The role of insulin-like growth factors in the development of prostate cancer.

Abstract

Introduction. Preclinical, clinical and population studies have provided robust evidence for an important role for the insulin-like growth factor (IGF) system in the development of prostate cancer.

Areas Covered. An overview of the IGF system is provided. The evidence implicating the IGF system in the development of prostate cancer is summarised. The compelling evidence culminated in a number of clinical trials of agents targeting the system; the reasons for the failure of these trials are discussed.

Expert Commentary. Clinical trials of agents targeting the IGF system in prostate cancer were terminated due to limited objective clinical responses and are unlikely to be resumed unless a convincing predictive biomarker is identified that would enable the selection of likely responders. The aging population and increased screening will lead to greater diagnosis of prostate cancer. Although the vast majority will be indolent disease, the epidemics of obesity and diabetes will increase the proportion that progress to clinical disease. The increased population of worried men will result in more trials aimed to reduce the risk of disease progression; actual clinical endpoints will be challenging and the IGFs remain the best intermediate biomarkers to indicate a response that could alter the course of disease.

Key Words: prostate cancer, IGFs, IGF-IR, blocking antibodies, tyrosine kinase inhibitors, clinical trials.

1. Introduction

Prostate cancer is one of the major epithelial cancers, having the highest incidence for men in 114 countries globally and the leading cause of cancer death in men in 56 countries [1]. Since the introduction of screening by the measurement of prostate specific antigen (PSA) in the circulation in the 1990's incidence rates rose rapidly in countries where such screening was adopted generally, such as Australia and the USA, and then declined as the prevalence of undetected cases was diminished. In other countries where such screening has been adopted more gradually, such as many European countries, the changes in incidence have been less marked. In countries where screening remains rare the incidence has been gradually rising [2]. Mortality from prostate cancer has been decreasing in many developed countries and this decrease has been attributed to early detection due to PSA screening and improved treatments [3]. The interpretation of such statistics is however far from straightforward and many confounding issues need to be considered. As the strongest risk factor for prostate cancer is age and the number of men living into old age is increasing in many populations, this clearly will increase the incidence of such cancers. In relation to incidence rate, this is largely affected by the method of detection and it is now widely recognised that screening with PSA results in considerable over-diagnosis. Over-diagnosis with screening results in many men being treated who would not have presented with symptoms during their lifespan and as treatment is commonly associated with complications, such as incontinence and impotence, this can result in considerable reduction in quality of life [4]. It has been estimated that screening results in 27 men being treated in order to save the life of one man [5]. The enthusiasm for screening in the USA led many to attribute the subsequent fall in mortality to early detection; however similar falls in mortality over the same time-span in countries without screening suggest this may be more complicated [5,6]. If mortality is presented as survival after diagnosis, then as adoption of screening results in a large increase in detected incident cases, this will misleadingly give the impression that there had been a major improvement in survival. But as many of those detected with indolent cancers would have not presented with symptoms and would not die from these cancers regardless of treatment, then there can be an apparent improvement in survival even if there were no actual change in mortality due to changes in treatment. The confusion over issues in relation to screening, over-diagnosis and over-treatment indicate that the critical challenge in relation to prostate cancer will be to identify men who will develop clinically significant disease and distinguish these from men with insignificant disease that will pose little or no danger to their life [7]. The high prevalence of indolent, clinically insignificant, prostate cancers is entirely consistent with the findings from numerous autopsy studies that have identified cancer in the prostates of men who died from unrelated causes [8,9]. The occurrence of indolent prostate cancers detected at autopsy has consistently been found even in men in their 20s, at an age when clinical disease is extremely rare, and to increase with age with up to 80% prevalence in men in their 80's [10]. The high prevalence has been found in all populations and appears unrelated to incidence of clinical disease. The prevalence of cancer found in prostates at autopsy were similar in black and white men in the USA despite a higher incidence of clinical disease and 2-3-fold

higher prostate cancer mortality in the black men [11,12]. The frequency of latent carcinomas in prostates of Japanese men found at autopsy were also similar to that of white men in the USA despite a much lower rate of incident disease and mortality in Japan [13]. Similar rates of prostate cancer at autopsy were also found in a comparison of Caucasian men from Russia and men from Japan [14]. The similarity in prevalence of prostate cancer detected at autopsy between different geographical regions is in marked contrast to the incidence of overall and age-specific clinical disease which show substantial variations regardless of the level of PSA-testing, with the lowest rates consistently seen in Asia [2]. Indeed, studies of migrants between different countries indicate that environment has little impact on the prevalence of latent cancers but a large effect on the rates of clinical disease. For example, Japanese that have migrated to Hawaii have a higher prostate cancer incidence and mortality than indigenous Japanese although the rates of latent carcinoma detected at autopsy were similar between the two groups [15].

A number of inferences can be made from these various observations: indolent occult cancers appear in the prostate as men age, such that they are common in most elderly men; there may be a long latency period between initiation and appearance of clinical disease and progression to clinical disease may depend on environmental or lifestyle factors. The high prevalence of occult prostate cancers and the potential effect of lifestyle on their progression to clinical disease is consistent with the recent mounting evidence indicating the ubiquitous accumulation of oncogenic mutations with age in rapidly turning-over epithelial tissues and the impact of lifestyle [16]. This means that in addition to the challenge of identifying the clinically relevant cancers there is also an opportunity: with many men diagnosed with sub-clinical cancers, these men present as a large cohort for the development of interventions to prevent these men with cancer from progressing to life-threatening disease. Of the modifiable lifestyle factors, a Western lifestyle and diet has been implicated in the progression of prostate cancer and considered as a factor in the geographical variations observed. Lifestyle factors including excess energy intake, physical inactivity, obesity and insulin resistance have all been associated with prostate cancer development and poor outcomes [17]. Indeed, a Western-type diet fed to mice increased the growth, histological grade and metastasis of prostate cancers in mice [18]. The realisation that lifestyle, and especially diet, may play an important role in prostate cancer development has focused interest on nutritional regulators. The insulin/insulin-like growth factor (IGF) system has been throughout evolution the most fundamental regulator of tissue growth and development according to nutritional status and we will review recent evidence of the involvement of this system in prostate cancer.

1.1. The insulin/IGF system.

Insulin and its precursor, proinsulin, are secreted from specialist endocrine β-cells in the pancreas in order to regulate the distribution and utilisation of nutrients, especially glucose, in response to ingested food arriving in the gut. Early in evolution gene duplication events gave rise to two further peptides, IGF-I and IGF-II, that share around 50% homology with proinsulin; indeed the IGF-II gene is still found on chromosome 11 adjacent to the insulin gene [19]. The IGF-

II gene has however evolved into an extremely complex genetic loci compared to that of insulin. The human IGF-II gene is driven by 5 promoters and controlled by a downstream imprinting control region between the IGF-II gene and that of H19 and which via differential methylation controls both genes [19]. In some tissues and at certain stages of development this ensures that only the paternal allele of IGF-II and the maternal allele of H19 are expressed. The regulation of IGF-II is further complicated by the presence of three IGF-II mRNA binding proteins (IMP1, IMP2, IMP3) that control the stability and translation of IGF-II mRNA and glucose regulated protein 94 (GRP94) a chaperone that ensures the correct folding of IGF-II peptide [19]. The IMPs and GRP94 are not specific exclusively for IGF-II but they are important components of the system and GRP94 appears essential for the secretion of IGF-II. In addition to IGF-II, the loci also gives rise to at least three long non-coding RNA (lnc-RNA), IGF-II-antisense, H19 and its antisense transcript, H91 and at least four micro-RNAs [19]. Two additional regulatory peptides are also derived from the locus, preptin and H19 opposite tumor suppressor (HOTS) [19]. In contrast to insulin, the IGFs are secreted from most cells throughout the body and have additional, broader regulatory functions [20]. Indeed, the IGFs are powerful mitogens and strong survival factors for most cell types [20]. The IGFs, however, also retain their insulin-like metabolic activity; indeed IGF-II was originally identified in bioassays as non-suppressible insulin-like activity responsible for more than 90% of the glucose-uptake stimulating activity found in serum [19].

The IGFs activate cell surface transmembrane tyrosine kinase receptors (IGF-IR) that are also very homologous to the insulin receptor (IR) and again these presumably arose from an early gene duplication event (figure 1). These receptors are expressed and cleaved into an extracellular α-subunit that binds insulin/IGFs outside the cells and a transmembrane β-subunit that contains the tyrosine kinase catalytic site within the cell. These two subunits then dimerise further to form a heterotetrameric complete receptor. The IGF-IR appears to be the primary IGF receptor as it binds both IGF-I and IGF-II and has a relatively low affinity for insulin. In contrast the insulin receptor is more complicated as it exists in two isoforms, due to alternative splicing of the α-subunit that alters the ligand-binding domain resulting in IR-A and IR-B. The classical insulin receptor appears to be IR-B, which predominantly binds to insulin and has a much lower affinity for IGFs. In contrast IR-A has an affinity for IGF-II that is comparable to that of insulin and a consideration of the relative abundance of IGF-II in the body, compared to the low concentrations of insulin, indicates that insulin would only activate IR-A in the immediate post-prandial state and for the majority of the time IR-A would act as an IGF-II receptor [21]. Although IGF-II binds to IR-A with a similar affinity to insulin, there is evidence that IGF-II results in a prolonged activation of ERK1/2 compared to insulin and this may contribute to a more mitogenic and less metabolic response [22,23]. The α-β-dimers of the IR and IGF-IR are so similar that they hetero-dimerise to form hybrid receptors, both IR-A/IGF-IR hybrids and IR-B/IGF-IR hybrids, depending on the relative expression of each receptor in any particular cell. These hybrid receptors appear to predominantly act as IGF-I receptors [24], but their pathophysiology is still far from understood.

Intracellular signaling is initiated when insulin/IGFs bind to the α-subunit of

these receptors inducing a conformational change that results in activation of the tyrosine kinase activity contained within the intracellular β-subunit. This then results in autophosphorylation of multiple intracellular sites that provide docking sites for the recruitment of a variety of adaptor proteins, including the insulin receptor substrates (IRS-1 to−4), Shc and receptor for activated C kinase 1 (RACK1). This then facilitates the assembly of signaling complexes that activate networks of signaling pathways. The two best characterized of these are the PI3K/Akt/mTOR/S6K and Grb2/SOS/Ras/Raf/MEK/ERK pathways [25]. The lipid kinase activity of PI3K, that recruits and activates Akt, is opposed by the lipid phosphatase PTEN (phosphatase and tensin homolog), a tumor suppressor gene, the expression of which is commonly suppressed in many cancers including prostate.

There is also a very specific IGF-II receptor (IGF-IIR) that is both structurally and functionally completely different from the IR and IGF-IR and is a single large transmembrane protein [26,27]. The IGF-IIR binds IGF-II with extremely high affinity and has very little affinity for IGF-I or insulin. Binding of IGF-II to the IGF-IIR is generally considered not to initiate any conventional intracellular signaling but results in internalization and targeting of IGF-II to the lysosomes for degradation. The IGF-IIR is therefore thought to act as a clearance receptor for IGF-II, tightly controlling the exposure of IGF-II to the other signaling receptors. In contrast to insulin, which is stored in large amounts in the pancreas, there are no intracellular stores of IGFs; however, a sophisticated system has evolved for maintaining extracellular stores of IGFs throughout the body due to their association with very specific high affinity binding proteins. There are 6 binding proteins (IGFBP-1 to −6) that bind to both IGF-I and IGF-II with high affinity but do not bind insulin. The IGFBPs are not related to the cell-surface receptors but they are structurally very closely related to each other although they have very distinct functional properties and are produced in different quantities and combinations in different tissues [28]. The primary effect of the IGFBPs is that they considerably slow the clearance of IGFs, enabling very high concentrations of IGFs to build-up. In the circulation when IGFs associate with IGFBP-3 and IGFBP-5, these binary complexes immediately bind to a further large glycoprotein, the acid labile subunit (ALS) that is present in excess. This ternary complex is then too large to cross capillaries and hence is retained in the circulation and further slows clearance. This enables the total IGF-I and IGF-II concentration in the circulation to accumulate to around 1,000 times higher concentration than that of insulin and while insulin levels fluctuate acutely in response to metabolic requirements, the circulating concentrations of the IGFs are very stable due to the very slow clearance of the IGF/IGFBP complexes [20]. Despite being expressed in most tissues, the majority of the IGFs present in the circulation originate from the liver, where the production of both IGF-I and IGFBP-3 are regulated by growth hormone (GH) but in addition they are also very dependent on nutritional status [29]. At the cellular level, both the IR and IGF-IR are optimally activated by just 1–2 nanomolar concentrations of IGFs or insulin; this indicates that there is a vast excess of IGFs in the body. The high, and very stable, concentrations of circulating IGFs therefore establish a large reservoir of metabolic regulators that is determined by the chronic nutritional status. The IGFs can be released from this reservoir in a controlled manner,

primarily by specific proteases that act on the IGFBPs and lower their affinity. This shifts the equilibrium and enables the IGFs to dissociate and then to bind and activate cell surface receptors [30]. This has important implications for cancers such as that of the prostate; these cancers only become life threatening when they invade and spread to tissues outside of the prostate, a process that depends on the proteolytic degradation of the extracellular matrix (ECM) to enable invasion and angiogenesis. This proteolysis can then also act on IGFBPs to mobilize the large reservoirs of latent IGFs [28]. In addition to their role in modulating the availability and activity of the IGFs, many other actions, independent of binding to IGFs have been described for the IGFBPs [28]. Some of the best characterised of these IGF-independent actions, and of particular relevance to cancers, are the effects of both IGFBP-2 and IGFBP-3 on cell survival and DNA-repair [28,31,32]. One of the critical signaling pathways activated by the IGFs is the PI3K/Akt pathway and IGFBP-2 not only binds to the IGFs to modulate their activity but it also binds to integrin receptors and suppresses the activity of PTEN, the phosphatase that switches off the PI3K/Akt pathway [33]. In this way IGFBP-2 controls not only the 'on'-switch but also the 'off'-switch for controlling cell signaling. PTEN is a tumor suppressor gene which when reduced has important implications for prostate cancer prognosis [34,35] and IGFBP-2 has been shown to be a novel suppressor of PTEN in prostate cancer cells [36].

2. Evidence from population studies implicating IGFs in prostate cancer.

Interest in aspects of lifestyle that could affect the development of prostate cancer led to epidemiological investigations of factors that could be measured in population studies and their association with prostate cancer incidence. A number of hormones were measured in blood samples that were collected from large population screens. Due to the potential for cancers to affect the levels of hormones; especially those affected by deteriorating metabolic status, it was always apparent that interpretation of retrospective cohort studies of men with prostate cancer could be confounded by reverse causality. Particular interest therefore focused on prospective epidemiology in which hormones could be measured in blood samples that were obtained from healthy men in studies with many years subsequent follow-up. This enabled the examination of which hormones affected the subsequent risk of the men developing prostate cancer. There is considerable inter-individual variation in the levels of most hormones within populations and therefore very large populations are required to show associations with disease. After initial reports from individual studies, collaborations were established to increase population size and enhance the power to show such associations. An international collaboration combining 18 prospective studies that included 3,886 men who subsequently developed prostate cancer and 6,438 control men found that there was no association between the circulating levels of any sex steroid, including several androgens, and the subsequent risk of prostate cancer [37]. In contrast a similar exercise with 12 prospective studies including 3,700 men who subsequently developed prostate cancer and 5,200 control men found that the circulating concentration of IGF-I was significantly associated with the subsequent risk of developing prostate cancer. Those men whose circulating IGF-I concentration was in the highest fifth of the population had a 38% higher risk of developing prostate

cancer ($p<0.001$) compared to those men whose IGF-I levels were in the lowest fifth [38]. This collaboration was subsequently updated with now 17 prospective studies including 10,554 cases and 13,618 controls with the same association with IGF-I confirmed and now indicating a 29% increased risk of developing prostate cancer for men with IGF-I levels in the top quintile [39]. A conventional meta-analysis of 42 studies, including 7,481 cases, found a significant increased risk of prostate cancer with a 21% increase in risk for every standard deviation (SD) increase in IGF-I concentration across the population [40].

The general high prevalence of occult prostate cancer in contrast to the geographical differences in prevalence of clinical disease and the changes in incidence of clinical disease in migrants between different regions of the world suggests that lifestyle and nutrition have a much greater effect on progression to clinical disease than on initiation. As described above, the circulating concentration IGF-I is a marker of nutritional/metabolic status and therefore the conventional epidemiology would predict that there would be a greater effect of IGF-I on progression to clinical disease than on initiation. There is some support for this: a study based on PSA-screening of a large population of 110,000 men identified 2,686 men with biopsy-confirmed asymptomatic prostate cancer found no association between the risk of these cancers and IGF-I. The odds-ratio (OR) per SD increase in IGF-I was 0.99 (confidence intervals (CI) 0.93-1.04) [41]. Furthermore, in an updated meta-analysis of population studies that were stratified according to whether the prostate cancers were detected by PSA-screening or detected by traditional clinical presentation; distinctions were then found between associations with circulating IGF-I according to the method of detection. In studies based on clinically detected prostate cancers a significant association with IGF-I was still found; however no significant association was found in studies based on screen-detected prostate cancers, the vast majority of which would have been occult cancers [41].

Indirect evidence for a role of IGF-I in prostate cancer has come from studies of associations with physiological and pathological conditions in which IGF-I is implicated. The GH/IGF-I axis plays a major role in childhood growth and the attainment of adult height and in a systematic review of 22 population studies most found that greater height increased the risk of prostate cancer by between 20-40% when comparing men in the tallest category with those in the shortest category [42]. In a recent large international consortium study with 6,207 prostate cancer cases and 6,016 controls men who were taller than 180cm had a 22% higher risk of prostate cancer (OR 1.22, CI 1-01-1.48) in comparison to men who were shorter than 173cm [43]. This study also analysed genetic variants in the growth pathways (including single nucleotide polymorphisms (SNPs) in the GH and IGF-I genes) and found the aggregate score of the genetic variants was associated with an increased risk of overall prostate cancer (OR 1.13) and high-grade prostate cancer (OR 1.15) [43]. Acromegaly results from pituitary tumours secreting high levels of GH and as a consequence circulating IGF-I levels are extremely high. In a recent study using national hospital episode and mortality data 2,495 men with acromegaly were identified and compared with a reference cohort of 4.3 million men with 30,000 prostate cancer deaths. For men with acromegaly the risk of a diagnosis of prostate cancer was increased by 33% (CI

1.09-1.63) and the risk for prostate cancer death was increased by 44% (CI 0.92-2.26) [44]. This observation may be confounded due to reports that serum IGF-I levels are associated with lower urinary tract symptoms (LUTS) in elderly men [45] and are increased in men with benign prostatic hyperplasia (BPH) [46]. In addition, acromegaly, a pituitary tumor resulting in increased serum IGF-I, in men is associated with prostate enlargement and with increased LUTS [47,48]. Prostate enlargement and LUTS may both increase the risk of investigation and detection of prostate cancer which could confound associations with acromegaly; however, this could also reflect a pathway with IGF-I increasing prostate epithelial proliferation and survival and increasing prostate size and the risk of cancer.

Studies with clinical follow-up can give the clearest evidence in relation to clinically important life-threatening prostate cancer. In a study of 909 men with PSA-detected clinically localized prostate cancer who were undergoing active monitoring, after a mean of 4 years follow-up there was some evidence of an association between their initial serum IGF-I level and their risk of signs of disease progression as assessed by a rapid PSA doubling time. For every SD increase in serum IGF-I their odds ratio risk of developing a rapid PSA doubling time was 1.34 (CI 0.98-1.81) [49]. In a study of 396 men with clinically advanced prostate cancer followed-up for a mean of 3.7 years with 66 cancer-specific deaths there was a significant association between serum IGF-I and mortality after controlling for the level of IGFBP-3 with a hazard ratio of 1.59 (CI 1.11-2.28) per SD change in IGF-I. However, in a larger prospective study of 2,424 men with a mean follow-up of 8.9 years with 313 prostate cancer specific deaths no association was found between prediagnosis levels of IGF-I and mortality [50]. An association between serum IGF-I and pathological Gleason score was examined in a study of 793 men who underwent radical prostatectomy with 272 control men with negative biopsies. An inverse association was found with low IGF-I levels associated with high Gleason score and an increase in Gleason score between biopsy and surgical specimen was most frequent in men with low serum IGF-I [51]. It was hypothesized that high-grade prostate cancers may develop independent of IGF-I.

Observational studies are prone to many potential confounders such as reverse causality; cancer is known to affect metabolic and endocrine status. This could explain associations between IGF-I and prostate cancer. In order to overcome such issues many studies have examined associations between genetic variants in the IGF-I pathway and prostate cancer. Gene alleles randomly assort at gamete formation and segregate randomly at conception to generate a genotype that remains throughout life. Associations between genotypes and prostate cancer cannot be explained by reverse causality and are generally not confounded by behavioral or environmental factors. In addition, a genotype may be more reflective of a lifetime exposure than a single measure of a serum or tissue sample. Initially studies examined associations with SNPs in the IGF-I and related genes. The increasing availability of data from genome-wide association scores then enabled the calculation of genetic scores from multiple gene variants that best predict exposures such as the circulating level of IGF-I that were then used

in Mendelian Randomisation studies. Of the studies examining single genetic variants most observed an association between genetic variance in IGF-I and the risk of prostate cancer although most were small studies lacking statistical power [52-57]. Of two larger studies, a consortium study with 6,012 cases of prostate cancer and 6,641 controls identified a SNP that was not associated with blood IGF-I levels but was associated with prostate cancer risk [56]. Another consortium study with 2,664 cases and 2,919 controls however found no association between the genetic variance that was related to blood IGF-I levels and prostate cancer risk [58]. A comprehensive Mendelian randomization study using genetic variants as instruments for circulating levels of IGFs and IGFBP-3 examined associations with prostate cancer risk using consortia data with 22,992 cases and 22,936 controls. The main finding was that there was considerable pleiotropy for the genetic instruments with the strongest instruments being SNPs in the IGFBP-1/IGFBP-3 region [59]. A meta-analysis of 18 individual studies also indicated that genetic variance in IGFBP-3 was significantly associated with an increased risk of prostate cancer [60]. The association with IGFBP-3 is entirely consistent with our current understanding of the physiology, as described above, with IGFBP-3 having a dominant effect on the circulating concentrations of both IGF-I and IGF-II. Significant associations were found between the genetic instruments and prostate cancer, particularly high-grade cancers; however, due to the pleiotropy, the evidence could only implicate the IGF-pathway rather than any specific component [59]. Consistent with the concept that the IGF-pathway may be particularly important for progression, many studies have found associations between genetic variance and clinical outcomes. In a study of 320 men post radical prostatectomy, IGF-I genotypes were associated with a 1.49- to 2.22-fold higher risk of advanced-stage prostate cancer and a genetic interaction between SNPs in IGF-I/IGF-IR was significantly associated with evidence of biochemical recurrence [61]. A study of 215 men with prostate cancer and bone metastasis polymorphisms in the IGF-I gene were associated with cancer-specific survival [62]. A large consortium study of 5,887 men with prostate cancer and 704 cancer-specific deaths found that genetic variance in the IGF-pathway were associated with mortality [50]. Interestingly the two SNPs with the strongest associations with mortality were in the IGF-II antisense gene and the somatostatin receptor 2 [50].

3. Evidence from Preclinical Studies.

There is overwhelmingly convincing preclinical evidence for a potential role for IGFs in prostate cancer from studies of prostate cells *in vitro* and a variety of animal models *in vivo*. At the cellular level IGFs have been consistently shown to stimulate the growth, metabolism, survival, migration and invasion of prostate cancer cells as we [63] and others [64] have reviewed earlier. The IGF-pathway appears to play an important role in many of the critical stages of cancer progression including supporting cancer stem cells, endothelial-to-mesenchymal transition(EMT), angiogenesis, immune escape, invasion and metastasis [65,66]. In addition, IGF-I has been reported to drive EMT of prostate cancer cells, a cellular de-differentiation that is key to enhanced migration, invasion and metastasis [67]. Furthermore, IGF-I stabilizes important integrin receptors on

prostate cells that determine cell attachment and changes in cell attachment are critical to cancer progression [68]. Genetic over-expression or silencing of either the IGF-IR or the IR in prostate cancer cells reduces growth, migration, angiogenesis and tumorigenesis in both cell lines and in mouse models [69,70]. In addition to its conventional role in cell-surface signaling, the IGF-IR has also been reported to localize within the nucleus of prostate cancer cells where it was reported to interact directly with chromatin to enhance the expression of a number of genes associated with prostate cancer progression [71].

A mouse model that better reflects human disease in contrast to xenograft models is the autochthonous Transgenic Adenocarcinoma of Mouse Prostate (TRAMP) model in which SV40 early gene expression is targeted to the prostatic epithelium, with expression starting at sexual maturity. The TRAMP mice initially develop prostatic intraepithelial neoplasia but ultimately develop adenocarcinomas that metastasise to other sites, primarily lymph nodes and lungs. Following androgen ablation 20-35% of TRAMP mice remain cancer free but 65-80% develop androgen-independent disease [72]. A comprehensive characterisation of the IGF-system in TRAMP mice revealed that prostatic IGF-I expression was increased in primary disease; in contrast expression of IGF-IR and IGF-IIR were not altered in primary disease but were dramatically reduced in metastatic lesions and in androgen-independent disease [72]. When TRAMP mice are fed a high fat diet there was an increase in incidence of tumors and increased mortality and this was associated with an increase in serum IGF-I and staining for the IGF-IR in the prostate [73]. The human population studies implicated a role for circulating IGF-I in promoting prostate cancers. This potential role has been tested in mice by crossing mice that develop prostate adenocarcinomas due to transgenic expression of the oncogene C-Myc with mice that are transgenic for over-expression of IGF-I specifically in the liver, generating high circulating IGF-I levels. The high circulating IGF-I in the mice resulted in an increased incidence and invasiveness of prostate cancers; there was also evidence that there was cooperation between the high IGF-I and c-Myc to promote IGF-IR expression in the tumors [74].

The development of the prostate gland is dependent on androgens and a reciprocal synergistic interaction between the IGF-system and the androgen receptor has been demonstrated to operate in prostate cancer cells. The expression of the IGF-IR receptor is up-regulated by androgens [75] and reintroduction of functional, but not mutant, androgen receptors into prostate cancer cells lacking this receptor results in increased expression of the IGF-IR [76]. The IGF-IR can also activate the androgen receptor in prostate cancer cells independent of the occupation of the receptor by androgens [77,78]. This androgen-independent activation of the androgen receptor suggested a potential mechanism for the development of castration resistant prostate cancer and indeed inhibition of the IGF-IR did enhance the inhibitory effects of castration in a mouse model [79]. The involvement of the IGF-system in the development of androgen-independence has also been implicated by reports of increases in IGF-I, the IGF-IR [80], IGFBP-2 [81] and IGFBP-5 [82]; all of which could promote progression in the absence of androgens. In multiple preclinical prostate cancer cell models, including a patient-derived castration-resistant xenograft (PDX) model, an IGF-neutralising antibody in combination with an androgen receptor

signaling inhibitor reduced the survival of prostate cancer cells more than either agent alone and the IGF-neutralising antibody inhibited the growth of the castration resistant PDX tumors in vivo [83]. Despite all of these preclinical indications, this however did not translate into any positive effect of combining IGF-IR blocking antibodies with androgen deprivation in a phase II clinical trial [84].

A number of oncogenes and tumor suppressor genes activate or interact with the IGF-pathway [85,86]. For example the breast and ovarian cancer susceptibility gene-1 (BRCA1) was found to regulate the IGF-IR in prostate cancer cells, suppressing the expression of the IGF-IR in androgen receptor negative cells but up-regulating it in androgen receptor positive cells [87]. Also of particular importance to prostate cancer are the frequently observed fusions between the TMPRSS2 gene and the ERG gene which links an androgen driven promoter with an oncogenic transcription factor [88]. The TMPRSS2-ERG fusion appears to be an early event in prostate cancer progression and to be related to aggressive disease [89]. The ERG transcription factor was shown to bind directly to the IGF-IR promoter and drive expression in prostate cells and the presence of the TMPRSS2-ERG fusion was shown to be associated with high IGF-IR expression in tumor samples from men [90,91].

The potential importance of IGF-II for the development of prostate cancer was demonstrated by a study in mice in which mutations had been created in the IGF-II/H19 imprinting control region that resulted in biallelic IGF-II expression [92]. Mice with biallelic expression of IGF-II showed an increase in the number and grade of multifocal prostatic intraepithelial neoplasia, a premalignant lesion, indicating that IGF-II promoted the rate of prostate cancer development. The factors that may induce loss of imprinting (LOI) resulting in biallelic expression of IGF-II are only starting to be unraveled but oxidative-stress has been reported to induce LOI in prostate cells in culture and in mouse models [93]. The plasticity of the epigenetic regulation of IGF-II/H19 was demonstrated by an increase in the expression of both IGF-II and H19 in the prostates of mice exposed to a methyl-deficient diet [94]. An additional mechanism of regulation of IGF-II was apparent with the report that the IGF-II antisense transcript can down-regulate the expression of IGF-II and act as a tumor suppressor in prostate cancer cells [95,96]. Additional effects of IGF-II have been suggested with the report that prostate cancer resistance to chemotherapy was mediated by IGF-II and that IGF-II can promote *de novo* steroidogenesis within prostate cancer cells [97].

Other products of the IGF-II/H19-locus have also been implicated in the development of prostate cancer. The long non-coding RNA H19 has been reported to down-regulate integrin receptors on prostate cancer cells and promote their mobility and invasion [98] and to promote 'stem-like' properties in prostate cells [99]. In addition, miR-675, derived from H19, has been reported to suppress prostate cancer metastasis by repressing the expression of TGF-β induced protein [100]. The other micro-RNA derived from within IGF-II, miR-483, has also been reported to promote the proliferation and invasion of prostate cancer cells [101].

Prostate cancer only becomes a lethal disease when it spreads beyond the confines of the prostate gland and one of the most common sites for metastasis is the bone with the incidence reported between 18-29% of men with prostate cancer in the USA [102]. The IGF-system appears to be critical for the development, growth and maintenance of normal bone [103,104]. Normal bones are maintained by constant dynamic cell growth and turnover in a local environment that is rich in growth factors. In this environment IGF-I is produced by osteoblasts, dendrocytes, osteoclasts and osteocytes and is stored with IGFBPs in the bone matrix [103]. This provides an ideal environment to attract tumor cells and for them to then proliferate [105,106]. The importance of IGFs to the metastasis of prostate cancer to bone was indicated by the ability of a neutralizing antibody to IGF-I and IGF-II to inhibit the growth of prostate cancer cells in human bone that had been implanted into severe compromised immunodeficient mice [107]. As IGF-II is by far the most abundant IGF present in bone a neutralizing antibody specific for IGF-II was shown to have a similar effect in the same model [108]. That the IGF-IR was mediating these effects was shown by the use of an IGF-IR inhibitor that potentiated the effects of simvostatin (a mevalonate pathway inhibitor) in inhibiting prostate cancer cells grown in co-cultures with mouse calvarial bone cells *in vitro* [109] and in a rat model of prostate cancer cells growing in bone [110].

The accumulation of evidence indicating an important role for the IGF-system in prostate cancer led to many preclinical studies investigating the effects of interventions targeting this system and these studies generally indicated a beneficial effect. With the IGF pathway having been implicated in a wide range of cancers a number of different strategies have been proposed to target the system. These include using antisense oligonucleotides or short interfering RNA to down-regulate the expression of IGF-I or the IGF-IR, dominant negative receptors, antibodies or small molecule tyrosine kinase inhibitors (TKIs) that block the IGF-IR. Several strategies have also been proposed that depend on sequestering the IGF ligands including antibodies that bind and neutralize the IGFs, both IGF-I and IGF-II, IGF ligand traps, recombinant IGFBPs and protease inhibitors that prevent the release of IGFs from IGFBPs [111]. Of these only the IGF-IR antibodies, IGF-IR TKIs and IGF neutralizing antibodies have been extensively developed for clinical application and these have been evaluated in preclinical models for prostate cancer. Antibodies blocking the IGF-IR have been reported to inhibit prostate cancer growth in cell lines and in mice, generally using xenograft models and either alone or in combination with docetaxel or androgen deprivation [79,90,112-116]. Additional encouraging results were also reported for IGF-IR TKIs in similar models [117-119] and in a rat model of bone metastasis [110].

4. Evidence from Clinical Studies.

Clinical evidence of the involvement of the IGF system in prostate cancer comes from many studies documenting derangements to the system in samples from men with prostate cancer and more recently from clinical trials of agents targeting the IGF-pathway.

The importance of IGF-I for the growth of the prostate gland is indicated by the association between IGF-I and BPH in normal men [46] and the increased prevalence of BPH in men with acromegaly [47,48]. The IGF system is perturbed in the prostate in men with BPH with increased expression of IGF-I, IGF-II and the IR [120]. The population studies, described above, indicate that high circulating IGF-I levels predispose to an increased risk of prostate cancer. A recent study analysed IGF-I concentrations in blood samples taken immediately before radical prostatectomy from 793 men and from 272 men who had negative prostate biopsies. Serum IGF-I levels were higher in the men with cancer, consistent with the prospective epidemiology, however they also found that in the men with cancer serum IGF-I concentrations decreased with increasing Gleason score of the cancers [51]. An increased grading of the Gleason score between the original biopsy and the surgical prostatectomy sample was also associated with a low serum IGF-I and the authors suggested that this may imply that the high grade disease may develop independent of serum IGF-I [51], although reverse causality cannot be exclude with disease progression resulting in lower serum IGF-I.

Consistent with the animal models, clinical studies have also reported loss of imprinting of the IGF-II locus. The loss of imprinting has also been reported to extend into adjacent normal prostate tissue and not simply be confined to tumor tissue [121]. The LOI of IGF-II in normal prostate tissue was reported to increase with age in both mice and humans and this was more extensive in men with prostate cancer [122]. These findings could imply either that an epigenetic defect in the tumor is transmitted in a 'field effect' to the surrounding normal tissue or, more likely, that a more widespread epigenetic defect predisposes the prostate to the development of a cancer as is suggested by the increase in cancer in mice with an engineered loss of IGF-II imprinting [92]. Consistent with these reports an early study reported increased expression of IGF-II mRNA, but not protein in prostate cancer tissue compared to that from men with BPH [123]. The significance of IGF-II imprinting has however been questioned by recent reports that IGF-II expression and protein levels are actually decreased in human prostate tumors compared to adjacent non-neoplastic prostate [124] or compared to tissue from men with benign prostate hyperplasia [125]. In both studies the expression of IGF-II was not related to the IGF-II imprinting status but was related to promoter hypermethylation [124,125]. The discordance between imprinting status and IGF-II expression or levels has also been reported in other tissues and in a study comparing IGF-II imprinting status and serum IGF-II protein levels in white blood cells from men with prostate cancer and control men [126].

There have also been several reports of perturbations to other components of the IGF-II/H19 loci. Expression of H19 has been reported to be decreased in human prostate cancers [127]. In a screen of cell-free microRNA that could be detected in urine samples, the one microRNA found to be abnormal in men with prostate cancer was the microRNA embedded within the IGF-II gene, miR-483 [128]. In a screen of microRNA isolated from lymphoblastoid cell lines derived from men in a study of families with a high risk of prostate cancer, miR-483 was one of 5 microRNAs associated with risk of cancer and a SNP associated with

miR-483 was also associated with risk [129]. These reports indicate that further studies of miR-483 are warranted. The levels of the IGF-II chaperone GRP94 were suppressed in a tissue microarray study of men with prostate cancer and in circulating tumor cells derived from prostate cancer patients [130]. The IGF-II mRNA binding protein, IMP3, has been reported to be over-expressed in prostate cancer tissue and to correlate with Gleason score [131-133] and to be associated with extracapsular extension, seminal vesicle invasion, lymphovascular invasion and lower PSA recurrence-free survival [132]. Furthermore, IMP3 measured in serum samples was raised in men with prostate tumors compared to BPH, particularly those with metastatic disease, and to be associated with poor cancer-specific survival [133]. These reports are interesting, particularly those for IMP3 but it should be remembered that IMP3 and GRP94 are not specific exclusively for IGF-II and hence these findings may not directly implicate the IGF-system.

The other component of the IGF-system that has been extensively investigated in human prostate cancers is the IGF-IR with conflicting findings. The conflicting findings can, at least partially be attributed to problems with specificity of antibodies used for immunohistochemistry. An early report indicated that IGF-IR mRNA and protein were decreased in prostate tumors compared to benign epithelium [123]. However more recent studies have generally found the IGF-IR to be increased in prostate cancer. A study that included a rigorous evaluation of different antibodies found that staining for the IGF-IR was increased particularly in men showing signs of disease progression [134]. In a further study from the same group high IGF-IR was associated with shorter recurrence-free survival [135] and that whereas the IGF-IR was localised to cell membranes in benign epithelium it was more prominently localised to nuclear/cytoplasmic regions in malignant epithelium [71]. In a study of tissue microarray from radical prostatectomy samples increased IGF-IR and IR were also found and either high IR or low PTEN were associated with increased risk of biochemical recurrence and the worst prognosis was when both the IR was high and PTEN was low [35]. In a large study of tissue from 805 radical prostatectomies increased IGF-IR or reduced PTEN were associated with worse prognosis [34]. In another large study of tissue from 769 men with advanced prostate cancer, the IGF-IR was increased in 29% and the IR increased in 10% of men and tumours with increased IGF-IR or IR showed increased cell proliferation and reduced apoptosis. In a follow-up, IGF-IR expression showed a borderline association with increased risk of lethal disease [136]. In contrast a study of samples from 270 men with more than 5 years follow-up reported that in men who were negative for the TMPRSS2-ERG gene fusion, high IGF-IR was associated with prolonged biochemical progression free survival [137].

With preclinical, clinical and population evidence for the involvement of the IGF system in prostate cancer, the pharmaceutical industry then proceeded with clinical trials administering agents designed to block the IGF-pathway. As described above many different strategies have been proposed and evaluated to varying extents. Technical and safety issues have limited the development of most of these strategies and only IGF-IR blocking antibodies , TKIs and IGF-neutralising antibodies have been evaluated clinically in men with prostate

cancer. The most studied clinically have been blocking antibodies against the IGF-IR. These have been evaluated in men pretreated with docetaxel [138] or in combination with docetaxel [139,140] or with androgen deprivation therapy [84,141] and as a single therapy preoperatively in men with localized disease [142]. There have also been more limited clinical trials with IGF-IR TKI and neutralizing antibodies [111,143].

Although most agents have generally been well tolerated; they can result in hyperinsulinemia and dose-limiting hyperglycemia, especially agents that also block the IR. In a trial in men with advanced prostate cancer, the IGF-IR-blocking antibody in combination with docetaxel resulted in more reported treatment-related severe adverse events than in the docetaxel-alone control arm [140]. More than expected adverse events were also observed in the arm of a trial in which an IGF-IR-blocking antibody was used in combination with an mTOR inhibitor compared to the latter alone [144].

As with all the clinical trials of agents targeting the IGF-pathway in all other types of cancer, the pharmaceutical industry considered that these trials demonstrated limited objective responses, and in some combinations increased treatment related adverse effects. This resulted in most, bar a few trials, being terminated. In some of the trials clear evidence of antitumor activity was observed in particular patients; this was most evident in trials in patients with Ewing Sarcoma, but even in that case only around 10% of patients showed a response [111]. There was no clear evidence for why particular tumors or particular patients showed signs of clinical response. With such a relatively small proportion responding and no biomarker available to predict potential responders the major pharmaceutical companies considered these were not sufficient to justify continuing trials targeting the IGF-pathway for any type of cancer.

Many different reasons have been proposed for the failure of agents targeting the IGF-system in clinical trials, despite the strong indications from preclinical evidence [111,143,145-147]. The actual reasons for the failure of these trials probably includes a combination of the suggested problems. One of the major issues is that all of these trials have, to date, been conducted in unselected patients and no other targeted cancer therapy has yet been found to be effective in unselected patients. The inclusion of many patients who do not respond for a variety of reasons, including some described below, dilutes any response that might have been observed in a subset of men. The reason that the trials were conducted in unselected men was due to a lack of convincing evidence for any potential predictive biomarker of response. Although there have been several suggestions, including levels of IGFs and their receptors; to date, there is not robust evidence for a biomarker that would predict which men would respond best to targeting the IGF-system. There are several reasons why some might not respond. The downstream signaling pathways activated by IGFs, especially the PI3K/PTEN pathway are commonly perturbed in prostate cancers by genetic, epigenetic and other alterations. If the downstream pathways are constitutively activated, then the cancer cells may be independent of interventions targeting upstream receptors. Another issue with trial design is that most have to be

conducted in men with advanced disease who have failed conventional therapies. It is now clear that in patients with advanced cancers considerable intra-tumor heterogeneity has developed. Therefore, even if the major clone initially responds to blockade of the IGF-IR, the presence of minor clones with autonomous activation of the PI3K pathway could result in ultimate treatment failure as these clones will continue to grow and eventually take over. Similarly, compensatory signaling activation by other receptor tyrosine kinases, for example EGFR/HER2, that activate the same downstream intracellular signaling could render cells independent of the IGF-system. In addition, blocking the IGF-IR can cause cell cycle arrest that could then limit the efficacy of specific cytotoxic drugs that act at different stages in the cell cycle; this could explain lack of response in trials of combination therapy. The IGF/insulin systems are endocrine pathways subject to feedback inhibition and blockade of the IGF-IR results in increased circulating IGF-I and insulin levels that could limit efficacy and in the case of short-acting TKIs may result in rebound activation of the pathway. The epidemiology, especially the autopsy findings, suggest that indolent prostate cancer is almost ubiquitous in elderly men and the evidence suggests that lifestyle and the IGF-system may be important in the initial progression from indolent disease to clinically relevant disease[16,147]. As already stated, most trials were initially in men with advanced disease where the cancer may already have acquired independence from stimulation by the IGF-system. Interestingly one of the trials in prostate cancer with the most promising results was using an IGF-IR blocking antibody in men with localized disease prior to prostatectomy [142]. It has to be recognized, however that conducting large-scale clinical trials in men with early disease with objective clinical endpoints would be very challenging as relatively few of these men show disease progression in the timeframe of normal trials.

These many reasons for lack of response are not mutually exclusive and in view of the recognized considerable intra-tumoral heterogeneity in prostate cancers it is likely that in any one man, many of these reasons combine to limit clinical response. The lack of predictive biomarkers and the problems of identifying objective clinical responses in men with early disease present difficult challenges that future research needs to address.

5. Expert Opinion.

Preclinical, clinical and population studies have provided compelling evidence that the IGF-system plays an important role in the development of prostate cancer. This led to many clinical trials of agents targeting the IGF system in men with prostate cancer. These trials have now been terminated due to a lack of convincing objective clinical response; a decision made by pharmaceutical companies based on the results of trials not just in prostate cancer but across a number of different cancers. These trials were, however, all in unselected patients. Promising results were obtained for prostate cancer in men with localised disease, but trials are unlikely to be resumed unless a convincing predictive biomarker is identified. Research into identifying such a predictive biomarker will continue although all of the most obvious components of the IGF

system, including the levels of IGFs and their receptors, have been evaluated without any providing convincing robust evidence.

The evidence however suggests that the IGF system provides such a strong advantage to the development of epithelial cancers that clones with constitutive activation of the pathway are selected during the evolution of most cancers, for example by activation of PI3K or suppression of PTEN. These clones are then less dependent on upstream activation of the pathway and contribute to the lack of efficacy of strategies blocking receptor activation and this also helps to explain why upstream components are poor predictive biomarkers. There are multiple compensating mechanisms and it is increasingly recognised that cell-signalling pathways are really intersecting networks. This means that it is also unlikely that a single downstream component will provide a robust predictive biomarker. In the future a more comprehensive understanding of signalling network activation may provide more predictive power but this is not on the immediate horizon.

The most critical general question in prostate cancer is how to differentiate the indolent cancers that are virtually ubiquitous in elderly men (and do not need treatment) from the aggressive cancers that can shorten their lives (and do need treatment). In addition, even though more conservative approaches to population screening are being adopted, there will be increasingly large populations of worried elderly men living with the knowledge that they harbour a prostate cancer. Initial studies investigating the IGF-system as a marker for differentiating the aggressive cancers that need intervention from the indolent cancers have not been promising. The role of epigenetic reprogramming is however an interesting aspect that may well provide some exciting new insights over the coming years. Changes to the imprinting of the IGF-II/H19 locus appear to be a common early occurrence in prostate cancer; although this does not appear to simply result in increased IGF-II activity as initially anticipated. The IGF-II/H19 locus is however a source of a large array of lnc-RNA, micro-RNA, antisense-RNA and potentially other translated peptides that appear to form a very complex integrated regulation system. Little is still known regarding many of these components and how they interact is only beginning to be understood. This will be an exciting new field for the future that could yield new targets for controlling prostate and other cancers.

The other area in which research into the IGF-system may be fruitful is in relation to lifestyle changes as a means to control cancers and maintain them in an indolent state in the growing population of elderly men. It is increasingly clear that many epithelial cancers, including that of the prostate, are yet another adverse consequence of the lifestyle that has been widely adopted in developed societies, in addition to the obvious consequences such as obesity and type 2 diabetes. It is now recognised that rapidly turning-over epithelial surfaces, such as prostate epithelium, acquire huge numbers of potentially oncogenic mutations and epigenetic changes as men age. As a consequence most elderly men will develop multiple indolent neoplastic lesions in their prostate gland, as confirmed by population studies particularly the autopsy studies, mentioned above, that indicate indolent prostate cancer is virtually ubiquitous in older men. There are multiple safeguards that prevent these lesions from progressing, including apoptosis, hypoxia and immune-surveillance. The local tissue environment

heavily influences the balance between the oncogenic drives to survive, proliferate and invade and these inherent defensive safeguards. The IGF system evolved as a critical component of the tissue environment signalling that the metabolic status is appropriate for growth and development. It is increasingly apparent that the IGF system also plays a similar role in many tumors and mediates many of the effects of nutrition and metabolic status on cancer progression. Over the coming years the IGF-system will be a key focus in the search for the most effective lifestyle interventions to control these naturally occurring indolent neoplastic lesions and allow men to live long healthy lives with their prostate cancer. Increasing use of screening will identify more men with prostate cancer, the vast majority of these will not develop into a life-threatening disease. The epidemics of obesity and diabetes will however increase the chances that these men may progress to clinical disease. There will therefore undoubtedly be increasing numbers of worried men and over the next years there will be a growth in trials of interventions to reduce the chance that these men develop clinical cancer. As most of these men will not suffer from their indolent prostate cancer any secondary preventative interventions will have to be devoid of adverse health effects. This will favour interventions such as diet, exercise and drugs with proven safety profiles such as those used to improve metabolic health, for example metformin. The challenge with such trials will be that so few of these men will show signs of disease progression over even the most ambitious timeframe of a trial, such as five years, that objective clinical responses will be difficult to monitor. The IGFs have a very robustly established relationship with prostate cancer progression and they will be used as an intermediate biomarker to indicate that the intervention has resulted in changes likely to affect the progression of the disease. There will be more studies characterising the nutritional changes that are most effective in modulating the IGF/insulin system that could reduce its effects on promoting cancer progression. As tumor heterogeneity is increasingly recognised as an impediment to many therapies and a leading cause of disease recurrence there will be increasing studies of factors that impact tumor heterogeneity. The IGF/insulin system and its downstream signalling pathways are prime factors that may impact the sustainment of tumor heterogeneity and importantly are potentially modifiable.

Article Highlights.

- The IGF system plays an important role in prostate cancer development. Evidence, particularly from population studies, indicates that the most important effect may be early in the progression from indolent disease to clinical cancer. This helps explain the lack of objective clinical responses in trials largely performed in men with advanced disease. It also provides opportunities as increasing numbers of men are diagnosed with indolent disease by screening.
- Clinical trials in men with prostate cancer of agents targeting the IGF-system are unlikely to resume unless convincing predictive markers are identified that could enable the selection of men most likely to respond.

- As IGFs are nutritionally dependent and they affect the progression of prostate cancer they provide an opportunity to monitor interventions aimed at reducing the risk for men with indolent disease. As most men will not suffer from their indolent prostate cancer any intervention will have to be safe from adverse effects. This favours nutritional/lifestyle interventions over pharmaceutical interventions. A better understanding of the nutritional determinants of IGFs will assist these studies. There are major differences in IGF-physiology between different animal species and these differences explain why IGF-levels are more responsive to nutritional changes in rodents compared to humans. These differences however limit the usefulness of animal models for such studies.

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Figure Legend.

Fig 1. Overview of intracellular signaling of the IGF system. At the cellular level IGF-I, IGF-II and insulin ligands interact with a family of signaling tyrosine kinase receptors: the IGF-IR and the insulin receptor IR, which exists in two alternatively spliced isoforms (IRα and IRβ). The IRβ has high affinity to insulin whereas the IRα to IGF-II. Upon binding of the ligands to the receptors signalling cascade is initiated. Conformational changes within the intracellular β-subunit result in autophosphorylation of insulin receptor substrates (IRS-1 to−4), Shc. This then results in activation of PI3K/Akt/mTOR/S6K and Grb2/SOS/Ras/Raf/MEK/ERK pathways. Such cascade of intracellular reaction culminates in increased cell proliferation, tumorigenesis, self-renewal, homeostatis and metabolism The lipid kinase activity of PI3K, that recruits and activates Akt, is opposed by the lipid phosphatase PTEN (phosphatase and tensin homolog), a tumor suppressor gene, the expression of which is commonly suppressed in many cancers including prostate.