both basal- and luminal cell-specific cytokeratins in both fetal and adult stages of prostate development as well as identification of intermediate cells in invitro cultures of primary prostate epithelium (Wang et al. 2001; Xue et al, 1998; van Leenders et al, 2000; Uzgare, A.R. et al. 2004; Garraway, L.A., et al, 2003; Tokar, E.J. et al, 2005). Several other studies have also suggested basal cells can differentiate into luminal cells in vitro (Robinson et al, 1998; Tran, et al, 2002; Liu, et al, 1997).

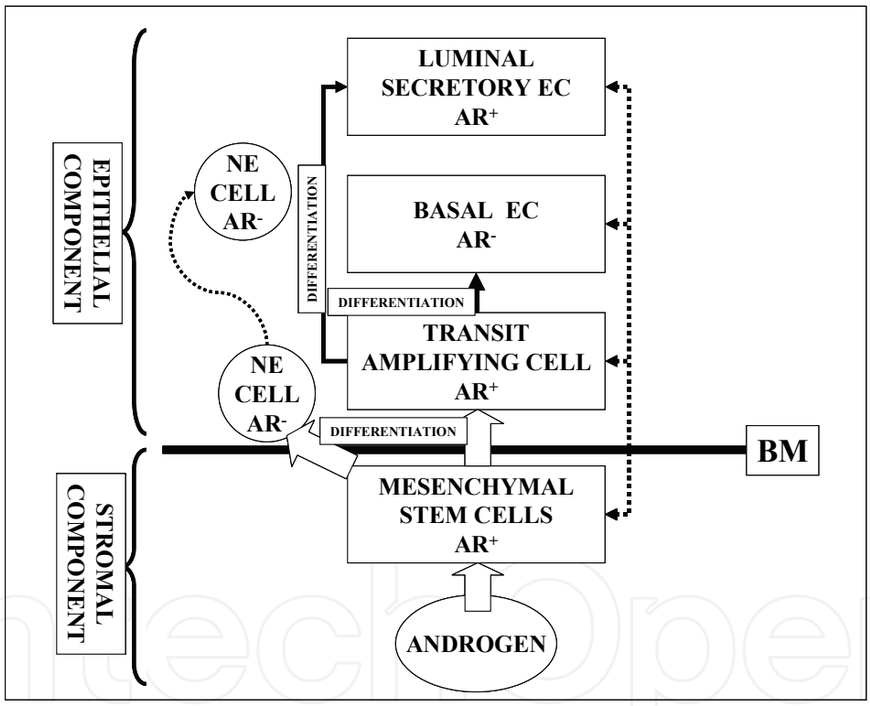


Fig. 3. **prostate developmental process.** Self-renewing prostate stem cells give rise to transit-amplifying cells of intermediate phenotype that may express both basal and luminal cell markers during their maturation. These cells theoretically possess transient self-renewal activity and produce large numbers of terminally differentiated secretory luminal cells. (*Adapted from Yin Sung et al, 2009*).

In human prostate adenocarcinoma, the majority of cancer cells express luminal cell-specific markers such as cytokeratin 8 (CK8), CK18, and prostate-specific antigen (PSA). Cells that solely express basal cell markers such as CK5, CK14, and p63 rarely observed (Okada, et al, 1992). This has led some investigators to suggest that prostate cancers are derived from luminal cell progenitor or mature luminal cell that has acquired self-renewal activity

through mutations (Lawson & Witte, 2007). However, some reports have indicated that prostate cancer may originate in an intermediate or transit-amplifying epithelial cell that precedes luminal cell differentiation (Verhagen, etal, 1992; Tran etal, 2002; Reiter etal, 1998). Identification of intermediate cells that co-express both basal and luminal cell markers (Verhagen, etal, 1992), as well as prostate stem cell antigen (PSCA), a presumed marker of normal late-intermediate prostate cells which is often up-regulated in prostate cancers (Tran etal, 2002; Reiter etal, 1998) have been also reported.

5. Prostate adenocarcinoma

Most prostate tumors are adenocarcinomas, sharing numerous common features with other prevalent epithelial cancers, such as breast and colon cancer. A distinguishing feature of prostate cancer is its intimate association with aging, and clinically detectable prostate cancer is not generally manifest until age of 60 or 70 (Abate-Shen & Shen, 2000). To identify specific gene expression patterns of prostate tumor epithelial and adjacent stromal cells, in a most recent study, researchers utilized Laser Capture Microdissection (LCM) analysis and identified nearly 500 genes whose expression was significantly different between epithelial and stromal cells (Gregg et al, 2010). One important finding was the differential expression of *WT1* in prostate cancer epithelial cells that suggests a potential role for *WT1* in prostate cancer. Several reports have shown that the androgen-insensitive prostate cancer cells increase the expression of IGF-1 and IGF-1R compared with the androgen-sensitive cancer cells (Krueckl et al, 2004; Nickerson et al, 2001). A recent study suggests that local secretion of IGF-1 in the prostate stroma mediates tumor-stromal cell interactions of prostate cancer to accelerate tumor growth (Kawada et al, 2006).

Although prostate cancers are phenotypically and behaviorly similar in many respect to luminal secretory cells, recent studies suggest that prostate cancer may arise from a more immature cell types located within the basal or luminal cell layer (Vehagen etal, 1992; Nagle etal, 1987; De Marzo etal, 1998; Bui etal, 1998). In addition, it is hypothesized that prostate cancer, like other epithelial and nonepithelial cancers, must arise from stem or progenitor cells rather than from a terminally differentiated cell type (De Marzo et al, 1998). In the prostate, p63, the p53 homologue, is expressed only in basal cells and most importantly p63 (-/-) mice do not develop the prostate (Signoretti, etal, 2000). This finding suggest that p63 is required for prostate development and support the hypothesis that basal cells represent and/or include prostate stem cells. Furthermore, the presence of surface integrins on prostate stem cells suggests that these cells share common pathways with stem cells in other tissues (Collins, etal, 2001). Basal cells also express the anti-apoptotic protein BCL-2 and BCL-2 expression may help cells resist apoptotic stimuli such as high TGFβ production resulting from androgen depletion (Kelly & Yin, 2008). Most recently, Howard Hughes Medical Institute (HHMI) scientists Owen N. Witte and his colleagues at the University of California, Los Angeles (UCLA) found that basal cells from primary benign human prostate tissue can initiate prostate cancer in immunodeficient mice (Goldstein etal, 2010). Moreover, it was shown that the cooperative effects of transcriptional factors and androgen receptor in basal cells results in loss of basal cells and expansion of luminal cells expressing prostate-specific antigen and alpha-methylacyl-CoA racemase. As a result, they concluded that histological characterization of cancers does not necessarily correlate with the cellular origins of the disease. It may be years before investigators will know whether the experimental model developed by Witte and his colleagues might have a similar impact on prostate cancer. However, scientists can at least

now begin to use the model to test suspected prostate cancer oncogenes systematically and in a more efficient manner with the goal of finding new targets for drug development. This chapter aims to outline recent concepts of stem cells role during the carcinogenesis process and bone metastses in prostate cancer.

6. Importance of androgens in prostate cancer initiation and progression

Prostate cancer development and growth is dependent on androgens and can be suppressed by androgen ablation monotherapy. However, due to the emergence of androgen-independent prostate tumor growth, prostate cancer recurs as androgen-insensitive and highly metastatic (Wang et al, 2007). There are two natural potent androgens in the mammal including humans. Although testosterone is the major androgen secreted from the testes, dihydrotestosterone (DHT) is the main androgen in the prostate to mediate the androgen action via the AR. Only one AR has been identifies so far, a member of the steroid/nuclear receptor superfamily, which is a ligand-dependent transcription factor. When androgens bind to the AR, this results in a conformational change within the AR, leading to the recruitment of co-regulators and transcription factors which mediate androgen-target gene expression. Although it is well known that androgens are important for prostate development and for the pathogenesis of prostate cancer, the precise mechanism as to how androgens control these processes are not yet fully understood. Furthermore, evidence for the direct modulation of androgen-AR actions by other hormones within the prostate cells emerging. For example, androgen actions in the prostate can be modulated by estrogens via estrogen receptors (ER). There are two known isoforms of the ER, ER_{α} and ER_{β} , which are both co-expressed with AR in the normal as well as tumors of the prostate (Zhu, 2005). Androgen-induced prostate epithelial cell proliferation is also regulated by an indirect pathway involving paracrine mediators produced by stromal cells, such as insulin-like growth factor (IGF), fibroblast growth factor (FGF), and epidermal growth factor (FGF) (Cunha & Donjacour, 1989; Byrne et al, 1996). In prostate epithelial cells, the androgenic signal engages secreted many cytokines which affects the prostate tumor microenvironment by inducing angiogenesis and stromal cell growth and differentiation (Zhu & Kyprianou, 2008).

Progressive prostate cancer is treated with androgen deprivation therapy, which causes an initial regression due to the androgen-sensitive nature of the vast majority of prostate cancer cells (Webster et al, 2005). However, a major problem in human prostate cancer is evolution of tumor cell populations toward androgen-insensitivity as well as resistance to apoptosis-inducing therapies and their tendency to metastasize. Prostate cancer preferentially metastasizes to the bone marrow stroma of the axial skeleton in up to 90% of patients and this is the principal cause of prostate cancer morbidity and mortality. This tendency arises from complexed molecular pathways that together lead to local invasion, extravassation and distal migration from the primary site followed by endothelial attachment, transmigration and site-specific metastasis. Androgen-induced prostate epithelial cell proliferation and differentiation is regulated by pathways involving paracrine mediators produced by stromal cells and this suggests that androgens are not sufficient to promote carcinogenesis. A key component of the search for new treatment strategies is an improved understanding of the differences between apoptosis-sensitive and apoptosis-resistant prostate cancer cells. Therefore, more effective therapies that can not only eradicate localized tumors but also prevent their metastasis are needed.

As is the case with normal prostate development, the growth of prostatic neoplasms is generally dependent on androgens, especially on 5a-dihydrotestosterone (DHT). Men castrated when young or men with inherited deficiency of 5a-reductase do not develop prostate cancer. Since the first observation (Hugginc & Hodges,1941), hormonal therapy remains the critical therapeutic option for advanced forms of prostate cancer. Multiple strategies have been used to reduce serum levels of androgens or interfere with their function via the androgen receptor (AR). However, the appropriate choice/timing and actual benefits of hormonal therapy in various situations still remain controversial (Figure 4).

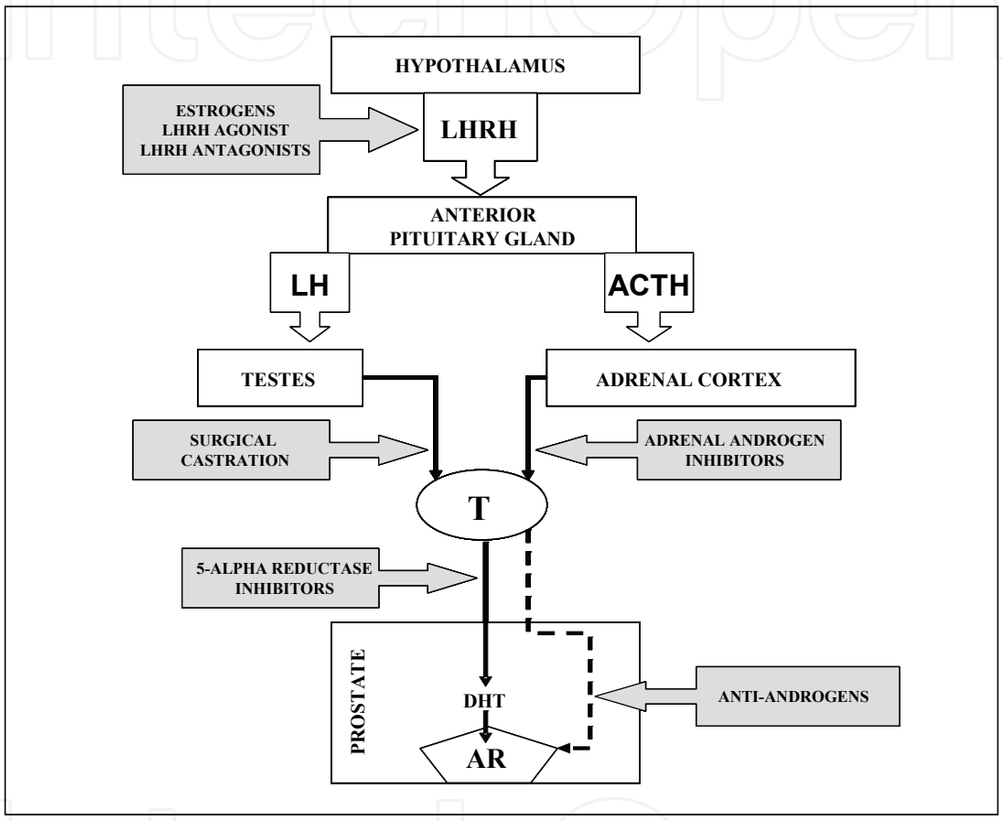


Fig. 4. **Current strategies for prostate cancer hormonal therapy.** LH-RH, luteinizing hormone releasing hormone; LH, luteinizing hormone; ACTH, adrenocorticotropic hormone; T, testosterone; 5α-R, 5α-reductase; DHT, dihydrotestosterone; AR, androgen receptor (*Adapted from Hiroshi et al (2004), THE prostate).*

7. Prostate cancer stem cells (PSCa)

In general, cancer stem cells can be defined as cells in the tumor with a tumor initiating potential. Normal stem cells are characterized by three properties: 1) capability of self-renewal; 2) strict control on stem cell numbers; 3) ability to divide and differentiate to generate all functional elements of that particular tissue (Bixby et al, 2002). Compared to normal stem cells, the cancer stem cells are believed to have no control on the cell number. Cancer stem cells form very small numbers in whole tumor and they are said to be responsible for the growth of the tumor cells (Sagar et al, 2007).

The cancer stem cell hypothesis was described more than 150 years ago (Virchow, 1860), but the new ideas came with the studies done in leukemia, where it was shown that a single cell with the CD34+/CD38- phenotype had the capacity of inducing the disease in NOD-SCID mice (Bonnet & Dick, 1997). More recently, cancer stem cells have been identified from solid tumors (Al-Hajj et al, 2003; Singh et al, 2003). There are several strategies to isolate prostate cancer initiating cells. The most common strategy used is identification of surface markers that share the same immunological profile with normal prostate stem cells. One of these markers is CD44, an adhesion molecule with multiple functions that appears to be important in tumor dissemination and metastasis (Draffin et al,2004; Naor etal, 2008; Ponta etal,2003). The CD44 cells also show properties of progenitor cells, and while these cells are AR^- they have the capacity to differentiate into AR^+ cells (Patrawala et al, 2006). Subsequent study has shown that $CD44^{high}/\alpha_{2\beta1}$ $integrin^{high}$ cells were more tumorigenic than $CD44^{low}/\alpha_2\beta_1$ $integrin^{low}$ cells when injected in immunocompromised mice (Patrawala etal, 2007).Putative prostate cancer stem cells have significant levels of telomerase, a ribonucleoprotein enzyme responsible for telomere elongation, indicating that they are an excellent target for telomerase inhibition therapy (Marian & Shay, 2009).

The progression to androgen-insensitive prostate cancer during androgen ablation therapy has led to speculation that prostate tumors may contain a small population of androgen-insensitive cells that survive and can expand in the absent of androgen (Litvinov et al, 2003). Since normal adult prostate stem cells (PSCs) are androgen-insensitive, it is reasonable to suspect they may be the source of these cells (Lawson & Witte, 2007). It has been described that the primary human prostate cancer cell subpopulation with the highest in vitro proliferative potential is negative for androgen receptor (AR) expression, and is suspected for normal PSCs (Collins etal, 2005). These cells also possess a $CD44^+\alpha_2\beta_1{}^{hi}CD133^+$ marker profile that is characteristic of normal human PSCs (Collins, et al, 2005; Richardson, et al, 2004). Utilizing several human prostate xenograft tumors and cell lines, it has been demonstrated that the $CD44^+$ cells, including PTHrP over-expressing PC3 cells, display enhanced proliferative activity in vitro and increased tumor-initiating and metastatic activity in vivo (Pacrawala, etal, 2006). These $CD44^+$cells are likewise AR^- and express higher mRNA levels of several stem cell markers including OCT3/4, BM11, β-CATENIN, and SMOOTHEND (Lawson & Witte, 2007). Human telomerase reverse transcriptase-imortalized primary human prostate cancer cell line has been shown to regenerate prostate tumors in mice that resembled the original patient tumor with respect to histopathology and Gleason score (Gu, etal, 2007). Regenerated tumors also contained basal, luminal, and neuroendocrine-like cancer cells, suggesting the clone of origin of the lines had multilineage differentiation capacity.

Common anticancer treatments such as radiation and chemotherapy do not eradicate the majority of cancer stem cells (Guzman et al, 2002; Jones etal, 2004). Cancer stem cells resistance to these therapeutics may be mediated by several stem cell-related mechanisms, including replication quiescence, activation of antiapoptotic pathways, and multi-drug transporter expression (Lawson & Witte, 2007). Androgen ablation therapies for invasive and metastatic prostate cancers may also spare prostate cancer stem cells (Litvinov etal, 2003). Research should therefore be aimed at developing therapeutics that can selectively target the prostate stem cell population rather than more differentiated prostate cancer cells. Clinical trials should likewise be designed to measure drug efficacy by examining their ability to eradicate prostate cancer stem cells rather than to measure bulk tumor regression (Lawson & Witte, 2007).

8. Multipotent stromal stem cells (MSCs) and their roles in the prostate cancer

Stem cells can be divided into three main categories: embryonic, germinal, and somatic. Embryonic stem cells originate from the inner cell mass of the blastcyst and are omnipotent, having indefinite replicative life span due to their telomerase expression (Soltysova, etal, 2005). Germinal stem cells are derived from primary germinal layers of embryo, and they differentiate into progenitor cells to produce specific organ cells (Sagar et al, 2007). Somatic/adult stem cells are progenitor cells as they are less totipotent i.e. less replicative life span than embryonic stem cells. They exist in mature tissues such as haematopoietic, neural, gastrointestinal and mesenchymal tissues (Sagar et al, 2007). The most commonly used adult stem cells are derived from bone marrow named haematopoietic stem cells, mesenchymal stem cells, and multipotent stromal stem cells (Kim et al, 2005).

Multipotent stromal stem cells (MSCs), or nonhematopoietic mesenchymal stem cell, were identified about 40 years ago (Friedenstein et al, 1974) in the bone marrow and were described as spindle shaped that proliferate to form colonies. These cells attach to plastic and are able to differentiate under defined *in vitro* conditions into multiple cell types present in many different tissues. The interaction between epithelial and stroma-forming non-hematopoietic bone marrow stem cells or multipotent mesenchymal stem cells (MSCs), such as fibroblasts, play a critical role in the development of both organs and tumors (Nelson & Bissell, 2006). This cross-talk is bidirectional and usually paracrine in nature. Multipotent MSCs have a fibroblast-like appearance that not only colonize numerous organs, but also are attracted to wounds and solid tumors especially. MSCs features include their ability to differentiate into cells of mesodermal lineage, such as bone, cartilage, and fat cells (Dominici et al, 2006). In addition, MSCs may transdifferentiate into cells of ecto- or endodermal lineages such as nerve, muscle, and epithelial cells (Ucelli et al, 2008). The plasticity of these cells, combined with their migratory potential and their preference for injured tissue, makes MSCs an ideal tool for therapeutic histogenesis (Brook et al, 2007). MSCs also enter tumors because cancer cells secrete chemokines that attract MSCs, and increase their migratory activity (Dwyer et al, 2007; Lin et al, 2008). In tumors, MSCs may alter the behavior of the cancer cells and may also differentiate to carcinoma-associated fibroblasts (CAF), which are known to be involved in cancer progression (Mishra et al, 2008). A recent report suggest that hMSCs enhance migratory potential of cancer cells by activating E-cadherin, a protease that down-regulates cell-cell adhesion and promoting cancer progression (Dittmer et al, 2009). Interestingly, MSCs have little effect on the migration of more aggressive breast cancer cells that already had lost E-cadherin. Instead, these highly aggressive cancer cells benefit from the interaction with hMSCs in a different way in that they aquire an increased potential to metastasize (Ditter et al, 2009; Karnoub et al, 2007). Yet, currently too little is known about hMSCs to get a clear picture of what the functions of hMSCs are in cancer progression. Among the many questions that remain are whether hMSCs act primarily on cancer cells as stem cells or as differentiated cells such as CAFs, and whether, under certain conditions, hMSCs may actually heal "cancerous wounds", which would explain why, in some cases, hMSCs suppress cancer growth (Dittmer, 2010).

9. Characteristics of multipotent stromal stem cells

MSCs and MSC-like cells have been identified to exist in and can be isolated from a large number of adult tissues, including the prostate, where they are postulated to carry out the

function of replacing and regenerating local cells that are lost to normal tissue turnover, injury, or aging (Chen & Tuan, 2008). There is no uniformly accepted clear and specific definitive phenotype or surface markers for the prospective isolation of MSCs. The minimal requirement for a population of cells to qualify as MSCs, as suggested by the International Society for Cytotherapy includes: (a) they must be plastic adherent under standard culture conditions, (b) they should express CD105, CD73, and CD90 and lack of expression of CD45, CD34, CD14, or CD11b, CD79α or CD19, and HLA-DR surface molecules, and (c) they should possess tripotential mesodermal differentiation capability into osteoblasts, chondrocytes, and adipocytes (Dominici et al, 2006).

Growth factors that have regulatory effects on MSCs include members of the transforming growth factor- beta (TGF-β) superfamily, the insulin-like growth factors, the fibroblast growth factors, the platelet-derived growth factor, and Wnts. Among these growth factors, TGF-βs, including TGF-β_1, TGF-β_2, and TGF-β_3, as well as bone morphogenetic protein (BMPs) are the most potent inducers to promote chondrogenesis of MSCs (Chen & Tuan, 2008). For hMSCs, TGF-β_2 and TGF-β_3 were shown to be more active than TGF-β_1 in promoting chondrogenesis (Barry et al, 2001). PTHrP also plays a regulatory role in MSC terminal differentiation. When human bone marrow MSCs from osteo arthritis patients were cultured in a 3-D polyglycolic acid scaffold in the presence of TGF-β_3, upregulated expression of collagen X was significantly suppressed by the presence of PTHrP whereas expression of other cartilage-specific matrix proteins was not affected (Kafienah et al, 2007).

MSCs are a source of soluble pro-angiogenic factors that act synergistically on endothelial cells to promote vasculogenesis and angiogenesis. These include: angiopoietin-1 (Ang1), vascular endothelial growth factor (VEGF), and growth factors such as platelet-derived growth factor (PDGF), fibroblast growth factor-2 & 7 (FGF-2/7), cytokines interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) as well as plasminogen activator (Honczarenko et al, 2006; Kinnaird et al, 2004). In addition, MSCs secrete chemokines such as IL-8, which is involved in the recruitment of endothelial progenitors (Honczarenko et al, 2006).

The ability of MSCs to migrate to tumor sites has encouraged investigation into the possibility of using these cells as gene delivery mechanisms (Studeny et al, 2004; Studeny et al, 2002). Naïve MSCs have been shown to inhibit tumor growth, prompting the use of these cells as tumor inhibitory cells in vivo (Khakoo et al, 2003).

The importance of cross-talk between cancer cells and other components of the microenvironment has been increasingly recognized. In vitro and co implantation models combining prostate tumor cells and hMSCs hold great promise as a system in which the interaction between tumor and stroma can be manipulated and studied. A better understanding of the interplay between hMSCs and the tumor cells will be important in developing strategies for improved treatment that take into account the influence of the microenvironment on tumor survival and growth.

10. Epithelial-stromal interactions in the prostate cancer

As with many other tissues, prostate formation is initiated as a consequence of interactions between epithelial and mesenchymal tissues. Chemokines, produced by tumor cell as well as by the stromal environment, and their Cognate receptors have been shown to regulate multiple steps during the prostate carcinogenesis (Vindrieux, et al, 2009). Because neoplastic foci arise in the epithelial compartment, the role of the stromal compartment in carcinogenesis has been relatively neglected. The role of epithelial-mesenchymal

interactions in prostate formation has been defined through elegant tissue recombination studies performed by Cunha and colleagues (Cunha et al, 1987; Cunha 1996). Their studies have led to the following principal conclusions:

1. Prostatic differentiation requires both epithelial and mesenchymal components.
2. Specificity for the mesenchymal component is relatively stringent.
3. Specificity for the epithelial component is relatively broad.
4. During prostate development, androgens initially act on the mesenchyme, and prostate does not form when urogenital mesenchyme is defective in androgen receptor.
5. Human epithelium and rodent mesenchyme (and vice versa) can be recombined to form prostate, supporting the validity of rodent prostate as a model for the human gland.

The interaction between epithelial and stroma-forming cells plays a fundamental role in the development of both normal organs and tumors (Nelson & Bissel, 2006). Recent studies suggest that cell of the microenvironment of solid tumors constitutes a permissive milieu for the induction, selection and expansion of cancer cells (Liotta & Kohn, 2001; Bhowmick et al, 2004; Maffini et al, 2004). Conversely, neoplastic cells may modify the microenvironment through cell communication proteins, in particular growth factors. Genetic profiling of solid tumors has shown abnormal gene expression in both cancer cells and cells from the microenvironment (Allinen et al, 2004). Elucidating the role of the microenvironment is a major concern in finding ways to disrupt this vicious circle and induce cancer cell apoptosis. Because interactions between the epithelial and stromal components are essential for all stages of normal prostate growth and development, it is likely that aberrant interactions play a significant role in prostate carcinogenesis (Abate-Shen & Shen, 2000).

Decreased E-cadherin expression is correlated with various indices of prostate cancer progression including grade, local invasiveness, dissemination into the blood, and tumor relapse after radiotherapy (Loric et al, 2001; Mason et al, 2002; Ray et al, 2006). In contrast, markers of a mesenchymal phenotype including N-cadherin, osteoblast-cadherin, and WAP-type four disulfide core/ps20 proteins (WFDC-1) are all up regulated by prostate cancer cells (Tomita et al, 2000; McAlhany et al, 2004; Jaggi et al, 2006). Increased levels of the extracellular domain of N-cadherin have also detected in the serum of prostate cancer patients (Derycke et al, 2006). The functional importance of decreased E-cadherin levels has also been demonstrated in prostate cancer cells with its inverse correlation with cellular motility and protease expression (Chunthapong et al, 2004). These changes in epithelial and mesenchymal markers and the loss of prostatic glandular architecture are consistent with the general differentiated phenotype of aggressive prostate cancer cells, although decisive evidence for EMT remains elusive (Hugo et al, 2007). The proof of principal for EMT in prostate cancer has emerged from studies using *in vitro* and *in vivo* models of prostate cancer progression. EGF can induce EMT in Du145 cells due to caveolae-dependent endocytosis of E-cadherin followed by transcriptional down regulation by Snail (Lu et al, 2003), and inhibition of EGF signaling restores E-cadherin levels (Yates et al, 2007). In contrast, loss of the epithelium-specific transcription factor prostate-derived ETS factor (PDEF), which is down regulated by TGFβ, induces EMT in PC3 cells (Gu et al, 2007). In addition, over-expression of PSA and kallikerin-related peptidase (KLK4), both potential activators of pro-EGF and latent TGFβ2, results in EMT in PC3 cells (Whitbread et al, 2006). While PSA and KLK4 are part of normal prostatic secretions, they leak into the tumor microenvironment due to the disruption of glandular architecture during cancer progression, suggesting a link between tissue architecture and EMT (Hugo et al, 2007). The cadherin profile and

invasiveness of prostate cancer cells correlates with androgen-insensitivity (Jennbacken et al, 2006), and the androgen receptor is also absent or lowly expressed in PC3 and Du145 cells. Therefore, it is likely that perturbation of the androgen receptor axis has a permissive effect on EMT as aggressive prostate cancer cells exhibit increased plasticity and loose their luminal epithelial phenotype, including androgen receptor expression during tumor progression (Hugo, et al, 2007). Cancer cells may also modify the microenvironment through cell communication proteins, such as cytokines, and MET has been recognized in a number of mesenchymal tumors. In prostate cancer, co-culture of DU145 prostate cancer cells with hepatocytes resulted in re-expression of E-cadherin (Yates et al, 2007). This is consistent with findings in clinical material, in which membranous E-cadherin was detected in hepatic metastasis using immunohistochemistry, and vimentin was absent in the tumor cells. In the Dunning prostate cancer model, mapping of FGF receptor-2(IIIb) in primary tumors, typically where the tumor cells were in contact with the stroma (Oltean et al, 2006).

11. Role of multipotent stromal stem cells in metastatic prostate cancer in the bone

The ability of prostate cancer cells to penetrate the basement membrane and then invade the interstitial stroma to initiate the metastatic process is largely mediated by proteolysis. It has been shown that CXCL12-CXCR4 interactions may play a role in the metastasis of prostate cancer to bone (Tiachman, et al, 2002), and the expression of CXCR4 and its interaction with CXCL12 may aid in facilitating the migration, invasion and matrix metalloproteinases (MMPs) expression by prostate tumor cells (Singh et al, 2004) .

Prostate and breast cancers show a high propenicity to metastasize to bone. Whereas breast cancer triggers preferentially an osteoclast reaction with bone resorption and consequent osteolytic lesions, prostate cancer elicits predominantly an osteoblast response resulting in osteosclerotic lesions, and preferentially metastasizes to the bone marrow stroma of the axial skeleton.(Mundy, 1997). Tumor-microenvironment interactions are crucial in bone metastases and genetic studies using laser captured microdissection and gene expression profiling of clinical specimens confirmed gene expression changes in prostate cancer cells and adjacent stroma (Gregg, et al, 2010). Co-culture of bone multipotent stromal cells with human prostate cancer cell line, LNCaP, induced permanent genetic, morphologic, and behavioral changes in LNCaP cells (Rhee et al, 2001). A recent study supports the concept of permanent genetic and behavioral changes of prostate cancer epithelial cells after being either co-cultured with prostate or bone multipotent stromal cells as three-dimensional prostate organoids or grown as tumor xenografts in mice (Sung et al, 2008).

1. **osteoblastic metastasis in prostate cancer**: Osteogenesis is achieved by differentiation of multipotent stromal stem cells into chondrocytes followed by endochondral ossification. Many stimulating factors have been identified with respect to osteogenesis in prostate cancer. There are three types of endothelin (ET-1, -2 and -3), which acts through the endothelin receptors Eta and ETb. They are synthesized in vascular endothelial cells and are involved in processes such as regulation of vascular endothelial tones and bone formation, amongst others (Clarke et al, 2009). It was shown that exogenous ET-1 induces prostate cancer proliferation and enhances the mitogenic effects of insulin-like growth factor and epidermal growth factor (Nelson, 2003). ET-1 production is a major factor in osteoblast overstimulation and osteogenesis (Guise &

Yin, 2003). Prostate epithelial cells produce ET-1 and its receptor, Eta is present throughout the prostate gland (Nelson et al, 1999). Experiments using an osteoblast mouse model (Guise & Yin, 2003) showed that tumors producing ET-1 act via Eta receptors on osteoblasts to stimulate accelerated osteogenesis. This abnormal activity is blocked by the ET-1 inhibitors (Nelson, 2003). Other osteoblastogenic factors include up-regulation of the Wnt pathways and production of cytokines such as bone morphogenetic protein, TGF-β, IGF, vascular endothelial growth factor, platelet-derived growth factor and MDA-BF (Logothetis & Lin, 2005). A further interesting aspect of the cytokine balance in prostate cancer metastasis relates to PTHrP, which is produced in prostate cancer bone metastases (Boyden et al, 2002). The prostate specific antigen (PSA) cleaves PTHrP and possibly shifts the prostate bone metastasis from osteolytic to osteogenesis (Cramer et al, 1996; Iwamura et al, 1996). In addition, PTHrP is known as an important local factor for osteogenesis by regulating chondrogenesis in a manner that attenuates chondrocyte hypertrophy (Amizuka et al, 2000). PSA can also cleave insulin-like growth factor binding protein (IGFBP-3), which in turn increases the level of IGF-1. This too would have the effect of shifting the axis of stimulation by the metastatic prostate cancer cells towards increased osteoblast activity (Cohen et al, 1994) (Figure 5).

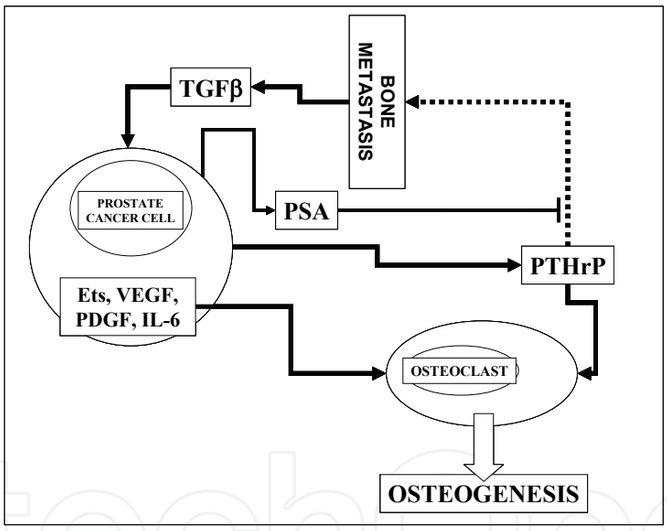


Fig. 5. Model of osteoblastic bone metastasis in prostate cancer.

2. **osteoclastic metastasis in prostate cancer**: It has been demonstrated that osteoblastic metastasis also involves considerable osteolysis (Reddi et al, 2003; Oades et al, 2002). Both the osteolysis itself and the factors released from bone matrix during bone resorption contribute to the vicious cycle of osteoblastic lesions (Clarke et al, 2009). Osteoclast recruitment, differentiation and activation by tumors are related to the osteoblast stimulation that results from osteoblastic over-expression of NF-κB (RANK ligand) and the production of osteoprotegerin (Jung et al, 2004). When PTHrP is present, osteoclasts differentiate in the absence of other stimulatory agents, suggesting that PTHrP plays a facilitating role (Clarke et al, 2009). On the other hand, androgen ablation increases osteoclastic bone resorption and bone loss (Smith et al, 2005; Krupski et al, 2004). The increased bone resorption due to androgen deprivation may result in a

more fertile environment for the development of bone metastasis. Furthermore, PSA is thought to contribute to prostate cancer metastasis through its protease activity and its ability to induce epithelial-mesenchymal transition and cell migration (Whitbread et al, 2006). Taken together, both osteoblasts and osteoclasts cooperate to actuate the settlement and growth of prostate cancer in bone (Ye et al, 2007) (Figure 6).

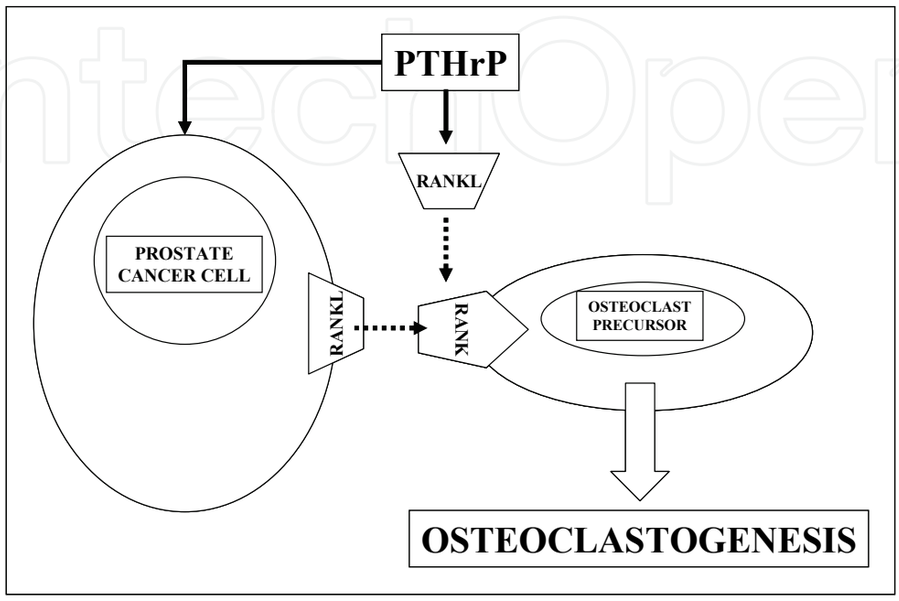


Fig. 6. Model of osteoclastic bone metastasis in prostate cancer.

12. Importance of parathyroid hormone-related protein in prostate carcinogenesis and bone metastases

PTHrP is produced by neuroendocrine, luminal, and basal stromal cells of the prostate and has been immunohistochemically identified in primary prostate cancer tissues (Iwamura ET AL, 1993.) as well as in higher levels in more advanced prostate carcinoma (Asadi et al, 1996). Additionally, it has been shown that expression of nuclear-targeted PTHrP can protect mesenchymal stem cells and chondrocytes (Figure 8) from apoptosis (Henderson et al, 1995). Other studies of androgen-sensitive LNCaP prostate cell lines *in vitro* provide interesting insights into potential mechanisms of PTHrP action. This cell line provides a good model for assessing the effects of PTHrP expression because the parental cell line produces no detectable PTHrP. Expression of full-length PTHrP in this cell line was protective against phorbol 12-myristate 13-acetate (PMA)-induced apoptosis, whereas the expression of NLS-deleted PTHrP in the same cells had no effect on apoptosis (Dougherty et al, 1999). This experiment confirms a previous study (Henderson et al, 1995) that PTHrP acts as an inhibitor of apoptosis.

In addition to anti-apoptotic role, PTHrP is produced by more than 90% of bone metastases (Powel et al, 1990), leading to the concept that local PTHrP production by cancer cells that reach bone promotes the bone resorption process, thus favoring tumor establishment and expansion. The experimental model that has provided the most support for this is one in which PTHrP-producing human breast cancer cells have established themselves and grown

as lytic deposits in bone after injection into the arterial circulation of immune-deficient mice (Yoneda et al, 1997; Guise et al, 1996).

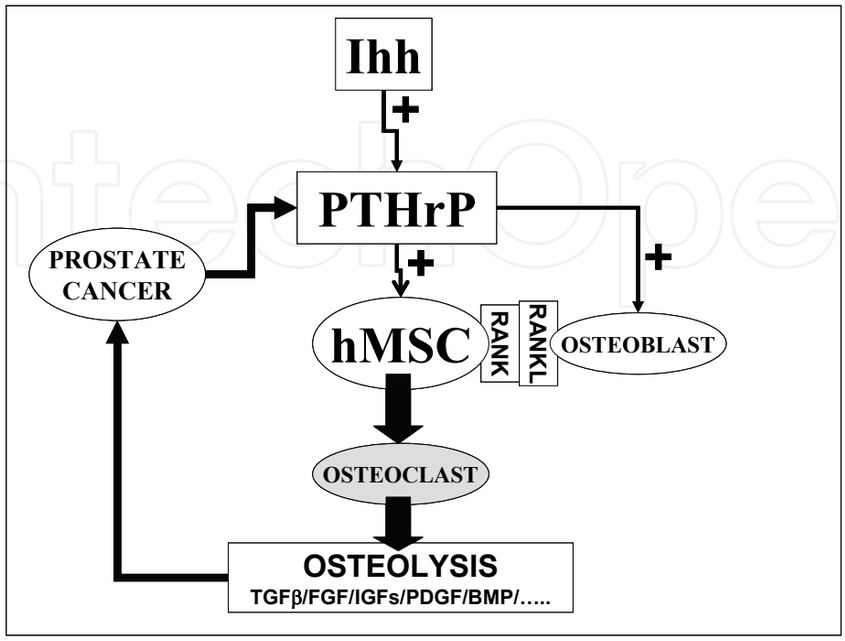


Fig. 8. Tumor cells produce PTHrP that stimulates hMSCs differentiation and osteoclast formation. Stimulated osteoclasts produce potent osteolytic factors that enhancing the effect of PTHrP.

PTHrP was originally discovered as a systemic humoral factor that is released by tumor cells and causes humoral hypocalcaemia of malignancy (HHM) (Suva et al, 1987; Wysolmerski and Broadus, 1994; Rankin et al, 1997; Grill et al, 1998). The hypercalcemic activity of PTHrP is based on its partial homology to parathyroid hormone (PTH) and by being able to bind to the parathyroid hormone 1 receptor (PTH1R) with equal affinity as PTH (Horiuchi et al, 1987; kemp et al, 1987; Juppner et al, 1991). Although PTHrP mediates its calcemic effects through PTH1R, there is evidence for a separate PTHrP receptor (Pearce et al, 1995). This is indicated by the observations that fragments not containing the N-terminal domain are present outside of cells, and that those fragments are able to interfere with cellular function when added exogenously (Soifer et al, 1992; Wu et al, 1996; Massfelder et al, 1997; Luparello et al, 2001). In particular, the mid-regional PTHrP (67-86) peptide, devoid of a functional NLS, has been shown to mobilize calcium through a phospholipase c-dependent pathway in squamous carcinoma cells (Orloff et al, 1996). This PTHrP domain is known to interact with an uncharacterized receptor, but different from the PTH1R in osteoblasts (Valin et al., 1997, 2001; Alonso et al., 2008). It has been previously demonstrated that PTHrP (107-139) can rapidly increase VEGF expression in human osteoblastic cells (Esbrit et al., 2000).

PTHrP is also expressed by non-transformed cells in almost all tissues (dePapp and Stewart, 1993) where it serves specific functions as an autocrine or paracrine factor (Moseley and Gillespie, 1995; Philbrick et al, 1996; Strewler, 2000). In embryogenesis, PTHrP plays an essential role in mammary gland and bone development (Vortkamp et al, 1996; Wysolmerski et al 1998). Disruption of the PTHrP gene in mice leads to fatal skeletal dysplasia (Karaplis et al, 1994; Karaplis and Deckelbaum, 1998). In the developing bone,

PTHrP secreted from periarticular perichondrium activates PTH1R on chondrocytes, therby preventing premature ossification (Vortkamp et al, 1996). The widespread expression of PTHrP in normal tissue was the first evidence that the protein had a role in normal physiology. Normal subjects do not have detectable circulating levels of PTHrP, suggesting that in normal physiology PTHrP acts as a local regulator or cytokine in the tissue where it is produced.

Recent evidence suggests the importance of parathyroid hormone-related protein (PTHrP) in tumor progression, androgen-insensitive and resistance of prostate cancer cells to apoptosis (Asadi et al, 1996; Asadi and Kukreja, 2005; Asadi et al, 2010; Wu et al, 1998; Gujral et al, 2001; Tovar and Falzon, 2002). PTHrP is a mediator of cellular growth and differentiation and is involved in mesenchymal-epithelial interactions in several tissues (Hardy, 1992; Van de Stolpe etal, 1993, Wysolmerski etal, 1994). It has been shown that PTHrP and the PTH/PTHrP receptor are expressed in cells of the adipocytic lineage and that PTHrP signaling by the cAMP-dependent PKA enhances MAPK activity, leading to phosphorylation of PPARγ, the master regulator of adipocyte differentiation, and thereby repression of the adipogenic differentiation program (Chan et al, 2001). Immunohistochemical studies have also identified PTHrP in a subpopulation of stromal cells located in the red pulp of the spleen, primarily in a subcapsular distribution (Funk et al, 1995). A most recent study has pointed out that in oral squamous cell carcinoma a suitable microenvironment has been provided for osteoclast formation not only by producing IL-6 and PTHrP but also by stimulating stromal cells to synthesize these proteins (Kayamori et al, 2010). Interestingly, BCL-2, an anti-apoptotic gene, lies downstream of PTHrP in a signaling pathway that regulates osteogenesis during development (Amling et al, 1997). It has been suggested that BCL-2 serves to regulate apoptotic cell death during embryonic development. In adult, BCL-2 expression is limited to renewing stem cell populations such as those found in prostatic glandular epithelia (Hockenbery et al, 1991). Osteoclastogenesis is a stromal-cell dependent process that is also mediated by PTHrP through receptor activator of nuclear factor κB (RANK)/RANK ligand and osteoprotegerin system (Clines & Guise, 2005). Tumor cells produce PTHrP, an osteoclastogenic factor, that stringers stromal stem cells to express receptor activator of NF-κB ligand (RANKL) which in turn binds to and activates osteoclast precursors and causing them to mature.

Since PTHrP over-expression correlates inversely with androgen sensitivity and results in resistance to apoptotic injuries in prostate cancer cells, it is important to control the level of PTHrP expression in these cells. Recent studies indicate that adenovirus E1A oncogene has strong tumor suppression activities that involve conversion of apoptosis-resistant cells to apoptosis-sensitive cells (Shisler et al, ; Cook et al, ; Yageta et al,; Breckenridge et al,; Shao,). Most recently, it has been shown that expression of the adenoviral E1A protein expression in apoptosis-resistant PC-3 cells sensitized these prostate cancer cells to TNF-α-induced apoptotic cell death. Furthermore, it was shown that the effect of E1A on PTHrP expression was through repression of the transcriptional activity of the PTHrP P3 promoter (Asadi et al, 2010).

PTHrP transcripts are translated into three different isoforms, PTHrP (-36/139), PTHrP (-36/141), and PTHrP (-36/173). They all contain the N-terminal signal sequence for entrance into the endoplasmic reticulum and the coding regions between residues 1 and 139 (Martin et al, 1991; Philbrick et al, 1996; Strewler, 2000). The isoforms PTHrP (-36/141) and the human-specific PTHrP (-36/173) products feature extended C-terminus (Dittmer, 2004) (Figure 7). The PTHrP protein is post-translationally cleaved at a number of dibasic sites

leading to the removal of the pre-pro sequence between -36 and +1 and to a limited fragmentation of the protein (Diefenbach-Jagger et al, 1995; Dittmer et al, 1996; Wu et al, 1996). These fragments contain one or more of the three functional domains which are the N- terminal (PTHrP 1-36), the mid region (PTHrP 38-94) and the C-terminal domain (PTHrP 107-139) (Dittmer, 2004). The mid-region domain is able to enter the nucleus. It contains a nuclear localization sequence (NLS) which allows PTHrP to accumulate in the nucleus and to bind to RNA (Massfelder et al, 1997; Henderson et al, 1995; Aarts et al, 1999). Nuclear targeting can be further achieved by residues 66-94 which is recognized by importin β (Lam et al, 1999a; Cingolani et al 2002). The mid-region sequence also holds a CDK 1(cdc2)/CDK2 phosphorylation site. Following its phosphorylation, PTHrP is retained in the cytoplasm suggesting that the activity of nuclear PTHrP is regulated by the cell cycle (Lam et al, 1999b; Dittmer, 2004). The C-terminal domain, also called osteostatin, is able to inhibit bone resorption and, thereby, antagonizes the action of the N-terminal domain of PTHrP (Fenton et al, 1994; Cornish et al, 1997). The C-terminal domain also harbors four potential targets for kinases at residues 119, 130, 132, and 138 whose mutation from a serine or threonine to an alanine blocked the mitogenic activity of PTHrP in vascular smooth muscle cells (Fiaschi-Taesch et al, 2004). The sequence between residues 140 and 173 has been shown to interfere with the nuclear localization of PTHrP and to raise the cAMP level (Goomer et al, 2000; Hastings et al, 2004). Previous studies have reported that the half-life of all three transcripts of PTHrP mRNA ranges from 30 min to more than 3h, depending on the cell type (Heath etal, 1995; Werkmeister etal, 1998, Benitez-Verguizas etal, 1999).

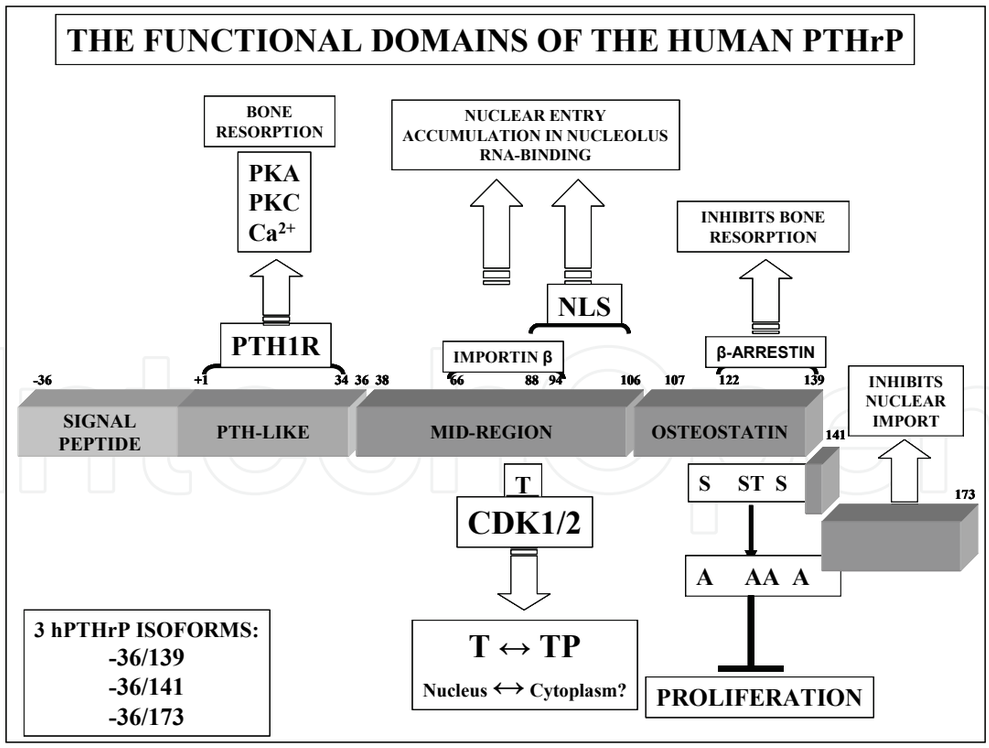


Fig. 7. **The functional domains of the human PTHrP protein.** T, Thr; TP, phosphorylation of Thr ; SSTS, residues :Ser^{119}, Ser^{130}, Thr^{132}, Ser^{138} ; AAAA, alanines; CDK, cyclin-dependent kinase; GPCR, G-protein coupled receptor (*Adapted from Dittmer, 2004*).

13. Therapeutic applications of multipotent stem cells (MSCs)

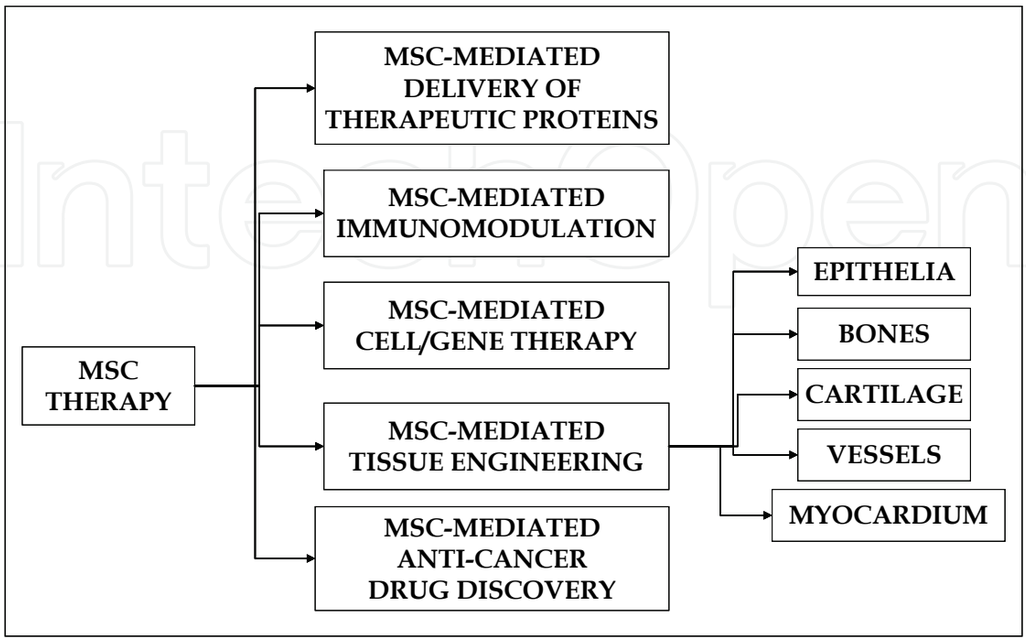
Despite significance advances in the field of gene therapy for cancer, two major obstacles remain that continue to limit the clinical potential of this approach: lack of tumor tropism of vectors; and stimulation of an immune response (Dwyer, et al, 2010). The fact that MSCs have a natural tropism for tumors and their metastases, can be differentiated into several different cell types in vitro, their relative ease of expansion in culture, and their immunologic characteristics clearly make MSCs and MSC-like cells a promising source of stem cells for tissue regeneration an cancer gene therapy (Lazennec & Jorgensen, 2008). Applications of MSCs in cancer treatment has gained considerable attention, with studies reporting engineered MSCs specifically targeting multiple tumor types followes by local secretion of therapeutic proteins. In a number of tumor models, MSCs expressing IFNβ has been shown to result in decreased tumor burden and increased animal survival (Studeny, et al, 2002; Nakamizo, et al, 2005;Kidd, et al, 2010). MSCs engineered to secrete IL-12 and embedded in a matrix adjacent to tumors were also reported to have a significant therapeutic effect (Eliopoulos, et al, 2008). MSCs expressing the hepatocyte growth factor antagonist NK4 in vivo were also found to prolong animal survival by inhibiting tumor-associated angiogenesis, lymphoangiogenesis and induction of cancer cell apoptosis (Kanehira, et al, 2007). Further, MSCs secreting IL-2 (Nakamura, et al, 2004; Stagg, et al, 2004), IL-12 (Eliopoulos, et al, 2008; Chen, et al, 2008) were shown to elicit an immunological reaction, and to stimulate inflammatory cell infiltration of the tumor tissue. Because MSCs are resistant to TRAIL-induced apoptosis, MSCs secreting TRAIL have been used in models of lung,breast,cervical and brain cancers in vivo, resulting in significant anti-tumor effects (Grisendi, et al, 2010; Loebinger et al, 2009; Mohr, et al, 2008; Kim, et al, 2008;Sasportas, et al,2009). The potential for MSC-mediated tumor promotion, however, is a significant concern and must be addressed.

14. Future research

At present, the cancer treatment is targeted at its proliferation potential and its ability to metastasize, and hence the majority of treatments are targeted at rapidly dividing cells and at molecular targets that represent the bulk of the tumor. This may explain the failure of treatments to eradicate the disease or the recurrence of the cancer (Reya et al, 2001). For tumors in which the cancer stem cells play role, three possibilities exist (Sagar et al, 2007): first, the mutation of normal stem cells or progenitor cells into cancer stem cells can lead to the development of the primary tumor. Second, during chemotherapy, most of the primary tumor cells may be destroyed but if cancer stem cells are not eradicated, they become refractory cancer stem cells and may lead to recurrence of tumor. Third, the cancer stem cells may immigrate to distal sites from the primary tumor and cause metastasis. Cancer stem cells are relatively quiescent compared to other cancer cells and do not appear to have the hyper-proliferation signals activated such as tyrosine kinase. These make the cancer stem cells resistant to the toxicity of the anti-cancer drugs, which traditionally target the rapidly dividing cells (Sagar et al, 2007). In addition, the tumor suppressor gene PTEN, polycomb gene *Bmi1* and the signal transduction pathways such as the Sonic Hedgehog (Shh), Notch and Wnt that are crucial for normal stem cell regulation, have been shown to be deregulated in the process of carcinogenesis (Galderisi et al, 2006; Groszer et al, 2001; Park et al, 2003). One approach to target the cancer stem cells may be the identification of the markers that are specific for the cancer stem cells compared to normal stem cells.

It has been suggested (Cunha et al, 1987) that during the embryogenesis of the prostate androgens do not initiate this regulation directly within the prostate epithelial cells. Instead, androgen ligand/AR interactions occur in embryonic prostatic stromal cells inducing these cells to synthesize and release soluble procrine factors in which their functions are to regulate the growth and development of prostatic epithelial cells (Cunha et al, 1987). Human adipose tissue-derived MSCs (hAT-MSCs) have been recently engineered, by retrovirus transduction, to express the suicide gene cytosine deaminase::uracil phosphoribosyltransferase (CD::UPRT). The ability of yeast cytosine deaminase expressing AT-MSCs (CD_y-AT-MSC) to convert the relatively nontoxic 5-fluorocytosine (5-FU) along with their ability to target tumor sites and micrometastases and to have a low immunogenic potential, makes thes cells a unique tool to convert prodrug to cytotoxic drugs directly within the tumor mass (Altaner, 2008). Previous results from *in vivo* experiments showed that CD_y-AT-MSCs, administered subcutaneously as a mixture with tumor cells, or intravenously significantly inhibited the growth of human colon adenocarcinoma (Kucerova et al, 2007) and human melanoma xenografts in nude mice treated with 5-FC (Kucerova, 2008). In a most recent study, the feasibility and efficacy of CD_y-AT-MSCs as cellular vehicle of the therapeutic gene CD::UPRT in the treatment of human prostate cancer has been tested (Cavarretta et al, 2010). It was demonstrated that AT-MSCs expressing fusion yeast CD::UPRT gene, when systematically administered in combination with the pro-drug 5-FC to human prostate tumor-bearing mice, were able to inhibit the prostate tumor growth (Cavarretta et al, 2010).

One possible therapeutic molecule for prostate cancer is interferon-β, which suppresses tumor cell growth by induction of differentiation, S-phase accumulation, and apoptosis (Dong et al, 1999; Qin et al, 1997). A most recent study describes the potential of genetically modified MSCs, constitutively expressing IFN-β in reducing tumor growth in a therapy model of prostate cancer lung metastasis (Ren et al, 2009). Targeted homing of MSC producing IFN-β, at tumor sites in the lungs was found to mediate anti-tumor effects by multiple mechanisms including induction of apoptosis, anti-angiogenesis and by increasing natural killer cell activity (Ren et al, 2009).



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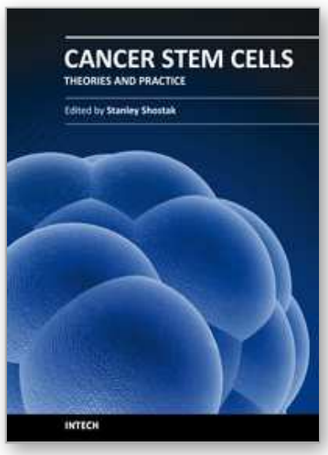

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Cancer Stem Cells Theories and Practice does not 'boldly go where no one has gone before!' Rather, Cancer Stem Cells Theories and Practice boldly goes where the cutting edge of research theory meets the concrete challenges of clinical practice. Cancer Stem Cells Theories and Practice is firmly grounded in the latest results on cancer stem cells (CSCs) from world-class cancer research laboratories, but its twenty-two chapters also tease apart cancer's vulnerabilities and identify opportunities for early detection, targeted therapy, and reducing remission and resistance.

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