Chapter 2

REVIEW OF LITERATURE
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The prostate gland surrounds the base of the urinary bladder and is composed of an extensive acinar/ductal network that contains large quantities of secretory material. Although these secretions contribute a substantial volume to the ejaculate, the function of this material or the prostate gland in general has not been clearly established (Coffey 1988). What is well known is that prostate is exquisitely sensitive to androgenic hormones. As one of the male accessory sex organs, it develops under the influence of and is maintained by testicular androgens.

Prostate is one of those rare tissues which grows in the later stages of life while most others tend to regress. Any medical advancement or health promotion strategy that increases longevity will also increase the incidence and overall prevalence of prostatic tumours (Carter and Coffey, 1990). Benign prostatic hyperplasia affects more than two-thirds of all males over the age of 50 and prostatic cancer is now the most common malignancy and the second leading cause of cancer death in males (Carter and Coffey, 1990; Carter et al. 1990).

The cause of abnormal growth of prostate is unknown. At present the therapy is based on cutting the source of androgens by orchiectomy (Huggins and Hodges, 1941), anti-androgens and inhibitors of 5-α-reductase, which convert testosterone into its active intracellular form dihydrotestosterone (DHT). Analogs of luteinizing hormone releasing hormone (LHRH) have also been employed (Borgmann et al. 1982; Ahmed et al. 1983; Smith 1984; Faure et al. 1984; Presant et al. 1985) as also active immunization against LHRH (Talwar et al. 1992a). The rationale of these treatments is to curtail the growth promoting effect of androgens. As the cancer cells are not killed or
eliminated, the treatment has to be sustained. It is also recognized that a variety of prostatic carcinomas are androgen independent, where androgen deprivation is of little avail. Antimitotic chemotherapy and radiotherapy are treatments common to all cancers and have benefits, limitations and side effects similar to those seen in other cancers. At present no specific drug or device is available to kill prostatic carcinoma cells in a selective manner.

2.1. Prostatic Growth and Polypeptide Growth Factors:

Differentiation of the prostatic epithelium is a complex process. The prostatic epithelium has generally been described as consisting of two different cell types (Cleary et al. 1983; Brawer et al. 1985; Nagle et al. 1987). The luminal glandular cells are highly differentiated and are often referred to as the functionally active prostatic secretory cells. On the other hand the basal cells are less differentiated and are located at the base of the epithelium. Their function remains largely unknown, although a function as reserve cells has frequently been suggested (Bazer 1980).

It has been observed that stromal-epithelial interactions and trophic factor(s) other than sex hormones play an important role in the normal and abnormal growth of prostatic cells (Cunha et al. 1983). The basis of the indirect positive involvement of androgens in prostatic growth is indirectly by the induction of synthesis of mitogenic factors which act upon prostate cells as autocrines or paracrines. This was indicated by the inability of androgens and ability of certain peptides to support proliferation of some populations of prostate epithelial cells in culture (McKeehan et al. 1984).

The search for substances stimulating prostatic growth in culture, receptors specific for such substances and growth promoting substances
produced by the prostate itself, has given rise to an extensive catalogue of factors. Thus, normal and abnormal prostate glands have been reported to produce and/or respond to and/or localize: growth hormone (Griffiths et al. 1991), LHRH (Fekete et al. 1989), osteogenic growth factors (Koutsilieris et al. 1987; Perkel et al. 1990), nerve growth factor (Chapman et al. 1981), insulin-like growth factor-I (Davies et al. 1988; Fiorelli et al. 1991), insulin-like growth factor-II (Matuo et al. 1988) and platelet-derived growth factor (Sitaras et al. 1988). Information has also been accumulated on members of epidermal growth factor (EGF), fibroblast growth factor (FGF) and transforming growth factor-beta (TGF-β) families and their relationships with each other and with androgens. Expression of some of these growth factors and their receptors is regulated by androgens and it appears that androgenic steroids may exert their growth and differentiation inductive activities in part via growth factor pathway (Thompson 1990). Epidermal growth factor has been detected in prostatic (Gregory et al. 1986) and seminal (Elson et al. 1984) fluids and is mitogenic for normal and malignant human prostate cells in culture (Peehl et al. 1989; Schuurmans et al. 1988a). High-affinity receptors for EGF have been found in normal, hyperplastic and cancerous human prostate tissues (Davies and Eaton, 1989; Fekete et al. 1989) and in human prostate cancer cell lines (Schuurmans et al. 1988a; MacDonald et al. 1990). Prostate cancer cell lines LNCaP (androgen-dependent), DU 145 and PC3 (androgen-independent), secrete EGF and TGF-α (Derynck et al. 1987; Connolly and Rose, 1989 & 1990). The later has significant sequence and structural homology with EGF and shows similar biological actions. TGF-α exerts its activities by interaction with EGF receptor (Lyons and Moses, 1990). Cultured human prostate cancer cell lines have been shown to contain high levels of TGF-α mRNA and to proliferate in response to TGF-α (Wilding
et al. 1989). This growth factor may also have a paracrine function, stimulating physiological activities such as angiogenesis, which impart a selective advantage towards malignancy. In recent studies with transgenic mice, TGF-α cDNA under the transcriptional control of inducible metallothionein-1 enhancer/promotor sequences produced significant epithelial hyperplasia and focal dysplastic changes that resembled carcinoma in situ in the anterior prostate (Sandgren et al. 1990).

Members of the biologically complex TGF-β family also appear to be involved in growth disorder of the prostate. Elevated levels of mRNA encoding TGF-β2 and basic fibroblast growth factor have been demonstrated in BPH as compared to normal prostatic tissues (Mori et al. 1990). These authors suggested that specific growth factors produced locally in the prostate may be involved in BPH development in an autocrine or paracrine fashion. Expression of high levels of TGF-β1 mRNA has been reported in human prostate cancer cell lines PC3 and DU 145 as well as Dunning series of transplantable rat prostate carcinomas (Wilding et al. 1989a; Steiner and Barrack, 1990). In the normal rat prostate and in androgen dependent prostate tumours, expression of TGF-β receptors and TGF-β1 mRNA seems crucial in activation of castration-induced programmed cell death (Kyprianou and Isaacs, 1989; Kyprianou et al. 1990). In most instances, prostate cell growth is inhibited by TGF-β1, although in some cases, TGF-β operates bifunctionally, being inhibitory at lower and stimulatory at higher concentrations (Shain et al. 1990). The mitogenic influences of epidermal growth factor and fibroblast growth factor are counterbalanced by the inhibitory effects of TGF-β. Stimulation of LNCaP cells by EGF and its
synergistic action with androgen could be inhibited by TGF-β (Schuurmans et al. 1988b).

On the basis of significant amino acid sequence identity, the product of the int-2 gene has been assigned to the FGF family (Moore et al. 1986; Abraham et al. 1986; Yoshida et al. 1987; Delli Bovi et al. 1987). Deregulated expression of int-2 can lead to profound epithelial cell-specific hyperplasia in the mouse prostate (Muller et al. 1990). In a transgenic model system, under the transcriptional regulation of a truncated mouse mammary tumour virus long terminal repeat the int-2 gene was expressed at high levels in the mammary glands of female mice and in the prostates of male mice. In the latter case, there was a reproducible enlargement of the prostate gland, characterized by hyperplasia of the prostatic epithelium. The epithelium proliferated remarkably, but the cells remained benign and could not be propagated in secondary host animals. Interestingly, mature human prostate contains substantial amounts of basic fibroblast growth factor (bFGF) mRNA and protein (Story et al. 1987; Mydlo et al. 1988a&b). In comparison to normal prostate, BPH tissue contains elevated levels of bFGF mRNA, suggesting that this growth factor may have a role in benign growth disorder of the human prostate (Mori et al. 1990). Both bFGF and acidic fibroblast growth factor (aFGF) have also been detected in rat prostate as well as in transplantable rat prostate tumours (Mansson et al. 1989). Although aFGF mRNA is abundant in the prostatic epithelium of immature animals, it disappears as the animals mature. Interestingly, expression of aFGF mRNA in the slow-growing, androgen-responsive Dunning R3327 PAP prostate carcinoma is specific to the mesenchymal cells of the tumour, leading to speculation that the mesenchyme-derived aFGF supports the growth of the malignant epithelium in a paracrine manner. In vitro studies have confirmed
that both aFGF and bFGF are mitogenic in cultured prostate epithelium as well as prostatic fibroblasts (McKeehan et al. 1984; Story et al. 1989) and provide evidence for a role of FGF-like factors in abnormal growth of prostate.

2.2. Prostatic Growth and Oncogene Activation:

Specific genetic changes and alterations in genetic expression are generally assumed to be associated with cancer progression. Like most other tumours, prostate cancers have been screened for activated or otherwise deregulated oncogenes. Aberrations in structure and expression of \textit{c-myc} and members of the \textit{ras} family have been detected circumstantially in human prostate tumours (Fleming et al. 1986; Viola \textit{et al.} 1986; Buttyan \textit{et al.} 1987; Peehl \textit{et al.} 1987; Eaton \textit{et al.} 1988; Nag and Smith, 1989; Carteret \textit{et al.} 1990a).

Using a series of monoclonal antibodies, it has been observed immunohistochemically that p21\textsuperscript{ras} is predominantly and uniformly expressed within the glandular epithelium of the normal human prostate (Chesa \textit{et al.} 1987). These authors have suggested that the significant levels of p21\textsuperscript{ras} in prostate adenocarcinoma and other solid tumours may reflect cellular differentiation rather than the transforming phenotype - a concept further supported by the demonstration of cytokeratin markers in human prostate adenocarcinomas similar to those present in normal differentiated luminal epithelial (Nagle \textit{et al.} 1987). Interestingly, luminal epithelium cell cytokeratins were also expressed in prostatic adenocarcinomas induced by \textit{ras} and \textit{myc} in the mouse prostate reconstitution model (Thompson \textit{et al.} 1989) which allows the introduction of genes, singly or in combination, via helper-virus free recombinant retroviruses into cells derived from the rudiment of the
fetal prostate gland (fetal urogenital sinus). These cells were then reconstituted and grafted under the renal capsules of normal isogenic male mice. In this model system, the \textit{ras} oncogene alone induced epithelial hyperplasia as well as dysplastic changes in the mesenchyme. The \textit{myc} oncogene alone produced hyperplastic changes in prostatic epithelium, whereas the \textit{ras+myc} combination induced predominantly malignant carcinomas. The \textit{myc}-induced phenotypic alterations may be important in the development of human prostate cancer, since in two separate studies, levels of \textit{c-myc} mRNA were found to be higher in human prostate adenocarcinoma versus BPH (Fleming \textit{et al.} 1986; Buttyan \textit{et al.} 1987). The analysis of steady-state mRNA from a series of human prostate cancer cell lines as well as transplantable rat Dunning prostatic adenocarcinomas demonstrated increased levels of \textit{c-myc} mRNA (Rijnders \textit{et al.} 1985; Cooke \textit{et al.} 1988). Elevated \textit{myc} expression is generally associated with cell proliferation, therefore modest increase in \textit{myc} expression could be circumstantial rather than causal in fast growing tumours.

\textit{HER-2/c-neu} gene represents a family of cellular genes involved in cell growth and differentiation. It encodes for a cell surface glycoprotein (p185\textit{neu}). The \textit{HER-2/neu} gene is the human homolog of the rat proto-oncogene \textit{c-neu} and was first identified in cDNA libraries by homology with the epidermal growth factor receptor (Coussens \textit{et al.} 1985; King \textit{et al.} 1985; Semba \textit{et al.} 1985). Since the initial observation on amplification of \textit{HER-2/neu} proto-oncogene in a human mammary cell line (King \textit{et al.} 1985), subsequent studies have shown that a higher percentage of human breast cancer patients present with an amplification of \textit{HER-2/neu} (Venter \textit{et al.} 1987; Slamon \textit{et al.} 1987 & 1989). Amplification of \textit{HER-2/neu} gene copy number and expression has now been reported to occur in many other human
malignancies, including salivary gland adenocarcinoma (Semba et al. 1985), non-small cell lung carcinoma (Weiner et al. 1990), adenocarcinoma of the stomach and kidney (Yokota et al. 1986), colon cancer (Cohen et al. 1989) and ovarian cancer (Slamon et al. 1989). Breast and ovarian cancer patients with amplification of HER-2/neu gene copy number or expression have a statistically significant reduction in overall survival (Slamon et al. 1987 & 1989).

All these findings imply that there may be properties of the HER-2/neu encoded protein tyrosine kinase (p185(neu)) that contribute to disease progression. Other data also suggest a central role for p185(neu) in aggressive human malignancies. It has been observed that over-expression leads to morphological transformation of NIH-3T3 fibroblasts as well as growth in soft agar, tumour formation in nude mice (DeFiore et al. 1987; Hudziak et al. 1987) and induction of cellular resistance to killing by activated macrophages and tumour necrosis factor-alpha (TNF-α) (Hudziak et al. 1988). In addition, transgenic mice that overexpress the activated c-neu proto-oncogene develop mammary carcinoma (Muller et al. 1988). Also as in the case of the activated c-neu protooncogene, monoclonal antibodies that down-regulate p185(neu) will inhibit the growth of NIH-3T3 fibroblast in soft agar and growth of breast tumour cells that demonstrate HER-2/neu overexpression (Drebin et al. 1985; Hudziak et al. 1987; Hudziak et al. 1988).

Monoclonal antibodies derived against the extracellular domain of p185(neu) also partially reverse the TNF-α resistant phenotype of breast tumour cells overexpressing the HER-2/neu gene product (Hudziak et al. 1989; Fendly et al. 1990). Monoclonal antibodies reactive with this receptor-like protein will inhibit the growth of experimental tumours induced by neu - transformed
cells in nude mice (Drebin et al. 1988). These observations point to a central role of p185 neu in regulating the growth of tumour cells that overexpress it and further suggest that this receptor-like protein may be an excellent target for therapeutic intervention. Not much information exists on the possible expression of this proto-oncogene in prostatic tumours. In a recent study, about 18% of the benign gland was found to be strongly positive for c-neu protein immunohistochemically (Mellon et al. 1992).

2.3. Prostate Markers and Prostatic Growth Disorders:

Antigenic molecules exclusively found in prostatic epithelium that could be used as specific tissue markers are actively being sought. Such molecules have great relevance both in diagnosis and therapy. Two known specific antigens of prostatic origin are prostate specific acid phosphatase (PSAP) and prostate specific antigen (PSA).

PSAP was one of the earliest serum markers for detecting metastatic spread of prostate cancer (Schulman et al. 1964; Nadji et al. 1980; Chiarodo 1991). PSAP has been extensively studied as a marker for prostatic cancer (Schulman et al. 1964; Mercer 1977; Yam 1984) and immunohistochemical demonstration of PSAP has been useful in determining the prostatic origin of metastatic adenocarcinoma (Nadji et al. 1980; Lippert et al. 1982). This marker has been augmented in recent years by prostate specific antigen (Chiarodo 1991).

PSA has been shown to correlate with tumour burden, serves as an indicator of metastatic involvement, and provides an excellent parameter for following the response to surgery, irradiation and androgen ablation therapy in patients with prostate cancer. PSA is a protein unique to prostate. Wang et al
(1979) reported isolation and purification of PSA from prostatic tissue which was both organ-specific and distinct from PSAP. Subsequently PSA was shown to be a useful serum marker in patients with prostatic carcinoma (Kuriyama et al. 1981; Takeuchi et al. 1983). The immunohistochemical localization of PSA (Nadji et al. 1981) can be used to determine the prostatic origin of a tumour. PSA is a serine protease of 34 kDa, belongs to the glandular kallikrein family (Clements 1989), and it exerts its proteolytic activity on the high molecular weight protein of seminal coagulates (Lilja 1985). The PSA gene is closely related to the human glandular kallikrein gene hGK-1 (Schedlich et al. 1987; Henttu and Vihko, 1989) and promoters of both the genes contain a putative steroid response element (Schedlich et al. 1987; Riegman et al. 1989). The physiological role of this protein is, however, not known.

Recently, a novel prostate specific membrane (PSM) glycoprotein Mr 100,000 has been detected by a prostate specific monoclonal antibody raised against the human prostatic carcinoma cell line LNCaP (Abdel-Nabi et al. 1992). The PSM antigen appears to have many interesting and potentially significant properties. As an integral membrane protein unique to prostatic epithelial cell, the antigen or perhaps a specific PSM ligand may serve as an excellent site for use in the imaging and/or targeting of metastatic deposits. Staining with a monoclonal (CYT-358) have been shown to exhibit a strong membrane location with LNCaP cells. Both benign and neoplastic prostate cells stained positively, with more intense staining seen with malignant cells. Lymph node and bone metastases also stain positively with the antibody, with the highest expression seen in hormone-refractory lesions (Axelrod et al. 1992). The molecular cloning of a full length cDNA encoding the Mr.
100,000 prostate specific membrane antigen has been done (Israeli et al. 1993). Antibodies directed against such unique antigen may be useful in tissue diagnosis and serve as new agents for the immunotherapy of prostate cancer.