Immunotherapy of prostate cancer: identification of new treatments and targets for therapy, and role of WAP domain-containing proteins

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Abstract
Prostate adenocarcinoma is present in over 80% of men over the age of 80 and is by far the most common cancer of men. Although radical prostatectomy is curative in early disease, the risks of incontinence and impotence can affect the quality of life of patients. Early intervention with localized immunotherapy represents a potential solution as lymphocyte infiltration does occur in prostate cancer lesions, and immunotherapy with dendritic cell vaccines can significantly increase survival in late stage disease. However, lymphocytic infiltrates in the cancerous prostates have an anergic character arising from the suppressive effects of the microenvironment resulting from a conversion of effector cells into regulatory T-cells. Although TGFβ (transforming growth factor β) and IL-10 (interleukin-10) are known to be strong suppressor molecules associated with prostate cancer, they are among many possible suppressive factors. We discuss the possible role of alternative suppressor molecules, including the WAP (whey acidic protein) homologue ps20 that is expressed on prostate stroma and other WAP domain-containing proteins in the immunosuppressive prostate cancer milieu and discuss novel immunotherapeutic strategies to combat this disease.

Background to prostate cancer
Prostate cancer is the most common cancer affecting men: it is so common that at least 80% of men who reach the age of 80 have localized cancerous lesions [1]. In many of these men, the cancer will remain localized so that it will not contribute towards mortality and is typically very slow growing. Many of these patients can be managed by active surveillance. However, active treatment with curative intent is recommended in younger men with more aggressive localized cancers. These include radical prostatectomy that can cause impotence and/or incontinence in some patients, thus affecting their quality of life [2]. Hormonal therapies in metastatic cancer, based on an inhibition of androgen receptor-mediated growth signalling in prostate cancer cells are also limited in efficacy as prostate tumour cells almost always become hormone resistant and begin to use growth signalling pathways that are independent of the androgen receptor in order to survive. Early administration of hormonal therapy after the failure of first-line treatment can be associated with a profound clonal selection of aggressive androgen insensitive variant cancer cells [3].

An alternative therapeutic option in other cancers such as melanoma and renal cell carcinoma has been the use of immunotherapy [4,5]. In these cancers, tumour-specific antigenic markers have been identified which are recognized specifically by T-cells. These markers include MAGE-3 (melanoma-associated antigen 3), MART1 (melanoma antigen recognized by T-cells 1), tyrosinase, gp100, syntaxin 4 and RAGE (receptor for advanced glycation end-products). Similar tumour-specific antigens have not been identified for prostate cancer, although there are antigens associated with increased risk such as PSA (prostate-specific antigen), PSMA (prostate-specific membrane antigen) and PAP (prostatic acid phosphatase). T-cells with specificity for these antigens have been detected in prostate cancer [6]. While prostate cancer has been thought to be poorly immunogenic, based on a number of recent studies, there is evidence that T-cells and NK (natural killer) cells do infiltrate the prostatic microenvironment. CD4+ and CD8+ T-cell infiltration are seen to a greater extent in patients with prostate carcinoma than in individuals with benign disease [7]. NK cells have also been observed in the infiltrate and are associated with a good prognosis for prostate cancer. Following these findings, the first Food and Drug Administration approved immunotherapy regimen (Provenge™) [8] has been used for patients with late stage castrate resistant prostate cancer. This treatment involves the isolation of leucocytes from patients and the expansion of dendritic cells that are then pulsed with PAP antigen and a homologue of GMCSF (granulocyte/macrophage colony-stimulating...
factor) to create activated antigen presenting cells that are injected back into the patient. The activated dendritic cells stimulate the production of effector T-cells with specificity against PAP which can circulate to the prostate and surrounding tissues to kill the tumour cells. This treatment can extend the life of the patients by up to 4 months, although the cost is approximately US$93000 per patient.

However, there is a major caveat with this treatment. When NK cells and T-cells infiltrate the prostate cancer microenvironment, they are exposed to potent immunosuppressive mechanisms that render them unable to kill tumour cells. Regulatory T-cells are frequently observed in prostate tumours with a CD4+CD25+FOXP3+ (FOXP3 is forkhead box P3) phenotype or a novel CD8+FOXP3+ phenotype [9]; it is observed that these latter suppressive cells arise by the conversion of CD8+ effector cells when they enter the prostate tumour microenvironment [10]. Where cells are not converted into a suppressive phenotype, they can still be rendered anergic. NK cells from prostate cancer infiltrates have also been shown to be inactive [11]. These findings indicate that although the Provenge vaccine will allow the creation of active PAP-specific T-cells that will be directed to the prostate, once they enter the cancerous prostate they may be rendered anergic or inhibitory.

### Immune checkpoints in the prostate cancer microenvironment leading to immunosuppression

There are a number of immune mechanisms that are thought to suppress T- and NK-cell function in the prostate tumour milieu (reviewed in [6]). Previous studies of the prostate cancer and other tumour cell milieu have highlighted TGFβ (transforming growth factor β) and IL-10 (interleukin-10) as potent immunosuppressive agents [12] and these are secreted by many cell types within the tumour microenvironment including T-effector cells, macrophages, tumour cells, stromal cells and regulatory T-cells. Recently discovered immune checkpoints also include the PD1–PD-L1 (programmed death 1 protein–programmed death ligand 1) coupling which will result in apoptosis of activated T-cells [13]. Activated effector T-cells express the PD1 receptor that ligates with PD-L1. In healthy individuals, PD-L1 is only expressed in monocytes and in macrophages of the lung, tonsils and liver. However, in patients with prostate cancer, PD-L1 is expressed on a small percentage of prostate cancer cells and on many types of cells in the prostate microenvironment including macrophages, stroma cells and other lymphocytes [14]. The inhibitory B7 family proteins B7x and B7-H3, which ligate with CD28 to inhibit T-cell activation, are also expressed on 99 and 93% of prostate tumour cells and the extent of expression correlates with poor survival in the disease [15].

The regulatory T-cell mediated CTLA-4 (cytotoxic T-lymphocyte antigen 4) binding to CD28 is already a well-known checkpoint that is now being targeted with anti-CTLA-4 therapy (Ipilimumab) [16], currently in clinical trials as a single agent and in combination with therapies such as Provenge.

### Role of WAP (whey acidic protein) domain-containing proteins in cancer immunosuppression

In recent years, members of a new family of proteins containing one or more highly conserved four-disulfide structural domain [WFDC (whey four disulfide core domain)] or WAP regions have been identified. The best characterized molecules are elafin, SLPI (secretory leukocyte peptidase inhibitor), ps20, Eppin, HE4 and Kal-1, with major functions including a homoeostatic control of inflammation and/or antibacterial or antifungal activity [17]. Cytokines and other mediators produced in pro-inflammatory environments, such as TNFα (tumour necrosis factor α), PGE2 (prostaglandin E2) and LPS (lipopolysaccharide) produced by bacteria initiate the expression and secretion of SLPI and elafin from epithelial cells [18].

There are NF-κB (nuclear factor κB) binding sites on WFDC1, SLPI and other gene promoters that encode WAP proteins (i.e. WFDC5, WFDC6, WFDC8 and WFDC12) [19], therefore indicating that these proteins are expressed upon NF-κB signalling, a common occurrence in inflammation and in tumour progression.

When WAP domain-containing proteins are expressed, they function to attenuate the inflammation that leads to their expression, and therefore they have many characteristics in common with immunoregulatory cytokines such as TGFβ and IL-10. In fact, both SLPI and elafin stimulate the production of TGFβ and IL-10 from activated macrophages [20]. The novel WAP proteins WFHKKN1 and WFHKKN2 are known to also bind members of the TGFβ family and thus may act to concentrate and localize these growth factors in inflammatory lesions [21]. TGFβ itself also stimulates the production of the protein WFDC1 (ps20) from cancer associated fibroblasts [22]. Originally, ps20 was shown to be a permissive factor in HIV and its expression is induced in activated CD4 T-cells [23]. In turn, ps20 can suppress infiltrating effector T-cells by inhibiting the production of IFNγ (interferon γ) [24] and can induce TGFβ production from endothelial cells [22].

As the tumour microenvironment is frequently of an inflammatory nature, it is not surprising that elafin and SLPI are expressed at high levels in many tumours. The expression of elafin, SLPI and other WAP domain-containing proteins such as HE4 also correlate with the progression of a number of aggressive cancers such as ovarian cancer, head and neck cancer and non-small cell lung cancer [28–29].

In prostate cancer, however, elafin, SLPI and HE4 gene and/or protein expression are observed to be decreased compared with normal prostate tissue or benign hyperplasia [19]. This is perhaps because, unlike ovarian and lung cancer, the inflammatory stages of prostate cancer may occur very early in the disease, often long before clinical diagnosis has
taken place. The majority of the carcinoma arises after the inflammation has abated. There is emerging evidence of the role of infection and inflammation in prostatic carcinogenesis [30] and bacterial infection induces expression of SLPI and HE4 in prostate epithelial cells [30a].

The other reason for a lower expression of the above WAP domain-containing proteins in cancerous cells is the possibility that these proteins may be mutated during carcinogenesis to create alternatively spliced forms that could aid further progression of tumours. The expression of ps20 is down-regulated on prostate stromal cells upon progression of prostate cancer, whereas its expression on epithelium is increased [31]. The ps20 gene detected on cancerous prostate epithelial cells has exon deletion and is a splice variant of ps20 expressed on prostate stroma [32]. HE4 is present in a number of normal tissues, but can undergo a complex series of alternative splicing events that can potentially yield five distinct WAP domain-containing protein isoforms [33]. It is therefore possible that the genes of elafin and SLPI may be alternatively spliced to give alternative protein isoforms in prostate cancer that have not yet been detected. The possibility of alternative splice variants of these proteins arising in cancerous cells and the functional differences between these proteins and their forms expressed in normal tissue is therefore critical to the understanding of their pleiotropic roles in tumour progression.

The expression of some WAP domain-containing proteins is also modulated by androgens and receptor signalling on prostate epithelial cells [34,35], and although this has not been investigated for ps20 or elafin and SLPI, this may also apply to these proteins.

Potential immunotherapeutic approaches
The current goal of immunotherapy in prostate cancer is to create an anti-tumour immune response in patients where cancer is extensive and metastatic. The immunological checkpoints such as PD1 and B7x above can occur at many stages of cancer progression, but if prostate cancer lesions are targeted with immunotherapy at an early stage, then early checkpoints such as WAP protein expression and other inflammatory cofactors can also be addressed. There are three major considerations to choosing the immunotherapeutic regimen in these patients. First, the immunotherapy has to effectively combat the immunosuppressive tumour microenvironment; our current findings indicate that IL-15, a potent expander of NK cells and CD8 T-cells, is an effective candidate cytokine when administered in the immunosuppressive prostate cancer effector-immune cell co-culture environment [36]. IL-15 also has potential to inhibit immunosuppression mediated by WAP domain-containing proteins due to its activation of STAT5a (signal transducer and activator of transcription 5a), which will inhibit the promoter region of these genes [37]. Secondly, research to identify potent immunotherapeutics has tended to focus on activation and proliferation of the effector cells at sites away from the prostate lesion, and as mentioned above, T-cells from the periphery activated through therapies such as Provenge® will become severely suppressed when entering the prostate. The immunotherapy should therefore be administered locally to the prostate and new technologies utilizing MRI (magnetic resonance imaging)/ultrasound guided needle targeting of prostate lesions can ensure a highly localized delivery of a therapeutic compound [38]. Thirdly, the compounds administered should be retained at the site of the lesion since the risk/benefit ratio of systemic immunotherapies such as IL-2 and anti-CTLA-4 is too great to be administered to early stage prostate cancer patients due to severe toxic reactions [39–41]. Technologies allowing retention of drugs, such as the membrane-targeting cytotoxic technology [42–44] previously used to retain drugs such as Mirococept in transplanted organs, and the use of polymeric drug delivery [45] will therefore play a key role in targeted localized immunotherapy for prostate cancer, while preventing collateral damage.

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