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Review

Interferon γ, a Possible Therapeutic Approach for Late-stage Prostate Cancer?

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Abstract. *Prostate cancer is the most frequently diagnosed cancer in men in Western countries. Clinically localized disease can be cured with surgery or radiotherapy, but once the disease has advanced or spread, there are no curative treatments. This review examines the potential utility of cytokines, in particular interferon gamma (IFNγ), in the treatment of clinically advanced prostate cancer.*

Prostate Cancer and Immunotherapy

The current treatment of prostate cancer largely depends on the stage at which the cancer is diagnosed. Due to the insidious onset of the disease, prostate cancer often presents at a late stage, where there may be extracapsular invasion and metastasis to surrounding tissue. By definition in low-grade tumours (more differentiated carcinomas) stromal invasion is present and as the tumour becomes more undifferentiated (higher Gleason grade), the risk of metastasis increases. Clinicians use prostate-specific antigen (PSA) level, histological information (such as Gleason grade) and imaging to calculate the chance of the patient having localized or locally advanced cancer. In patients with prostate cancer which has extended outside the prostate, initially to the seminal vesicles or local lymph nodes, curative treatment becomes less feasible (1). In late-stage carcinomas the treatment is often palliative and tumours can become unresponsive to hormonal treatments within a median period of 18 months (2). However, there has been an emergence of new therapeutic strategies and chemotherapeutic agents. These include second-line hormonal therapy (3), chemotherapy (4), vitamin D analogues (5, 6), immunotherapy (7), COX2 inhibition therapy (8), granulocyte macrophage-colony stimulating factor (GM-CSF) therapy (9-11), dendritic cell therapy (9, 12), gene vaccination therapy (13), inhibition and/or blockade of growth factor receptors or growth factor receptor pathways (14) and inhibition of neo-angiogenesis (15).

Many of these treatments display antiproliferative and anti-angiogenic properties *in vitro* and may decrease PSA levels *in vivo*, but none yet have produced significant prolonged survival in patients with late-stage prostatic disease. This is exemplified in the case of immunotherapy with interferons. Stimulation of the body's immune system by enhancement of endogenous processes or by exogenous administration of cytokines has been a popular approach to anticancer therapy. Enhanced cytokine signalling by either of these means has been shown to instigate antiproliferative (16-19), proapoptotic (17, 19-23), antimetastatic (17, 18, 24, 25), immunostimulatory (26) and antiangiogenic (20, 27) effects. By virtue of these effects, cytokines could be seen as the ideal tumor suppressors, however, it has been noted that many tumours display a lack of immunogenicity possibly due to a lack of major histocompatibility complex (MHC) class 1 expression (26, 28). Prostate cancer exemplifies this: the prostate cancer cell line LNCaP is unresponsive to interferons due to epigenetic silencing of Janus kinase 1 (JAK1) expression, a protein required for interferon signal transduction (29). In addition, prostate cancer metastases, from which the LNCaP cell line is derived, express little or no MHC class 1 protein (30). It has been postulated that this lack of immunogenicity is an important event in tumourigenesis as it allows the cancer cells to evade host immune responses (30, 31). Tumor-mediated immunosupression may also occur at the systemic level as elevated levels of cytokine receptors identified in cancer patient serum have been found to bind to and inhibit cytokine activity (32).

Several studies have examined the effects of cytokines, particularly interferons, on prostate cancer cells. Interferons are proposed to be the ideal tumour suppressor as they are highly specific in differentiating between normal and neoplastic cells. These cytokines are known to have cytotoxic

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Table I. *Clinical trials of IFNα combination therapy.*

Treatment	No. of participants	Response rate	>50% PSA reduction	Reference
IFNα, IL-2 and dendritic cells primed with prostate-specific membrane antigen (PSMA)	13	31%	23%	40
caIFNα, Retinoic acid	14	15.3%	7.7%	41
IFNα, TNFα	10	38%	18-87%	22
IFNα, 5-fluorouracil	28	0%	14%	42

IL-2: Interleukin-2, TNFα: tumor necrosis factor α.

and immunomodulatory effects on cancer cells, not only at the primary site, but also at a cellular level in disseminated cancer (33). Interferon alpha (IFNα) has been the most extensively studied of the interferons in relation to prostate cancer. IFNα is produced by lymphocytes, lymphoblasts and macrophages and is part of innate immunity, activating macrophages and monocytes, stimulating natural killer (NK) cells and enhancing antigen expression by MHC1 (26). *In vitro,* IFNα has been found to inhibit growth of the prostatic cell line DU145 by limiting progression from the G1- to the S-phase of the cell cycle in a p53- and pRb-independent manner (16). In xenograft mouse models of prostate cancer, IFNα has been shown not only to have an antiproliferative effect, mediating a 37% reduction in tumour volume, but also an antimetastatic effect in decreasing lymph node metastases. This is possibly due to an increase in the ratio of E-cadherin to matrix metalloprotease (MMP)-9, as the ratio of these proteins has been suggested to be an indicator of metastatic risk (17). In addition IFNα and β have been observed to increase androgen receptor levels *in vitro*, possibly demonstrating a method of restoring androgen sensitivity in late-stage tumours (34).

Clinically, IFNα is in therapeutic use for renal cell carcinoma, ovarian cancer, melanoma, hepatocellular carcinoma and transitional cell carcinoma, and has undergone phase I and II trials, for use in prostate cancer (17, 22, 26, 35-38). In the largest of these trials IFNα treatment produced a response rate of 5% and a >50% reduction in PSA in 23% of patients (39). This minimal beneficial effect was however, accompanied by high toxicity, with patients experiencing weight loss, fatigue, malaise, central nervous system toxicity, leucopenia, nausea and vomiting. This study concluded that patients cannot tolerate the high doses of IFNα required to produce a beneficial effect in prostate cancer (26). Subsequently, to limit the dose of IFNα required, many clinical trials have been conducted combining IFNα with various other treatments (Table I).

As Table I demonstrates, the greatest cytokine-based therapeutic effect in prostate cancer was achieved by a combination of two cytokines and co-administration of primed dendritic cells was able to further stimulate the host immune system. Patients within these trials, however, did still experience varying levels of toxicity. However, a recent study has indicated that IFNα increased androgen receptor levels in prostate cells, possibly increasing androgen-stimulated growth (34). Further investigation will be required to ensure the benefits of IFNα treatment outweigh the side-effects.

The therapeutic potential of interferon beta and gamma (IFNβ, IFNγ) in prostate cancer has been studied far less than that of IFNα. IFNβ is produced by fibroblasts and epithelial cells and, like IFNα, has an antitumour activity (26). *In vitro*, IFNβ has been found to suppress the growth of androgen-independent JCA-1 cells, established from a primary prostatic tumour site prior to antihormonal therapy, by 15-30% and when combined with the antitumor agent Onconase, by 42-51% (18). In mouse prostate cancer xenograft models, IFNβ suppressed tumour and metastasis formation by 80% and eradicated tumours in 20% of mice (27) and suppressed growth by 90% when transfected into the tumour site (43). This effect may be due to the anti-angiogenic properties of IFNβ in reducing the expression of MMP's and pro-angiogenic factors (17), as prostate tumours from mice treated with IFNβ contained fewer microvessels and a greater degree of apoptosis than untreated tumours (27). IFNβ is in clinical use for renal cell carcinoma and has been used in clinical trials for advanced prostate cancer (44). However, 65% of participants in that study were unable to complete treatment due to disease progression and those who did continue showed no response in terms of tumour volume, spread or PSA level, and they also suffered various side-effects such as shivers and fever (44).

IFNγ is a type II interferon produced predominantly by CD4+ lymphocytes and NK cells that affects cells by signalling through the IFNγ receptor (IFNγR), Janus kinase-signal transducer and activators of transcription (JAK-STAT) pathway. Activation of this pathway by IFNγ leads to the transcription of interferon-inducible genes containing gamma-activated sequence (GAS) elements, *via* the ISGF3γ transcription complex, which consists of a 48-kDa DNA, binding protein (p48) and two signal transducing activator

of transcription (STAT) proteins, STAT1 and STAT2 (45, 46). In humans, the IFNγ-inducible genes thought to be involved in prostate cancer progression are located on the 10q 23-26 and 17q 21 chromosomal loci, regions which are commonly deleted in prostate cancer (47). Studies have found this region to be deleted in 30% of prostate cancer samples (49) and it is thought to encode tumour suppressor genes (47, 48). A recent paper indicated that deletion of interferon-regulatory factor 8 (IRF8) in region 16q24 in various carcinomas, including these of the prostate, leads to a decrease in the tumour suppressor activity of IFNγ (49).

Molecular Effects of IFNγ in Prostate Cancer Cells

The cell surface effects of IFNγ in prostate cancer cells include increased adhesion *via* modulation of cell adhesion molecules (CAMs) such as P-cadherin, α integrins and intracellular adhesion molecule 1 (ICAM1); increased density of cell receptors; clustering of receptors into large lipid raft structures or "signalosomes"; and an increase in the display of proteins signalling the status of the cell (*e.g.* MHC-1, tumour-associated antigens) (50). Through modulation of these various cell surface proteins, including CAMs and receptor tyrosine kinases (RTKs), cytokine treatment can alter several prostate cancer attributes closely associated with tumour invasion and the acquisition of a metastatic phenotype. In addition, IFNγ has been found to reduce the binding affinity for bone matrix stroma in prostate cancer cells, indicating that this cytokine may be effective in reducing the secondary skeletal tumours common in prostate cancer (51). In the prostate cancer cell lines PC3 and LNCaP, *in vitro* exposure to IFNγ enhanced Fas-mediated cell death, rising from 40% to 60% . In a mouse model of prostate cancer, IFNγ treatment lead to a three-fold increase in apoptosis in the primary tumor (23). This study suggests that IFNγ acts to sensitize prostate cancer cells to the effects of Fas inhibition.

In mouse models of metastatic prostate cancer, xenografts in treated mice showed a distinctly smaller tumour volume than those left untreated, even at the lowest dose of IFNγ (47) and mice lacking IFNγ progressed to metastasis quicker than those expressing the cytokine. The IFNγ-negative mice were as susceptible to tumour metastasis as mice lacking NK cells (24). In a recent study, intratumoral innoculation of mice xenografts with adenovirus-mediated IFNγ gene significantly reduced growth without toxicity (52). IFNγ may be of therapeutic use for prostate cancer where it could be beneficial in late-stage and hormone-refractory disease by retarding invasion and metastasis. The finding that mice lacking the IFNγ receptor develop spontaneous tumours more rapidly than those with functional receptors indicates that IFNγ is involved in both immune surveillance and retardation of invasion and metastasis, making this cytokine a true candidate for tumour/metastasis suppressor status (47, 48).

Interferon Signalling

A recent study resulted in the identification of key constituents of the IFNγ signalling pathway that appear to exhibit differential expression patterns between normal and neoplastic cells when stimulated with IFNγ. In accordance with the published literature, this study suggested that cultured prostate cancer cells, 1542 CP3TX, exhibit differential expression of certain molecules in the IFN signalling pathways in comparison to their normal isogenic counterpart, 1542 NP2TX, specifically the transcription factor p48 (48). The pattern of induction of p48 expression following interferon stimulation observed in this study was in agreement with that seen in a recent study of transitional cell carcinoma of the bladder, where IFNα treatment led to a low level of p48 expression which could be restored by pre-treatment with IFNγ (53). Combinatorial use of IFNγ and α has also proved effective in increasing p48 and causing subsequent growth inhibition in renal cell carcinoma (54). Defects in the activation of p48 have been noted in the hepatocelluar carcinoma cell line MHCC97, where mutations possibly affecting the stability of the protein were shown to negate the antiproliferative effect of IFNα (55). In clinical observations, the presence of p48 staining in patients with hepatocellular carcinoma was predictive of poor survival (38). This may suggest that cancer cells gain a selective advantage by suppression of the expression or activity of p48, thereby decreasing sensitivity to the immunomodulatory effects of IFNs, which include growth inhibition, increased adhesion and heightened immunosurveillance. In general, it has been noted that IFNγ is capable of elevating p48 levels in cancer cells unresponsive to other cytokines and, as a subsequent effect, cancer cells become sensitive to the growth inhibitory effects of IFNα (53, 56). This may suggest that IFNγ could be used as an adjuvant to enhance the effects of IFNα in growth retardation of prostate tumours.

gp96

The alteration in interferon signalling in cancer cell lines has been found to include the modulation of proteins on the cell surface, including gp96 and annexin II (AII). In a recent study it was observed that overexpression of gp96 in 1542 CP3TX cells could be decreased significantly by treatment with IFNγ. An opposite pattern of gp96 expression was observed in 1542 NP2TX cells, as in this cell line, stimulation with IFNγ appeared to increase the expression level of gp96 and its isoforms. These results supported the previous finding that gp96 is up-regulated in the cancer cell line and add a novel dimension in the discovery of the production of previously unreported gp96 isoforms in normal prostate cells, following stimulation with IFNγ. In their normal resting state, the prostate cancer cells appear to overexpress a single form

of gp96, a feature known to be associated with avoidance of apoptosis (57, 58). In these cancer cells on stimulation with IFNγ, there is an apparent decrease of the expression of this single gp96 isoform and a failure to produce the multiple isoforms of gp96 observed in immunoblotting of normal cells. Although the immuno-logical activity of gp96 has yet to be characterized, gp96-associated peptides constitute the antigenic repertoire of the source tissue. As such, the multiple isoforms may be required as part of the functional role of gp96 as a molecular chaperone involved in the transport of antigenic peptides to the MHC class I proteins on the cell surface. In Xenopus frogs, gp96 mediates a potent immune response involving MHC class Ia proteins (59). The gp96-mediated antigen display has been demonstrated to elicit a CD8-specific T-cell response and therefore possible anti-tumour immunity (60). The multiple isoforms of gp96 seen in the normal cells could possibly represent the result of glycosylation events, as a recent study observed differences in the gp96 monosaccharide composition between normal and cancer cells from rat and cell line prostate cancer models. Additionally, a further decrease in gp96 glycosylation was observed in cells with more aggressive cell phenotypes (61). Down-regulation of gp96 glycosylation may be selectively advantageous for the cancer cells in avoidance of the immune response, a mechanism also observed in bacterial infection by *Orientia tsutsugamushi* (62). Suppression of gp96 is a potential negative effect of IFNγ treatment, as it is possible that the MHC I display of tumour-associated antigens may be adversely affected.

Annexin II

AII has been reported to have a potential role in cell invasion (63-68) but has been found to be expressed at the same level in both normal and cancer prostate cell lines. However, cell surface expression of AII in 1542 CP3TX and NP2TX appears to be suppressed by IFNγ in a surface-specific manner, which was also observed in a second isogenic pair of cell lines 1532 NP2TX and CP3TX. The apparent surface- specific suppression of AII by IFNγ also appeared to result in a decrease in the invasive capacity of cells expressing AII. This finding was in accordance with the research of Falcone *et al*., which demonstrated that matrix invasion and degradation was dependant on cell surface AII expression in macrophages (63). The role of AII in invasion has been further suggested by transfection of an AII expression vector into LNCaP cells, which appeared to increase invasive capability. However, the vector was a dual construct also encoding green fluorescent protein (GFP), which could interfere with the expression and normal biological function of AII. AII may have a significant role in prostate epithelial cell invasion, possibly through interaction with endopeptidases and extracellular matrix components (69-72).

The apparent ability of IFNγ to reduce AII expression on the cell surface has a potentially therapeutic application, as it may reduce the invasive potential of prostate cancer cells. In a study using prostate cancer xenografts in mice, Shou *et al*. found that with IFN treatment the rate of progression to metastasis decreased (47). However, it appears that the loss of surface-expressed AII is accompanied by a reduction in lipid raft structures in the cancer cells. These lipid raft structures are known to contain many of the signalling factors, receptors and MHC molecules required for interaction with the host immune system (73). In normal cells, the response to IFNγ treatment has been shown to involve the formation of signalosomes, as was seen in this study. In cancer cells, however, this cytokine appeared to reduce the quantity of lipid rafts and no signalsome formation was noted. Exogenous interferon treatment could therefore be potentially detrimental as it may impede detection of cancer cells by the host immune system.

Appraisal of the Therapeutic Benefit of IFNγ

IFNγ treatment may prove beneficial as an adjuvant pre-treatment before exposure to further cytokine-based therapy, to limit proliferation and retard the invasive potential of cells in prostate cancer. However, it has also been demonstrated that this cytokine may affect proteins, such as gp96, and structures, such as lipid rafts, involved in cell–immune system interactions. Therefore, the potential benefits of IFNγ treatment may be negated by the effect of the cytokine on immune system interactions. As such, treatment with IFNγ may cause a lack of detection of cancer cells and potentially increase the disease load. However, several proteins have been identified that show apparent differences in response to cytokines between normal and cancer cells. These proteins could therefore become targets for therapeutic intervention in their own right. For example, gp96 has been used previously as an antitumour vaccine in a rat model of prostate cancer, administration of host-derived gp96 limited both the occurrence and growth of prostate tumours, indicating that treatment with this vaccine could be of prophylactic or therapeutic use in prostate cancer patients (74). Clinical trials would be required before this could be considered a viable treatment option. AII expression could provide a potential drug target to limit remodelling of the basement membrane by cancer cells. If effective, such treatment may be of particular use in mid- to late-stage prostate cancer in limiting further spread of the disease.

In summary, proteins with the potential utility in the treatment of prostate cancer have been identified, though further characterisation of these proteins is required before their clinical application can be realised. Morevover, IFNγ may be of therapeutic benefit in the suppression of prostate cancer, but the effects of this cytokine on cell–host immune system interactions may limit its clinical application. Further research of these mechanisms may indicate the molecular basis for the evasion of host responses necessary for tumourigenesis.

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