

The biological nature of prostate cancer – a basis for new treatment approaches

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Introduction

The statistics for prostate cancer make alarming reading and set undoubted challenges for oncological research. In the Western world, where the incidence is highest, a man has a 10–11% chance of developing clinically apparent prostate cancer, and a 3–4% chance of dying from the disease [1]. Worldwide the incidence of prostate cancer is rising annually by 2–3% [2]. In 1986 a quarter of a million cases of prostate cancer were diagnosed and at least half a million cases were expected by the turn of the last century [2,3]. In many countries prostate cancer is now the second leading cause of cancer-related death [4] and in Northern Europe it has already taken number one position as the leading cause of cancer-related death in males [4]. The incidence has increased recently, largely due to better and earlier detection, but also because of the general aging of the world's population and hence an increase in the proportion of men aged over 65 years old in whom the disease is known to be prevalent. In the USA, although signs are that the rate of increase in incidence is falling slightly, it is predicted that in the next 50 years the number of deaths from this disease could increase by 50% [5]. Translated into actual figures these statistics mean that, in the USA in 1999, there were an estimated 179,300 new cases and 37,000 deaths [6]. Similarly, in Europe, assuming that age-specific rates of prostate cancer remain at 1980 levels, the number of men aged over 65 years with prostate cancer is expected to rise from 79,453 in 1990 to 92,240 in 2000. The rise will be most pronounced in those countries with the greatest increase in life expectancy – France, Germany and Spain – and will be further exacerbated as the post-war 'baby-boomers' reach their fifties [5].

Despite its high incidence, knowledge and understanding of the pathophysiology of prostate cancer remains rudimentary. A clearer understanding of the biological nature of the disease could have a real impact on its management. Much progress has been made towards understanding the development and progression of prostate cancer and the factors, which drive the development of androgen independence.

There still remain many enigmas about the pathophysiology of prostate cancer. Evidence indicates that the disease is present histologically many years and even decades before clinically significant prostate cancer can be detected. Sakr et al (1993) showed that prostatic intraepithelial neoplasia (PIN) and foci of histological cancer are present at an early age [7]. They concluded that the factors which initiate clinically significant prostate cancer must occur at a young age and that clinically relevant prostate cancer must develop over a much longer period of time than originally postulated. [7]. There is also some data that suggests a higher prevalence of PIN in African-American men than in Caucasian men [8]. However, it still remains unclear why one in four men aged 40–50 years have evidence of foci in the prostate gland while

only 1:2000 will go on to develop cancer. Cell turnover is known to be high in prostate cancer; even higher than that observed in testicular cancer. However, disease progression is slow; a man may have malignant cells in the prostate gland for years before the disease becomes clinically significant.

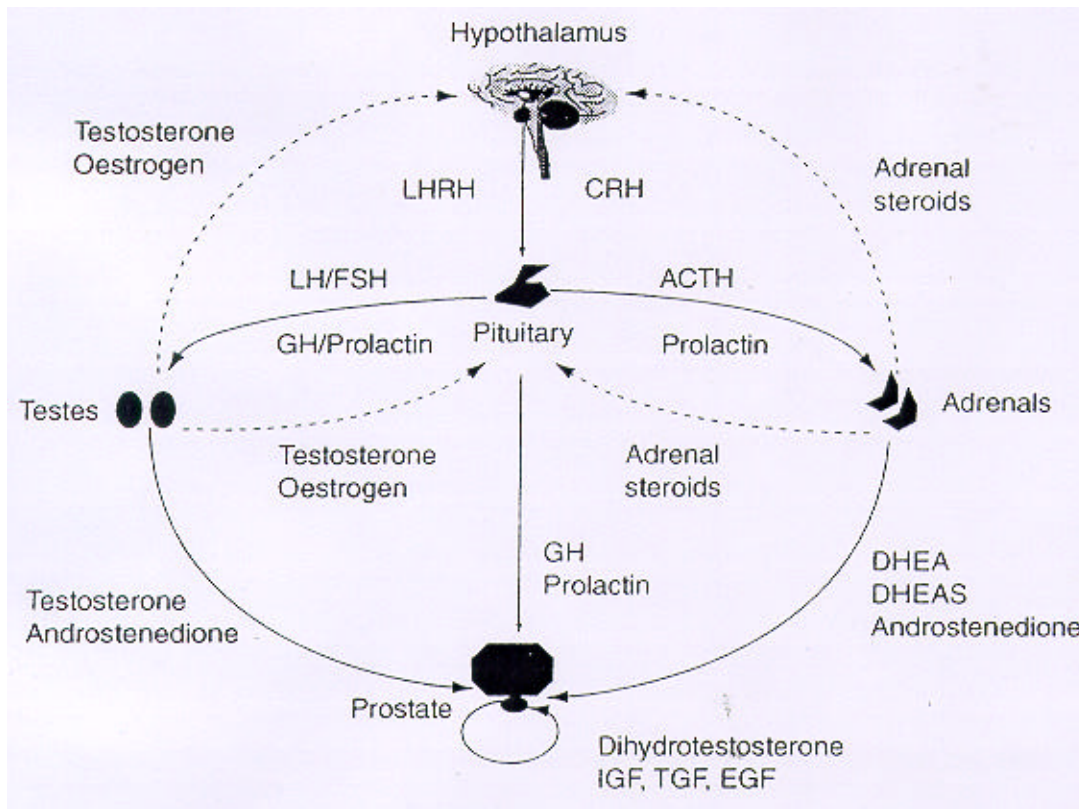
In terms of management, the lack of known markers of tumour aggressiveness to help select patients for more or less aggressive management means that many patients are either over-treated or under-treated. In addition, most patients progress to androgen independence, in which case they become unresponsive to hormonal intervention. At this stage, treatment options are limited to chemotherapy with its concomitant side effects and distressing negative impact on quality of life. Today the factors involved in the development of androgen independence are still being elucidated and therefore it is unclear how to intervene to prevent or delay the process.

Optimal treatment relies on a clear understanding of the disease process, however, it is apparent that there remain significant gaps in knowledge about prostate cancer. A better understanding of the disease is difficult to acquire for the practicing clinician when there is a paucity of reviews summarising the pertinent literature in a way, which is meaningful and on a practical level. This review aims to fulfill this need - to summarise the key facts about the pathophysiology of prostate cancer and highlight their relevance for treatment practice. Although, it is not possible to complete current understanding, but by providing practicing clinicians with the most recent evidence on the biological nature of the disease, we hope to help facilitate more rational decision-making in the treatment of prostate cancer.

Cell biology of the normal prostate

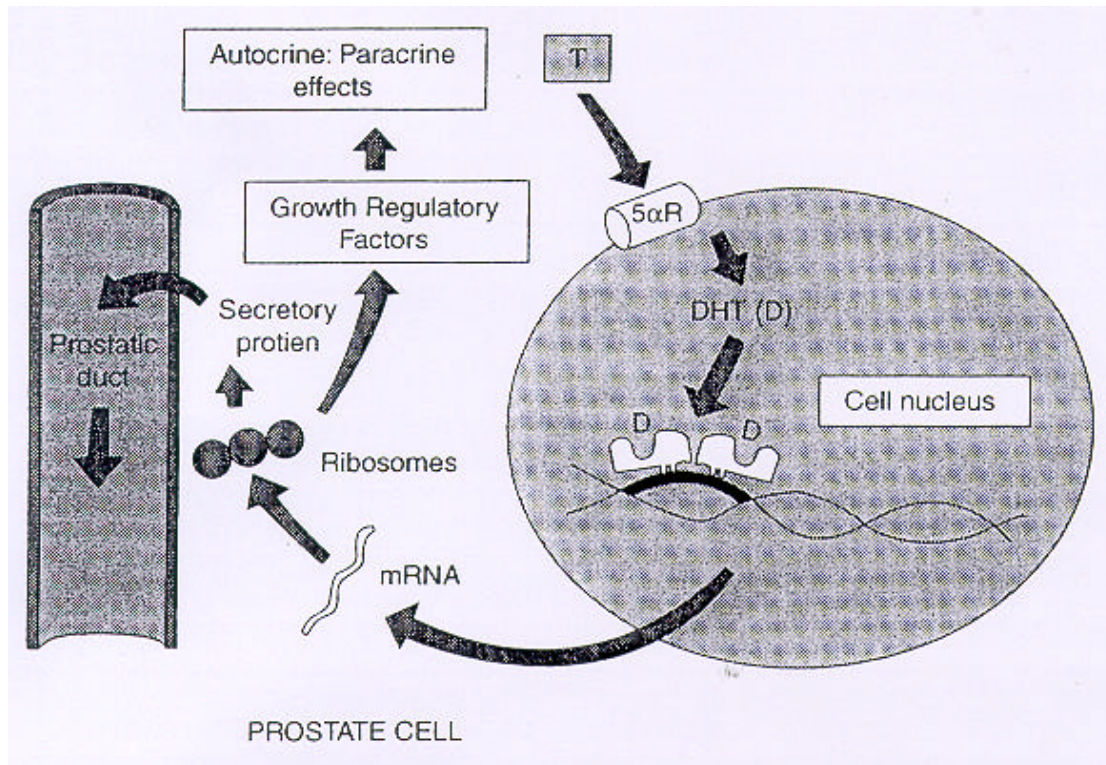
The epithelium of the prostate gland is under the hormonal control of androgens, the production of which is dependent upon the hypothalamic-pituitary-testicular axis (Fig. 1). The hypothalamus episodically secretes luteinizing hormone releasing hormone (LHRH) causing the release of the gonadotrophins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland. LH in turn stimulates testicular Leydig cells to produce the androgen, testosterone, which represents 95% of total androgens produced. The adrenal glands produce the remaining 5% of the body's androgens, under the control of adrenocorticotrophic hormone (ACTH) released from the anterior pituitary gland. The adrenal androgens, androstenedione and dehydroepiandrosterone, are converted to testosterone in peripheral tissues and the prostate gland.

Fig. 1. Hormonal pathways controlling the prostate gland: the hypothalamic-pituitary-gonadal axis



Only a small percentage (about 3%) of circulating testosterone remains free; the majority circulates in the body bound to high-affinity globulin or low-affinity albumin. In the prostate, free testosterone diffuses directly into the epithelial or stromal cells where it is converted into the functionally active androgen, dihydrotestosterone (DHT), by the action of 5- α reductase enzyme system located on the nuclear membrane. DHT becomes bound to the androgen receptor, which after a conformational change that leads to receptor dimerisation, is transported into the nucleus. Inside the nucleus of the cell it binds to the target genes and initiates transcription [9], and thereby ultimately controls the regulation of the cell cycle, cell growth and differentiation [10] (Fig. 2).

Fig. 2. Mechanism of androgen-cell receptor system

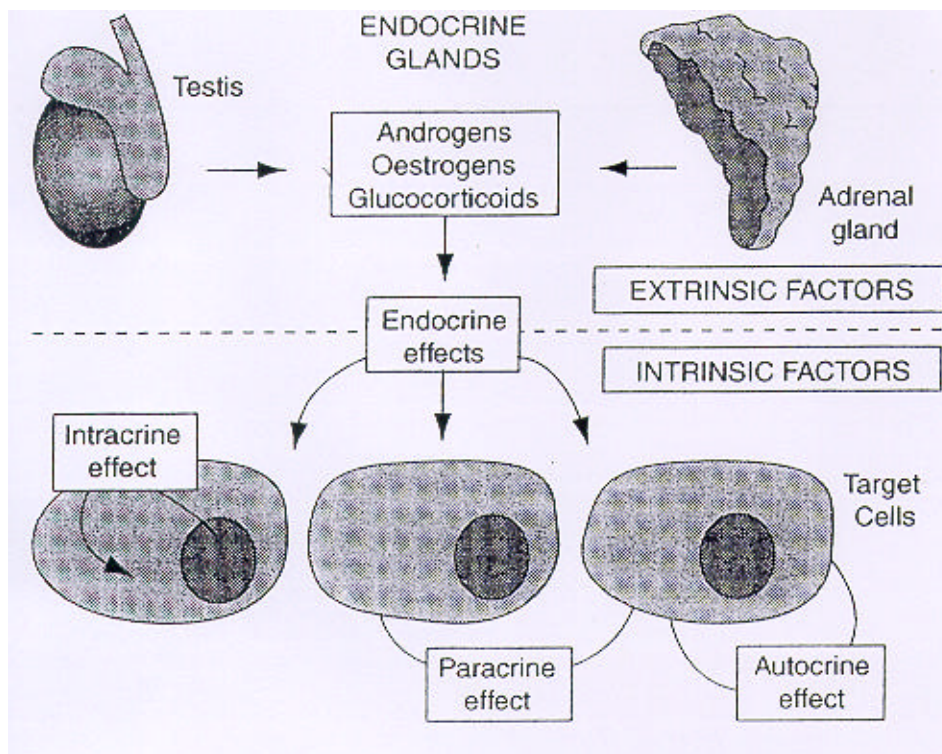


The prostate is not only under endocrine control; the homeostasis of the organ is maintained by several other regulatory factors. Knowledge of these other systems is necessary to arrive at an understanding about what causes prostate cells to become malignant and how to effectively treat the disease.

The connective tissue base or framework of an organ is referred to as the stroma. In the prostate, the interaction between the stroma and epithelial tissue appears to be an important aspect of growth regulation [11]. Epithelial cells are mostly secretory, with the basal component comprising less than 10%. It is the basal cells that are believed to contain a subset of stem cells. Androgen ablation mostly affects the secretory epithelial cells, and can reduce their cell numbers by 90%. In addition, there are the neuroendocrine cells, that are secretory but it has also been postulated that they may act in an autocrine/paracrine fashion. Since neuroendocrine cells do not contain cytokeratin, commonly found in the basal cell layer and urothelium, it has been suggested that these cells are of a different origin from other prostatic epithelial cells [9,12,13,14]. In a recent study, human prostate neuroendocrine cells were found to represent a cell lineage of their own, being of neurogenic origin and therefore distinct from the urogenital sinus-derived prostate secretory and basal cells [13]. The secretory epithelial and neuroendocrine cells may interact in a paracrine fashion with the stroma [5,15].

Paracrine and autocrine communications are localized versions of endocrine control. Whereas endocrine control, primarily via hormones, is effected by molecules secreted into the circulation and transported a considerable distance to the target tissue (e.g. testosterone), paracrine communication is essentially local and restricted to binding to the receptors of adjacent cells. Autocrine secretions on the other hand, stimulate the very cells that secrete them. In normal circumstances, neither paracrine nor autocrine secretions enter into the general circulation (Fig. 3 [16]).

Fig. 3. Endocrine, paracrine and autocrine secretions [16, with permission]



Androgen-dependent tissue growth factors are produced in the stromal cells of the prostate and act on adjacent stromal cells and epithelial cells in a paracrine fashion. They are concerned with the regulation of epithelial proliferation [16]. These include epithelial growth factor (EGF), insulin-like growth factor (IGF), transforming growth factors ($TGF\alpha$, β), and fibroblast growth factor (FGF). Growth factors can have a stimulatory or an inhibitory role to play in the regulation of cell proliferation, and alterations to their secretion and receptors, and a switch between paracrine and autocrine communication, may be contributory factors to the development of prostate cancer [17,18].

Neuroendocrine cells, with the dual properties of endocrine cells and neurons, are widely distributed in normal prostate tissue. There are open and closed cell types. The open cell type has extensions at their

apex that connect with the lumen, and dendritic processes that extend between adjacent cells, resting on the basal lamina and in close topographical relationship with nerves. It is thought that via a variety of secretory products they form a communication network involved in cell regulation [9,19,20, 21].

The main product, chromogranin A, is an excellent marker of neuroendocrine cell differentiation in prostate carcinoma [12] and it also serves as a generic marker of the neuroendocrine cell population. Other commonly found secretory products include serotonin, neuron-specific enolase, calcitonin and other members of the calcitonin gene family, such as calcitonin-gene-related peptide, ketacalcin, a thyroid-stimulating-like peptide, somatostatin and PTHrP [22–27]. Neuroendocrine tumour cells are found at all stages of prostate cancer and are ‘freely’ dispersed throughout the tumour. Independent groups of researchers have shown that neuroendocrine cells lack or do not express the androgen receptor [20,28,29,30]. Several reports have shown an increased number of neuroendocrine tumour cells in advanced tumour stages, high grade versus low-grade tumours and, especially after androgen suppression therapy during tumour progression [12,31].

Apoptosis and angiogenesis are also important in the progression of prostate cancer. It has been postulated that apoptosis is induced when a cell begins to receive confused growth signals as a result of exposure to radiation, chemoradiation, or abnormal cell contacts and changes in growth factors. In prostate cancer, the withdrawal of androgen results in apoptosis in androgen-sensitive cells. Apoptosis is regulated by the ratio of BAX protein, which induces apoptosis and Bcl-2, which suppresses it; overexpression of Bcl-2 blocks apoptosis so that it occurs less frequently in tumours, which express this oncogene.

Angiogenesis enables new blood vessels to grow from existing vasculature. This provides the tumour cells with a blood supply enabling them to grow and proliferate. The process is activated by fibroblast growth factor, platelet-derived growth factor, vascular epidermal growth factor, tissue growth factor beta1 and angiogenin and it is inhibited by thrombospondin and interferons. The tumour suppressor protein p53 increases transcription of thrombospondin and it is postulated that mutation of the p53 gene may result in a reduced production of thrombospondin and corresponding increased angiogenesis.

Prostatic intraepithelial neoplasia

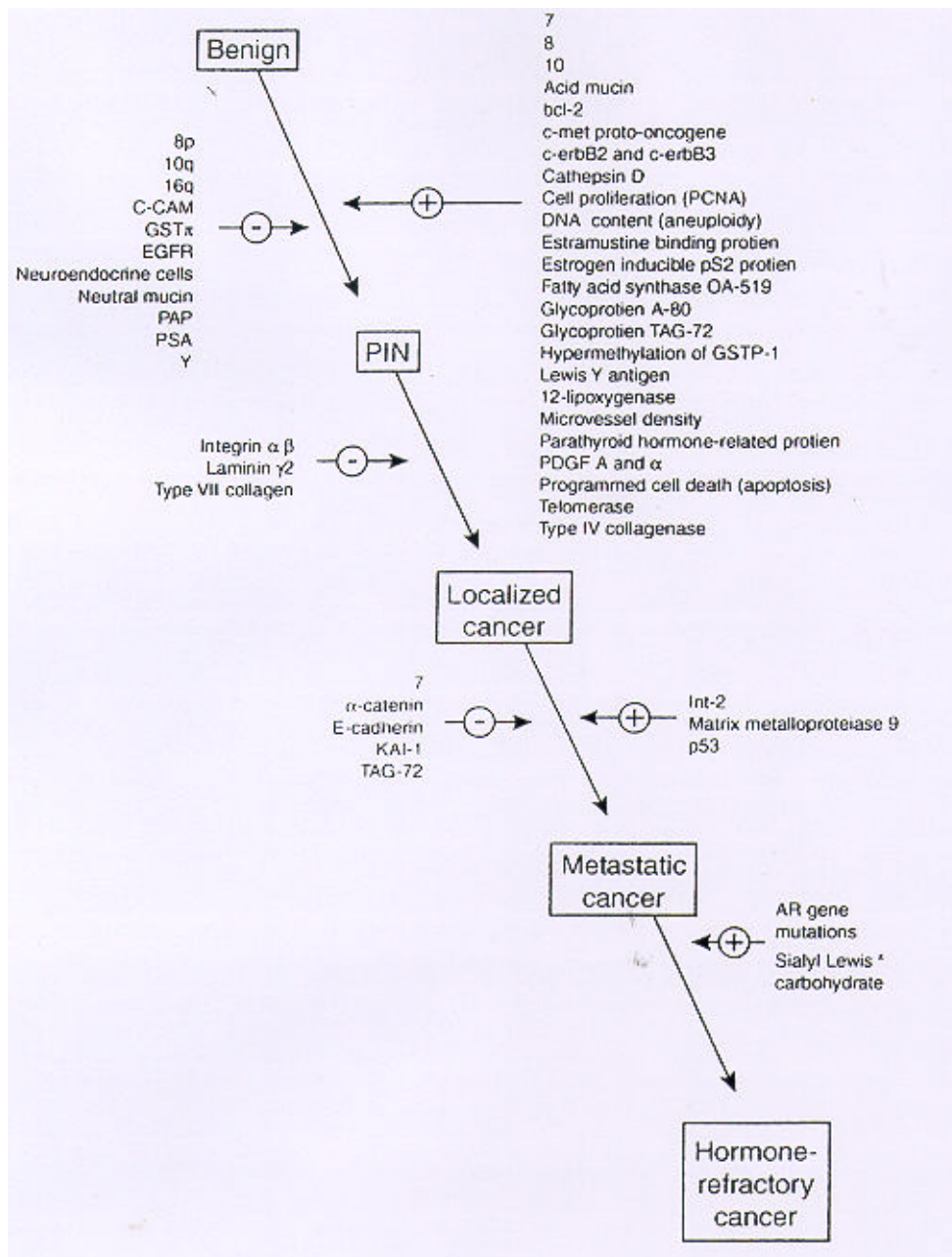
An overwhelming proportion (99%) of all prostate cancers are adenocarcinomas, and the majority (70%) appears in the peripheral zone of the epithelium. Malignancy occurs due to the breakdown of local regulatory control and the development of a degree of autonomy on the part of the cancerous cells, such

that they no longer react or respond to the normal signals. A condition called PIN appears to be a pre-malignant lesion that is associated with progressive abnormalities and this represents an intermediate step between the benign and invasive (malignant) state indicative of the early stages of release from normal growth control [32]. Two grades of PIN are identified (low grade and high grade). High-grade PIN is characterised by high expression of EGF, TGF- α , and c-erbB-2, together with a low expression of FGF receptors, all similar to that found in cancer. This is in contrast to the situation found with either low-grade PIN or a benign condition of the prostate called benign prostatic hyperplasia (BPH). There is also a high level of cell proliferation outside the basal cell layer, and several phenotypic alterations to cells in PIN lesions. These include the development of a more anaplastic morphology, nuclear pleomorphism, increased cell motility and angiogenesis. PIN, like prostate carcinoma, is more frequently located in the peripheral zone of the prostate gland [33]. There is now convincing evidence that PIN is a precursor of prostate carcinoma [34], and that it precedes prostate cancer by 10 years or more [35].

Loss of regulatory control through genetic changes

The alteration of a normal cell into the malignant state represents its escape from normal regulatory controls, and a new set of cellular capabilities develop. The enormity of these changes that enable a cancer cell to move, leave its original organ, invade new tissue and develop cannot be underestimated (Fig. 4 [32]). The fact that most cancers eventually produce cells with these capabilities highlights the survival advantage of malignant cells. Even so, it has been reported that only one in every 100,000 cancer cells that break way from the primary and enter the circulation will survive [9].

Fig. 4. The route a prostate cancer cell takes to establish metastases [32]



The foundation of these major changes to a malignant state can often be linked to alterations in the genetic make-up of the cell [36]. These changes may not be uniform in the primary tumour and this will result in phenotypically distinct metastases being produced [9]. This might explain the variety in response seen between patients to various types of hormonal therapy. Several genes and oncogenes are involved with these alterations in cellular regulation and, as discussed below, mutations in many of them appear to be behind the appearance of the androgen-independent state in prostate cancer (Table 1). It is probable that these mutations are already present in a subset of cells of the primary tumor, which has been described as “biologically heterogeneous and genetically unstable” [37] and that cells with these mutations become the dominant type through clonal selection [38].

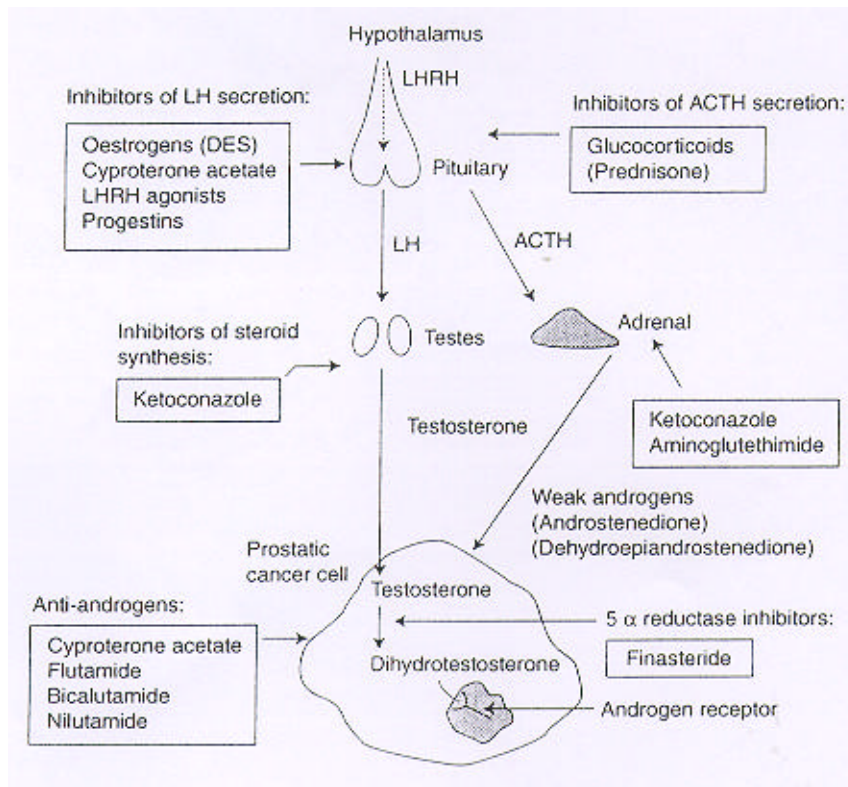
Table 1. Genes relevant to prostate cancer

<i>Genes</i>	<i>Function</i>	<i>Role in Prostate Cancer</i>
<i>Ras oncogenes</i>	A group or family of oncogenes; mutations in the ras oncogenes cause abnormal proliferation of affected cells	
<i>Bcl-2 proto-oncogene</i>	Produces the protein Bcl-2 that regulates apoptosis in balance with another protein BAX	Overexpression in androgen-independent tumors, prevents apoptosis
<i>p53</i>	Sometimes called ‘guardian of the genome’, in normal cells its transient expression allows it to coordinate changes in proliferation and apoptosis	Mutation removes restraint on cell proliferation and angiogenesis
<i>c-myc oncogene</i>	Codes for transcription factors that amplify regulatory signals	
<i>retinoblastoma</i>	In normal cells Rb codes for a phosphoprotein that inhibits proliferation at G ₁ /S boundary	

Prostate cancer – the progression to hormone independence

As the prostate is under hormonal control, when a tumor develops it should be possible to control or inhibit its growth by manipulating the normal hormonal environment of the organ. The fact that prostate cancer is hormone responsive has been known for a long time, and the withdrawal of androgen induces apoptosis in normal and malignant prostate epithelial cells alike. Androgen responsiveness is probably a stochastic process (continuously variable), but it is most convenient to define three phenotypes: androgen-dependent, androgen-sensitive and androgen-independent. In the early stages, when the tumor is localized in the prostate the androgen-dependent cells are predominant, and the various therapies aimed at androgen depletion are successful. Their sites of action are shown in Fig. 5 [39].

Fig. 5. Control of normal prostatic cell growth and site of action of therapies [39]



Androgen depletion is not usually able to eliminate all the cancer cells and over time, via selective growth of pre-existing clones, an androgen-independent population emerges and ultimately predominates. This progression to androgen-independence also appears to involve adaptive up-regulation of genes that help cells survive and grow after androgen ablation, together with mutation to the androgen receptor and its interaction with other transcriptional factors.

Patients who develop resistance to androgen withdrawal therapies do so not because of a loss of androgen receptors as these continue to be expressed [40]. Instead, mutations occur in the androgen receptor gene; as it is x-linked, only one allele needs to be mutated for phenotypic alteration [41]. Somatic point mutations, all in the hormone-binding domain, have been found in 50% of metastatic lesions of androgen-independent prostate cancer; they were not detected in tissues taken from primary cancers [42]. These mutations probably provide a selective growth advantage to a pre-existing clone of androgen-independent cells, which can begin to predominate and spread under conditions of androgen-deprivation [43,44]. A high number of mutations are found in the bone metastases of patients who are no longer responsive to endocrine therapy [42]. Some mutant receptors are responsive to progesterone and oestrogen [41]. These results match in-vitro observations in which point mutations in the androgen receptor

binding domain in a human prostate cell line had high binding affinities for steroid hormones (oestrogen, progesterone) and anti-androgens, both steroidal (cyproterone) and non-steroidal (flutamide, nilutamide) [43]. Once bound to the receptor, these compounds all had the same stimulative effect as androgens. The authors of this in-vitro study argued that the presence of such mutations *in vivo*, acting as partial agonists to antiandrogens, might explain the flutamide withdrawal response discussed below [44].

It appears that down-regulation of the androgen receptor gene does not occur in hormone-independent prostate cancer cells. Instead, amplification takes place, which allows the receptor to become super sensitive to very low levels of androgens, and this could be the reason why these tumour cells are able to multiply with incomplete androgen blockade, when only the testicular source of androgens has been removed [45]. Over amplification of the gene in prostate cancer cells has been shown after androgen withdrawal [46]. One-third of hormone-independent or refractory cancers have amplification of the androgen receptor gene, suggesting that this and overexpression (adaptive 'up-regulation') are involved in the emergence of the hormone-independent state [47].

Not all the changes that induce androgen independence are due to alterations to the androgen receptor; androgen receptor mutants are found in only a subset of cells. Recently, the role of the deregulation of apoptosis in the development of androgen-independence has received a lot of attention. Apoptosis is an essential process for success in killing prostate cancer cells and ultimately reducing the volume of the primary and number of metastatic lesions. It is regulated by a balancing act between two or more protein products, including one from the suppressor BAX and the other from the Bcl-2 proto-oncogene. Following castration-induced androgen deprivation, androgen-dependent prostate cells undergo apoptosis, but androgen-independent prostate cells do not. This ability to avoid cell death appears attributable in part to an overexpression of Bcl-2. This mutational event is known to inhibit apoptosis and is highly correlated with cancer progression and androgen-independence; mutations are infrequent in early stage cancer, but frequently detected at the hormone-refractory stage [47–49]. One study showed that the number of cells in tumours exhibiting these high levels of expression increased from 7% in the localised tumour, to 17% in metastatic cells that were still androgen-dependent, to 67% in tumours that were androgen-independent [50]. Although Bcl-2 protein is not involved directly in regulating cellular proliferation, it can do so indirectly via its involvement in the regulation of microtubule function, which is essential for cell division [51]. Several chemotherapeutic drugs used for hormone-refractory prostate cancers, are inhibitors of microtubule

formation. For example paclitaxel and vinblastine are able, via a process of phosphorylation, to inactivate Bcl-2 and induce cell death [52].

Other genetic mutations also play a part in the progression of prostate cancer. The introduction of an activated *Ras* oncogene into a prostate cell line will bestow androgen-independence [53]. Mutations to the tumour suppressor *p53* gene have a low frequency in early-stage or organ-confined prostate cancer, but the frequency increases with disease progression and is highest when the cancer becomes hormone-refractory [47,53,54]. Focal *p53* expression in the primary tumour is related to the probability of disease recurrence following radical prostatectomy [47]. The *p53* gene is concerned with the regulation of the cell cycle, apoptosis, and has a stimulatory effect on thrombospondin production (an angiogenesis-inhibitor). Mutation would affect all these areas, removing the restraint on cell proliferation and encourage angiogenesis and metastatic spread. Another 'tumour suppressor' is the retinoblastoma (*rb*) gene. Its inactivation also appears to be connected with tumour progression and again, is associated with the loss of a restraining influence on proliferation [55]. Other research has highlighted the existence of metastasis-suppressor genes in prostate cancers and in particular, that decreased expression of KA11 and CD44 is correlated with an increased malignant potential [56].

Peptide growth factors are secreted in normal prostate cell tissue, and act as regulators (both stimulators and inhibitors) of cell growth and function. Changes in their relative balance are implicated in the progression of prostate cancer. EGF is a mitogen produced in the normal prostate and acts as a paracrine stimulator of stromal and epithelial cells. It appears that, in androgen-independent tumours, autocrine stimulation may become more important which, via EGF, could also lead to unrestrained growth [57]. Interestingly, it has been reported that there is an overexpression of EGF receptors in the neuroendocrine phenotype of tumour cells [27].

Other growth factors that show alterations as prostate cancers progress include TGF- β and IGF I and IGF II. TGF- β in normal conditions is a growth inhibitor. However, as prostate cancer progresses, the cells become less sensitive to this controlling influence, which may be due to a loss of TGF- β receptors in the tumour [58]. The influence of IGF also appears, like EGF, to change because of a move towards autocrine stimulation, with the result that in androgen-independent situations, the cells can proliferate in an uncontrolled manner [59].

Treatment options for hormone-independent prostate cancer

Despite initial success with androgen ablation therapies, it is still true for the vast majority of cancers that they will grow and progress. How can these changes be used to ensure the best benefits for prolongation of survival, while maintaining a high quality of life for the patients? At this stage, unfortunately, treatment with curative intent is usually not an option. A change in prostate-specific antigen (PSA) level is the most accurate and popular method of monitoring response to therapies both in a clinical trial setting and in routine practice. A decline in PSA value of more than 50% is generally considered to represent a response to therapy in hormone-refractory disease [60]. Furthermore this decline appears to have a bearing on survival, as patients with a PSA reduction $\geq 50\%$ have significantly longer median survival times than those with a PSA reduction of $< 50\%$. However, PSA does not always reflect response to treatment in advanced prostate cancer, especially not hormone-related prostate cancer. Therefore, an additional surrogate marker, such as chromogranin A, which reflects neuroendocrine differentiation in mainly hormonal prostate cancer, is usually needed [61].

It is important to determine whether patients who have disease progression following the failure of surgical or medical castration (e.g. with luteinizing hormone releasing hormone agonists [LHRHa] alone) as first-line hormonal therapy, have castrate levels of testosterone. Orchiectomy does not always abolish testosterone production, probably because not all the testicular tissue was removed [61]. The addition of an anti-androgen at this point will produce a response in approximately a third of the patients, but it is usually only short lived, generally between 3 and 6 months [62]. After the second failure, the anti-androgen should be discontinued, as its withdrawal at this point is associated with a significant clinical benefit in about a third of patients. This phenomenon known as ‘endocrine withdrawal syndrome’ generally lasts 4 to 6 months, and is associated with a fall in PSA levels over this period. It was first described in patients receiving flutamide [63], but has since been reported with bicalutamide [64], steroidal antiandrogens (e.g. megestrol acetate) [65], and oestrogens (DES) [66]. Mutations in the androgen receptors probably alter responses to anti-androgens, for example, by increasing their sensitivity [67]. Although the molecular basis for this phenomenon is not established, anti-androgen withdrawal is a recommended treatment option for patients who develop hormone-refractory disease. This syndrome should be taken into account when designing future trials [68]; it may be that in some past studies, responses attributed to an experimental drug were actually due to the prior withdrawal of anti-androgen before starting the trial [44].

When anti-androgens are withdrawn, it is recommended that treatment with LHRHa should continue, as there is evidence they are of benefit, even though theoretically the cancer is no longer androgen-dependent. This is based on two observations. Firstly, tumour cells may become reactivated if testosterone levels rise again following the removal of a block on their synthesis by the presence of the LHRHa [69,70]. Secondly, androgen-sensitive cells persist in the treated tumours [70,71]. Two recent retrospective trials comparing survival times between those who continued androgen deprivation (either by means of LHRHa or orchidectomy) and those who did not, reached opposite conclusions. In one trial, patients had a median survival advantage of 2–6 months following androgen deprivation [72]; in the other, no survival advantage was demonstrated [73]. However, the retrospective nature of the trials makes definitive interpretation difficult in view of possible selection bias and the absence of stratification of patients by prognostic factors. Results from prospective randomized trials are needed to answer these questions, but general clinical practice is to maintain existing androgen ablation treatment.

Despite the fact that androgen-independent cells become more and more prominent, there is a great deal of heterogeneity amongst tumours that have reached the hormone-refractory stage and patients will retain hormone-sensitivity to a greater or lesser extent [74]. In view of this, it is worth considering additional or secondary hormonal manipulations for patients who have failed previous hormonal intervention. This can be approached in different ways.

Based on the knowledge that different antiandrogens interact in different ways with the androgen receptor, and have variable half-lives and chemical structures, studies have examined the benefits of employing a second anti-androgen after failure with the first. Some success, as measured by a PSA response, has been found in patients who were given bicalutamide after flutamide withdrawal [75]. The converse situation has not yet been reported.

Steroidal anti-androgens, megestrol acetate and cyproterone acetate, can also be used at this point. These drugs achieve an effect in three ways: they competitively block androgen binding to its receptors; they inhibit LH secretion from the anterior pituitary; and they prevent steroidogenesis (Fig. 5). A recent trial comparing low-dose and high-dose megestrol acetate in hormone-refractory patients showed an objective response in less than 2% of patients. However 14% of patients had a PSA response, which was associated with a tendency towards improved survival (17.4 months versus 12.7 months). The overall median survival was reported as similar to historical controls [76].

There is a likelihood of some residual hormone-sensitivity, so further adrenal androgen suppressors could also be tried at this point. The two main choices, aminoglutethiamide and ketoconazole, are both inhibitors of adrenal and gonadal steroidogenesis. However, their use has sometimes been limited because they prevent all forms of steroidogenesis, not just androgen synthesis, and their administration requires corticosteroid supplementation. Side effects associated with ketoconazole mean that liarozole is often recommended instead. Nevertheless, reports looking at their use at the time of anti-androgen withdrawal have shown encouraging results. PSA response rates of 48% in patients treated with aminoglutethiamide and hydrocortisone at the time of anti-androgen withdrawal, were considerably higher than those achieved in the group undergoing anti-androgen withdrawal alone [77]. Even higher PSA response rates (80%) have been reported for patients already receiving aminoglutethiamide and hydrocortisone before flutamide withdrawal [67]. In addition, PSA response rates of 65.5% have been reported in a group of 50 patients treated with ketoconazole and hydrocortisone after flutamide withdrawal [78].

Corticosteroids have also been assessed for their use as a possible therapeutic option for patients with hormone-refractory prostate cancer [74]. It is believed that two mechanisms of action may be involved: an anti-inflammatory role can be seen in improved pain relief, and a reflex inhibition of adrenal steroidogenesis, which has obvious consequences on adrenal androgen production. A recent randomized trial compared the steroid, prednisone, with the combination of prednisone and the cytotoxic antibiotic mitoxantrone, in patients with hormone-refractory prostate cancer. There was no difference in survival between the two groups, but the combined therapy group had better quality-of-life scores. A decrease in PSA levels of over 50% was reported in 22% of patients who received only prednisone [79]. Some work has been done looking at the efficacy of glucocorticoid administration while controlling for a possible anti-androgen 'withdrawal' effect, but with conflicting results [80]. Any therapeutic benefit with corticosteroids should always be treated with caution as they have often been reported in conjunction with other agents, and an individual benefit cannot always be attributed to the corticosteroids *per se*. However, a corollary to this is that their impact should always be considered when designing trials of combination therapies that include them because they themselves have a bearing on PSA progression, quality of life and possibly even on objective response [80].

Oestrogens delivered intravenously at high doses as cytotoxic agents have had limited success in second-line hormonal therapy. General worsening of morbidity, and cardiovascular side effects have been reported [81]. More recently the benefit of total androgen ablation with either orchidectomy or the

LHRHa, triptorelin, plus the antiandrogen, flutamide, has been compared with parenteral oestrogen in the treatment of 915 patients with prostate carcinoma. This study indicated that the two treatments had comparable efficacy and cardiovascular safety. The authors concluded that parenteral oestrogen should therefore remain a therapeutic option in prostate carcinoma since it is a more cost-effective option compared with total androgen ablation [82].

Delaying the progression to hormone-refractory disease

LHRHa have one great advantage over orchiectomy in androgen ablation; their use is reversible. The huge benefit this may have to the treatment of patients has only recently been realised and developed in the past few years. This has given rise to the concept of intermittent therapy in which patients intersperse periods of therapy with therapy-free periods. The rationale behind its introduction is based on the observation that repetitive induction of apoptosis can be produced by the periodic interruption of androgen-blockade therapy [83]. This is probably due to the fact that when androgen levels rise after anti-androgen withdrawal, the prostate epithelial cell's sensitivity to androgen withdrawal returns because it is forced into the normal pathway of differentiation [84,85]. The hope is that maintenance of the apoptotic potential through successive periods on and off treatment will be reflected in a delay to tumour progression. This therapeutic approach has been the subject of two recent reviews [86,87]. Early reports have been encouraging and some patients have been able to remain off therapy for up to 40 weeks before a rise in PSA level appears [83]. This study also indicated that intermittent therapy was as good as continuous androgen blockade with respect to the median time to progression and survival. Prospective randomised phase III trials are underway to compare intermittent with continuous therapy and to study their effects on survival. The long-term benefits are not known. Short-term, the benefits include an improved quality of life (with an improved sense of well being and a return to potency as the testes begin to function and produce testosterone), reduced cost of treatment, and less cumulative drug toxicity. In addition, the retention of the androgen-dependent state of the tumour opens the possibility of interspersing with other treatment [87].

Ideally, androgen withdrawal therapy should be continued for the period that is necessary to maximise apoptosis of the tumour and then stopped at the point just before an androgen-independent cell appears. If therapy were withdrawn too early, cells programmed to undergo apoptosis would survive as the testosterone levels increased again. After withdrawal there is an immediate and rapid fall of PSA levels as androgen-regulated PSA gene expression ceases, followed by a more gentle decrease as the tumour

volume declines due to apoptosis [86,87]. The PSA nadir can take up to 8 months to achieve in most patients with stage D2 prostate cancer. Timing the re-introduction of therapy is rather more problematic and until more is known and possible prognostic indicators identified, it is rather an individualised matter and down to a balancing act between various clinical factors. For example, a clinician will take into account: pre-treatment PSA levels, PSA velocity, tumour stage and current symptoms. At present, insufficient trials have been done to address these factors and provide information on the length or the number of cycles feasible, although multiple cycles have been reported [87]. The optimal candidates for intermittent androgen suppression (IAS) are probably not those with stage D2 prostate cancer, but rather patients with biochemical failure after treatment with curative intent, i.e. radical prostatectomy or radiotherapy, and locally advanced disease.

Chemotherapy and palliative care

Once the patient no longer responds to any of these second-line hormonal alternatives (as signified by rapidly rising PSA levels, LDH suggestive of visceral metastases, or increase in number or size of metastases) then the patient is truly in a hormone-refractory condition. The only non-experimental option available now is chemotherapy. This treatment modality, so successful in many other types of cancers, is usually of limited benefit to patients at advanced stages of prostate cancer. This is partly due to the fact that most patients are extremely ill by the time this therapy is introduced. Recently, however, certain combinations have shown limited promise in clinical trials. For example, mitoxantrone in combination with prednisone was superior to prednisone alone in terms of pain control and the duration of control (a median of 43 weeks versus 18 weeks, respectively). The combined drug group had a higher probability of PSA reduction, and better quality of life. However, no difference was found in overall survival [79]. A trial comparing mitoxantrone and hydrocortisone versus hydrocortisone alone showed similar results [88]. Other drugs commonly used at this stage include established agents, such as estramustine and etoposide, as well as more recently developed drugs like the anti-microtubule taxanes (paclitaxel, docetaxel), and retinoid acid metabolism inhibitors (liarozole); many of these are undergoing comparative clinical trials [89–93].

More recently the role of somatostatin in the pathophysiology and treatment of cancer has been explored. Somatostatin is a family of regulatory peptides produced by neuroendocrine, inflammatory and immune cells throughout the central nervous system and in most major peripheral organs. In addition, many tumour cells, when activated, produce somatostatin. Two bioactive peptides are produced by different

somatostatin cells – SST-14 and SST-28. These peptides act in several different ways on five somatostatin receptor subtypes found in varying densities in the brain, gut, pituitary, endocrine and exocrine pancreas, adrenals, kidneys, thyroid and immune cells as well as a number of tumour cell lines, including prostate.

The function of somatostatin depends on its location in the body. It can act as an endocrine hormone, in paracrine/autocrine regulation or as a neurotransmitter. The diverse effects of somatostatin can largely be explained by inhibition of two important cell processes: cell secretion and cell proliferation. Endogenous somatostatin is produced locally and is rapidly inactivated, thereby minimizing any unwanted systemic effects. In contrast, exogenously administered somatostatin produces a wide range of effects because it activates multiple target sites of action. Therefore, a number of selective peptide somatostatin analogues have been developed for clinical use. More recently, selective non-peptide agonists have been developed for four of the somatostatin receptor subtypes. This is an important advance since it could help shed further light on the physiological functions of the different receptor subtypes and could be the starting point for the development of orally active therapeutic compounds with selective action on specific somatostatin receptor subtypes. Peptide antagonists have also been discovered for SSTR2 and SSTR5 receptor subtypes.

Somatostatin has antiproliferative actions in normally dividing cells, such as the intestinal mucosal cells, immune cells and inflammatory cells and in solid tumours, including prostate tumours. It prevents cell proliferation by inducing cell cycle arrest and apoptosis. These effects are believed to be mediated by somatostatin receptors on tumour cells and indirectly by receptors on non-tumour-cell targets which inhibit the secretion of hormones and growth factors involved in promoting tumour cell growth, inhibiting angiogenesis, promoting vasoconstriction and modulating immune cell function. Four receptor subtypes are involved in induction of cell cycle arrest via protein tyrosine phosphatase (PTP)-dependent modulation of MAPK associated with induction of retinoblastoma tumour suppressor protein and p21. One receptor subtype (SSTR3) is believed to trigger apoptosis and to activate p53 and the pro-apoptotic protein BAX. It is clear, therefore, that somatostatin has an important role in tumour development and in the future there may be a potential role for somatostatin analogues in the treatment of the disease [94].

The question of whether to continue with endocrine therapy during chemotherapy has received attention. A recent report has shown that in some cases, re-starting LHRHa (goserelin) or oestrogen (DES) therapy in patients receiving chemotherapy produced a response as measured by falls in PSA levels. This study concluded that continued androgen deprivation was essential for some patients to achieve a response to chemotherapy [95].

At this stage palliative care, to maintain the highest possible quality-of-life, is of paramount importance. Pain management using analgesics and radiotherapy can help control the inevitable pain from bone metastases, although there is concern about myelosuppression and the possible effects this may have if chemotherapy is introduced later [96,97]. The addition of the radionucleotide, strontium, to external beam radiotherapy appears to prolong the time to relapse and a further course of radiotherapy in patients with hormone-refractory metastatic prostate cancer. This translates into significant lifetime cost savings even accounting for the additional cost of strontium to the treatment regimen [98]. Bisphosphonates, powerful inhibitors of osteoclast-mediated bone resorption, are promising new agents for the treatment of painful bone lesions in prostate cancer patients [96,97]. Preliminary evidence from phase II trials indicates that bisphosphonates might convey a benefit to patients with hormone-refractory prostate cancer [99].

Novel therapies

It will be apparent from this review that there is no clear and standard treatment for metastatic prostate cancer, particularly after the failure of first-line therapy. We have seen how recent research developments in molecular biology and pathophysiology of prostate cancer is advancing our understanding of the rationale behind the success of existing therapies. It will also, hopefully, lead to the development of entirely new therapeutic modalities, to help inhibit tumour cell proliferation.

Novel approaches currently being tested in early clinical trials include angiogenesis inhibitors, immunological therapies, gene therapy and differentiation therapies (Table 2). Interference in growth factor mediated pathways is another new strategy in the treatment of cancer (Table 2). For example, suramin can block the binding of several growth factors to their receptors. Newly developed somatostatin analogues may also be useful agents in the treatment of prostate cancer [100,101]. Potential mechanisms of antitumour action include suppression of circulating levels of trophic hormones and growth factors as well as direct effects at the tumour level, potentially involving autocrine/paracrine mechanisms.

Table 2. Future management options for prostate cancer

<i>Biological Area</i>	<i>Rationale</i>	<i>Developmental Stage</i>	<i>Comment</i>
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<i>Growth factor inhibitors</i>			
Suramin	Binds several growth factors (FGF, EGF, TGF β 1,PDGF); blocks receptor binding. This interferes with autocrine loops, cell migration, invasiveness, angiogenesis and EGF-stimulated cell proliferation. It also blocks signal transduction by growth factor and inhibits receptor signaling and tumor promotion	Subjective response in 30%, objective response 15%; duration response <1 year; another trial 75% response rate. Recent randomized study controlling for use of hydrocortisone has shown promise	Concomitant use of hydrocortisone confounds results; relative merits of drug are controversial. Toxicity (fatigue, renal impairment, axonal neuropathy) may limit use. Diarrhoea a major problem in long-term use
Somatostatin	Somatostatin analogues: reduce levels of endocrine (IGF-1) and paracrine growth factor products	Somatostatin analogue (<i>somatuline</i>): early results have been mixed	
Bombesin/GRP	Bombesin/GRP-like peptides can stimulate the growth of human prostate cancer. Therefore, binding of bombesin receptor antagonists or new targeted cytotoxic bombesin analogues could be considered for therapy		
Serotonin antagonists	Serotonin (5-hydroxytryptamine, 5-HT), a known mitogen, is involved in the growth of prostate tumour cells and may a target for treatment		
<i>5α-reductase inhibitors</i>			
Finasteride Episteride	DHT is by far the most important stimulus for prostatic cell proliferation; its production involves the enzyme 5 α -reductase. Finasteride is a competitive inhibitor that does not bind to androgen receptor	Finasteride plus flutamide has reduced PSA levels, but not to levels of medical or surgical castration. As monotherapy, has shown ability to delay PSA rise after radical prostatectomy	5–10% incidence of impotence and ejaculatory problems; 5 year therapeutic values show no other serious side effects. Possible prophylactic role; removal of DHT has little toxicity

Table 2. Future management options for prostate cancer (continued)

<i>Biological Area</i>	<i>Rationale</i>	<i>Developmental Stage</i>	<i>Comment</i>
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<i>Differentiation agents</i>	Activate peroxisome proliferator	Trials are ongoing
Phenylacetate	activated receptor-gamma and inhibit	
Phenylbutyrate	cell proliferation in androgen-dependent cell lines; phenotypically differentiates some into nonmalignant form	
Troglitazone	Diabetes drug that binds peroxisome proliferator activated receptor-gamma	

<i>Inhibitors of cyclin-dependent kinases</i>	A family of cyclin dependent kinases mediate progression of the cell cycle.	Phase II studies underway
Flavopiridol	Favopiridol shown to decrease cell proliferation by halting cells in G1 or G2 <i>in vitro</i> and mouse xenograft model.	

Table 2. Future management options for prostate cancer (continued)

<i>Biological Area</i>	<i>Rationale</i>	<i>Developmental Stage</i>	<i>Comment</i>
<i>Apoptosis activators</i>	Castration-induced apoptosis is preceded by increase of intracellular calcium		
Nifedipine	Blocks influx of calcium and inhibits apoptosis		
Calcium ionophores	Elevate intracellular calcium levels, rapidly induce apoptotic cascade		
Paclitaxel	Induces bcl-2 phosphorylation and apoptosis		
Bcl-2 oncogene	Overexpression prevents apoptosis	Use of anti-sense TRPM-2	Elevated levels of clusterin and bcl-2 may aid the survival of prostate cells in an androgen-depleted environment. This could be exploited to augment the benefits of intermittent therapy
Clusterin	Anticytolytic protein encoded by TRPM-2 gene. Highly expressed in cells undergoing apoptosis; androgen withdrawal promotes its up-regulation	2 would inhibit clusterin production	
<i>Antiangiogenesis agents</i>	Inhibit the factors that promote angiogenesis		
Thalidomide		Thalidomide and TNP-470 in clinical studies	
TNP-470			
Endostatin		Endostatin and angiostatin: still being tested on animals	
Angiostatin			

Table 2. Future management options for prostate cancer (continued)

<i>Biological Area</i>	<i>Rationale</i>	<i>Developmental Stage</i>	<i>Comment</i>
Cell motility	Enzymes that facilitate migration of	Marimistat: phase II	
Matrix metalloproteinases (Marimistat)	tumor cells through extracellular matrix.	studies planned	
Gene therapy	Various genes can cause death of the cells into which they are introduced, either directly or by stimulating immune mechanisms. The key to this approach is to develop strategies that allow insertion of specific genes into prostate cancer cells. One method is to use viruses that seek cells producing prostate-specific antigen to insert these "suicide" genes		
<i>Immunotherapy</i>	Induce immune cells to cause tumor regression, e.g via an immunostimulatory gene or specific tumor antigen. PSA is a promising target molecule for induction of a specific immune response; the PSA gene is expressed predominantly in prostatic tissue	Recombinant vaccinia virus encoding for PSA gene is in development	
	Peptide-based and dendritic cell vaccines, which enhance the immune system	In clinical trials, 1/4 of patients in both Phase I & II studies showed some improvement and the disease remained stable in another 1/3	

Key: fibroblast growth factor (FGF); platelet-derived growth factor (PDGF); vascular epidermal growth factor (EGF); tissue growth factor (TGF β 1); insulin growth factor (IGF); dihydrotestosterone (DHT); prostate-specific antigen (PSA)

Conclusions

A combination of factors may trigger the development of the hormone-resistant stage of prostate cancer. Androgen ablation, introduced as an initial means of controlling the cancer, ultimately selects for a population of androgen-resistant cells, which become the dominant type in the tumour. This resistance is due to mutations of the androgen receptor that alter its sensitivity to hormones and allow it to spread and resist further hormonal intervention. These mutations also interfere with the process of apoptosis, an essential event for reducing tumour burden.

Several hormonal interventions can still be tried at this point, however, as the tumours do retain a degree of hormone-sensitivity. Therefore, it is advised that LHRHa are continued and that anti-androgens are withdrawn to maximise this potential. Encouraging results have been reported with this approach. In addition the philosophy of intermittent androgen ablation may become more popular if studies can show that it is of benefit in slowing progression of the disease. However, it remains an unavoidable fact that advanced prostate cancer is at present an incurable disease. Although many recent advances have prolonged the duration of PSA response and delayed the time to disease progression, it is still the case that patients with advanced prostate cancer have poor survival prospects. A greater knowledge and understanding of the pathophysiology of the disease and the biology behind the development of the hormone-refractory condition will hopefully lead to improved therapies.

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