Chapter 16

DISTILLING THE PAST – ENVISIONING THE FUTURE

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The foregoing contributions make it patently clear there has and continues to be an "explosion" of burgeoning new technology and resulting information in cellular and molecular biology of prostate cancer. It is noteworthy that there is also a renewed interest in the under appreciated area of immunology with the immune response as a prospective biological marker and the use of immunotherapy. However, this knowledge has thus far out distanced our ability to assimilate and translate it to bring to bear on the complexities and dilemmas faced in meeting the "challenge" of the enigmatic face of prostate cancer. We are therefore of the opinion that the key to the "challenge" of prostate cancer is the translation of basic knowledge into more efficacious preventative, diagnostic and therapeutic end points.

1. NATURAL HISTORY

As a first priority, it must be acknowledged that the natural history of prostate cancer, as considered by Penson and Albertsen (1), remains as enigmatic as ever. Studies, cited by Penson and Albertson (1), and elsewhere in this Volume, have shown that a substantial proportion of men will survive long-term, and free of disease progression, even in the absence of any treatment. In the current era where, increasingly, men are diagnosed on the basis of a biopsy prompted by a marginally elevated level of prostate-specific antigen (PSA), it is likely that an even higher proportion of men diagnosed require no treatment (2). In the recently published study of Finasteride

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as a preventative agent for prostate cancer, the design anticipated a 6% incidence of prostate cancer in the control group of patients. What they actually found was a 24% incidence (3). On first thought this may appear to be a reflection surely, that we are getting better and better at picking up insignificant, or latent prostate cancer since it is obvious that 24% of the male population do not develop clinically significant prostate cancer. However, we would perhaps concur, as suggested by Welch et al. (4) that "... the cellular abnormality that pathologists call prostate cancer is far too prevalent to be consistently clinically important." And, "How much prostate cancer is found seems to be directly related to how hard it is looked for." (4). In addition, we have substantial uncertainties about the best treatment, even for patients with a higher likelihood of having "significant" disease; there is a conspicuous lack of high quality clinical trials in this area. Two ongoing studies – the European Randomised Trial of Screening for Prostate Cancer (ERSPC), and the UK ProtecT study (5), will shed light on the utility of screening and, perhaps on the optimum form of curative treatment. Analysis of the further follow up of a recent randomised trial conducted by the Scandinavian Prostate Cancer Study Group (6) reporting estimated 10-year results has shown an overall survival benefit for patients treated by radical prostatectomy rather than by watchful waiting, together with a significant difference in prostate cancer-specific mortality and in the rate of developing metastatic disease in favour of patients treated by surgery. The significance of this is, at the moment, unclear particularly as its magnitude could not easily be translated into the expected benefits of treatment in today's patient population.

And so, the time, if not already past due, has come to recognize and change the standard of care for patients with prostate cancer to reflect the facts that:

- Aggressive treatment for localized low-grade disease is not usually warranted.
- Prostate cancer beyond localized low-grade disease is potentially a systemic disease and must be managed with that in mind and that,
- Therein treatment should ideally include attention not only to the location of the tumor at detection, but to its biological properties.

Therefore, having set "metastasis as a therapeutic target" let us in that which follows endeavour to bring into perspective ongoing observations within each of the topics covered.

2. GENES AND METASTASIS

Initially, we can directly attack the tumor cell genotype. A greater understanding of the molecular basis of metastasis will itself generate a range of

new targets (7). In addition to CpG methylation, pointed out by Maitland (7) as the most commonly recognized and observed mechanism for selective gene silencing, histone modifications, a second prominent type of epigenetic regulatory mechanism, is closely related. By way of example the Vitamin D receptor by which 1, 25 $(OH)_2$-vitamin D_3 acts to exert cell-cycle regulatory antiproliferative effects, is regulated by histone modification. Indeed, clinical trials with histone deacetylation inhibitors are now underway. The significance of epigenetic silencing of select genes can contribute to cancer initiation, progression, invasion and metastasis.

Of a number of methylated genes in prostate cancer which correlate with clinicopathological features and may be critical to the ability of tumor cells to invade and metastasize, E-cadherin may be selected as a prototypical example. The role of E-cadherin in the biology of the prostate, its partners, signalling mechanisms and implications in the progression and metastasization of prostate cancer have been meticulously covered in respective overviews by Hendrix et al. (8) and Davies et al. (9).

Of further interest in relation to the E-cadherin/catenin complex considered by Davies et al (9), is that in addition to its known immunohistochemical expression in the prostate, recent studies by Kuefer et al. (10) have reported the identification of an 80 kDa fragment of E-cadherin in the serum of patients with prostate cancer. Although not disease-specific, nor related to tumor burden, the highest serum levels were observed in patients with advanced hormone refractory metastatic prostate cancer. Parenthetically, at an optimized cutoff, high expression of the 80 kDa serum fragment at the time of diagnosis was associated with a significantly increased risk of late biochemical failure at 3 years after radical prostatectomy.

For those interested in CpG hypermethylation and alterations during the progression of prostate cancer to metastasis, the reader is referred to the recent review by Yegnasubramanian and Nelson (11).

Mention and, albeit brief, comment on the following genes and proteins identified through the technique of gene expression profiling described by Maitland (7), is appropriate given their potential to identify tumors that are on an aggressive path toward the development of metastasis and given that, in select instances, they may in themselves serve as potential therapeutic targets.

Metastasis-associated gene 1 (MTA1), is involved in transcriptional silencing and is overexpressed in metastatic compared to localized prostate cancer, suggesting it plays a role in cancer progression to the metastatic state (12). MTA1 silencing is mainly dependent on histone deacetylases and is another example of epigenetic silencing via histone modifications.

Expression of JAGGED1, a NOTCH receptor ligand, which plays a role in epithelial to mesenchymal transitions (requisite for the migration of cancer

cells) in cancer is significantly increased in metastatic vs. localized prostate cancer or BPH (13). JAGGED1, as well as other genes is increased in other cancers, and, therefore, its role, as suggested by Santagata et al. (13) in distinguishing indolent from aggressive prostate cancer must be viewed with caution.

Survivin, an inhibitor of the apoptosis gene family and overexpressed in numerous cancers, has been linked to accelerated relapses, hormone refractory disease and unfavorable outcome. Given the association of differentiation of neuroendocrine (NE) cells in relationship to the growth and progression of prostate cancer, notably hormone refractory tumors, the identification of survivin and its overexpression in NE cells (14) is noteworthy.

In maintaining homeotic gene expression, two groups of proteins referred to as polycomb and trithorax play a key role in the transcriptional maintenance or memory system of the host. Dysregulation of this system can lead to malignancy. Among the polycomb proteins, there are genes involved in control of cell growth and division. Therein expression of Enhancer of Zeste homolog 2 (EZH2), a transcriptional repressor, has been found to be significantly higher in hormone-refractory, metastatic vs. localized prostatic tumors or normal prostate (15) suggesting that overexpression "portends aggressiveness and metastasis" (15). Studies of EZH2 to date have shown it to be the best predictor of clinical outcome; and have demonstrated EZH2 has a role in mediating cell proliferation and transcriptional repression contributing to the lethal progression of prostate cancer (15). Within this context, it may, pending further evaluation, be considered as a marker to distinguish indolent from aggressive prostate cancer.

In searching for further proteins influencing metastasis of prostate cancer, hedgehog – a cell signalling molecule that drives normal development and regeneration in the prostate (and in several other tissues) is significantly upregulated in prostate cancers that have been observed to metastasize (16, 17). Development of methods to selectively target hedgehog may prove useful in inhibiting the progression of prostate cancer.

Among investigations toward understanding the role of serine proteases upregulated by androgens in the metastatic process of prostate cancer, several recent studies have looked at the TMPRSS2 gene. *In situ* hybridization studies showed localization of TMPRSS2 to basal cells (18). However, given its presence in the normal, as well as the malignant prostate and in other tissues, e.g., colon and lung, the initial hopes for diagnostic and therapeutic targeting of TMPRSS2 in the absence of its other interacting macromolecules would seem to be questionable. It is noted however, that

as TMPRSS2 is expressed on the cell membrane, as well as being released therefrom, it could function as a receptor for other proteins and/or act in an autocrine manner. In this regard, TMPRSS2 has been observed to activate the protease activated receptor (PAR)-2 expressed in the prostate which increases the levels of matrix metalloproteinases (MMPs)-2 and -9 –key proteases contributing to the metastization of tumor cells (19).

With up to 35% of prostate cancer patients treated for organ confined disease having a local recurrence which may eventually lead to metastatic disease, a key question is whether the metastasis comes from pre-existing micrometastasis or from persistent disease remaining locally. Therefore, in addition to the identification of tumors that are on an aggressive path toward metastasis, the ability to detect micrometastatic disease is critical. From this perspective, Kufer et al. (20) have described a novel sensitive multimarker nested RT-PCR capable of detecting individual expression of human melanoma-associated antigens (MAGE [also named cancer-testis antigens])-A genes. Members of the MAGE-A gene family, MAGE-1, -2, -3/6, -4 and –12 have been observed in rare disseminated tumor cells in the blood and bone marrow of patients with prostate cancer. Patients with an exceptionally high risk of metastatic disease, defined by clinical prognostic factors, were significantly more MAGE positive than lower risk patients (20).

Further, within the context of predicting clinical outcome, increased phosphorylation of the serine/threonine kinase Akt (Ser^{473}) [phosphorylated Akt (pAkt)] and decreased phosphorylation of extracellular signal-related kinase (ERK; Thr^{202}/Tyr^{204}) [phosphorylated ERK (pERK)] have been shown by Kreisberg et al. (21) to be an excellent predictor of poor clinical outcome. In terms of distinguishing an indolent vs. a would-be aggressive cancer, phosphorylation of Akt alone or together with ERK may, pending further study, be a useful biological marker of a clinically aggressive cancer.

In commenting on various signaling pathways and signaling elements, we should make brief mention of toll-like receptors (TLR) (22). TLR, which activate innate and adaptive immune responses are, in addition to being present on immune cells, expressed on tumor cells from a variety of tissues. Recent studies by Zheng et al. (23) have demonstrated sequence variants of the TLR4 gene in association with the risk of prostate cancer. The association of sequence variants in TLR4 and risk for prostate cancer is particularly interesting when placed in context with observations that chronic infection and inflammatory processes, hallmarks of several diseases, including prostatitis, prone to progress to cancer, are mediated in part by the recoginition of various stimuli by TLR (24). The significance of this

in relation to evasion of immunosurveillance and metastasis may be seen from studies in which activation of TLR4 signaling in tumor cells has been observed to induce the synthesis of soluble factors which protect tumor cells from cytotoxic T-lymphocytes (CTL [25]).

As noted, there are a number of genes overexpressed in prostate cancer. However the majority of genes identified are also expressed in several common malignancies and thereby not specific for prostate cancer. Additionally, many of the genes identified may be reflective of the "output of hyperactive cells rather than the molecular machinery driving (the) metastasis" (26) and/or in themselves induce no or only a modest response, however when co-expressed with other genes be highly relevant. Therefore, as the question has been raised by Eccles and Paon (26): "How can we use gene profiling to find genes casually linked to metastasis?"

Recent observations, e.g., with vimentin by Singh et al. (27), in studies of its overexpression in an androgen-independent model of prostate cancer, provide an example of the role of the necessity for co-expression with other genes. In the case in point, transfected induced overexpression of vimentin needed the co-expression of cytokeratins intermediate filaments to confer the invasive and metastatic phenotype.

3. THERAPEUTIC TARGETS

3.1 Gene Therapy

Against this backdrop, Eccles and Paon (26) have suggested that from approaches investigating gene-expression profiles in combination, "we might eventually move closer to the ultimate goal of individualized targeted therapy". Additionally, and as logical as it may be, we must remember that while it is one thing for a gene to be expressed, we must also pose the questions: is the protein there and is it functional?

Gene therapy has yet to overcome the formidable problems of delivery, particularly in patients with disseminated disease, but there may, nonetheless, be other ways of using it in prostate cancer, for example by preventing disease progression in patients with localised disease (28). Nonetheless, using gene therapy as a means of recruiting the immune system is a strategy with inherent advantages in this respect, although data which suggests that tumors produce a cytokine repertoire that induces tolerance, or indeed apoptosis in cytotoxic T-cells, might yet defeat this approach (29, 30, 31), unless as suggested herein by Satoh et al. (32), the object of gene therapy itself is to reverse this (32).

3.2 Dietary Supplements

In continuing, one might target specific aspects of prostate cancer cell biology. Herein, initial thoughts perhaps turn to diet and hormone sensitivity.

The link between diet and prostate cancer, initially suggested by Armstrong and Doll (33) based on international differences in mortality rates and the national average intake of fats, has been considered herein by Jiang (34). In the interim of the observations by Armstrong and Doll (33) some case-control studies have shown a significant association between various measures of fat intake, most notably saturated fat, monounsaturated fat and alpha-linolenic acid with advanced prostate cancer, while others have not.

With the focus on the metastatic process, Jiang (34) has provided a meticulous look at the role of polyunstaturated fatty acids (PUFA) by examining their effect on the essential steps in the metastatic cascade toward the formation of tumor metastases. This analysis has illustrated the impact of PUFA and their potential therapeutic value on the metastatic process.

In keeping with the observations of Jiang (34), but with a focus on the general parameter of prostate cancer risk vs. metastasis, Bidoli et al. (35), in one of the largest case-control studies of diet and prostate cancer to date, have shown a decreased risk of prostate cancer (OR 0.8) in association with PUFA.

Not related to PUFA, but a possible *caveat* from the study by Bidoli et al. (35), for further investigation, was the association of high starch intake and an increased risk for prostate cancer (OR 1.4). As a possible explanation, Bidoli et al. (35) suggest the glycemic overload may be compensated by an increase in insulin-like growth factor-1 (IGF-1) known to be associated with prostate cancer. Within the context of IGF-1 and diet, the recent study by Kelavkar and Cohen (36) has shown overexpression of the fat-metabolizing enzyme, 15-lipoxygenase in prostate cancer, which contributed to cancer progression by regulating IGF-1 receptor expression and activation.

3.3 Molecular Mechanisms of Hormone Resistance and Optimization of Hormone Therapy

With reference to hormone sensitivity, there is an urgent need to understand the mechanisms whereby prostate cancers become hormone refractory (37). This might result from the recruitment of alternative “rescue” signal transduction pathways (38, 39, 40, 41, 42) or by subversion of androgen receptor (AR)-mediated signalling, e.g., using a different repertoire of

co-activators and co-repressors (43, 44), including the cytokine IL-6 (45). Additionally, autocrine and/or paracrine mechanisms may, in lieu of, or in concert with mutations and/or amplifications of the AR, activate pathways, producing responses of the AR downstream (46), e.g., studies by Yeh et al. (47) and Wen et al. (48) have reported that overexpression of the tyrosine kinase, HER-2/neu (ErbB-2) in prostate cancer promotes androgen-independent survival and growth of prostate cancer cells and activated AR transcriptional function through the downstream mitogen-activated protein kinase or Akt pathway. Given the role of HER-2/neu signaling, it is a natural target to disrupt AR function. However, clinical trials using humanized monoclonal antibodies to HER-2/neu (Herceptin) only and in combination with docetaxel have been problematical due to the variable overexpression of HER-2/neu (49). In an attempt to obviate this problem a humanized monoclonal antibody, referred to as Pertuzumab, that targets the role of HER2 as coreceptor has been developed (50). Pertuzumab binds to a different epitope of the HER2 ectodomain than Herceptin and sterically hinders ligand-dependent heterodimerization of HER2 with other HER receptors. This results in inhibition of signaling by HER2-based heterodimers in cells with low and HER2 expression. Pertuzumab has shown antitumor activity in preclinical models of prostate cancer. However, a recently reported phase II study with this agent was negative (51).

Reviewed by Shelley et al. (52), the full potential of our existing modalities of hormone therapy has yet to be completely determined, and this deserves continued attention (53, 54, 55). Herein, the naturally occurring pathway of RNA interference (RNAi), a unique form of post-transcriptional gene silencing, may have application. Utilizing a prostate-specific vector expressing small interfering RNAs (siRNAs) from the PSA promoter –a RNA polymerase II promoter, Song et al. (56) recently demonstrated androgen-dependent and tissue-specific siRNA-mediated gene silencing in the androgen-dependent prostate cancer cell line, LNCaP. Biologically, the significance of this was evidenced by alteration of apoptotic activity via inhibition of the apoptosis-related regulatory gene. Additionally, by way of further example of siRNA interference, inactivation of TWIST, a highly conserved basic helix-loop-helix transcription factor, levels of which correlate with Gleason grade and metastasis in androgen-independent prostate cancer cells resulted in increased chemodrug-induced apoptosis and suppression of invasiveness (57). These observations provide impetus for further study of the potential effectiveness of siRNA-mediated gene silencing for the treatment of prostate cancer.

3.4 Proliferation

Concurrently there are many approaches, simply directed against proliferation, which may be of benefit in prostate cancer, just as they may be in many other cancers (58, 59, 60).

In addition to the loss of the tumor suppressor –PTEN, as considered by Newman and Zetter (58) and subsequent constitutive activation of the Akt pathway leading to activation of mitogenic and pro-survival signaling molecules (as one of the ways of undermining cell cycle control in the control of the progression of prostate cancer to androgen-independence), the pivotal transcriptional factor, nuclear factor kappaB (NFκB) has been shown to prevent cell death by apoptosis in PC-3 and DU-145 cell lines (61), Interestingly, blockade of NFκB activity in PC-3 xenografts in nude mice inhibited angiogenesis, invasion and regional lymph node metastasis (62). The observation that NFκB is constitutively activated in the bone metastasis-derived PC-3 cell line (63) further implicates its role in bone metastasis as also considered previously herein by Hoffman (64) and Clarke and Fleisch (65).

Of particular further interest with reference to the role of NFκB in metastasis are observations by Ayala et al. (66) in perineural invasion (PNI) in prostate cancer. A key process for the extracapsular spread of prostate cancer, studies of PNI in an *in vitro* model of PNI and of human prostate cancer tissue microarrays prepared from patients who underwent radical prostatectomy, demonstrated increased proliferation and decreased apoptosis of perineurally localized cancer cells in association with up-regulation of NFκB and its downstream targets, PIM-2 and defender against death 1.

The foregoing and related observations by others supports the rationale for inhibitors of NFκB presently in development and/or in clinical trials for the prevention and/or treatment of prostate cancer (67).

In addition to the cell cycle markers considered by Newman and Zetter (58) shown to have a direct role in dysregulation leading to metastatic prostate cancer, one of the earlier noted oncogenes, c-myc (also mentioned by Maitland [7]) exhibits increasing amplification with transition through PIN to metastatic prostate cancer (68) and with increasing Gleason score (69) and mortality. However, as several genes are also located at the 8q24 amplicon, which is amplified, the role of c-myc in prostate cancer has been unclear. However, recent studies have shown that: i) transgenic models overexpressing c-myc in the prostate have a concentration related progression towards malignancy and ii) c-myc in a model of human prostate cancer is sufficient to induce carcinogenesis (70).

4. TUMOR MICROENVIRONMENT

One area, which is proving to be of particular importance in prostate cancer, and in understanding mechanisms of invasion and metastasis, is the dynamic relationship between the tumor and the host, i.e., the tumor microenvironment (71, 72, 73, 74, 75, 76, 77).

Observation of the plasticity of tumor cells by Hendrix et al. (8), whereby the dynamic interplay between tumor cells and their microenvironment may determine their function and fate, *ergo*, vasculogenic mimicry and the acquisition of the malignant phenotype further demonstrates that: i) tumor cells do not grow in isolation and ii) that the inter- and/or intratumoral microenvironment are important to modulation of gene expression and the phenotypic properties of tumor cells and tumor-derived factors in extension and refinement of earlier studies pointing to the role of the microenvironment and factors therein in tumorigenesis (78). The environment, while not inducing malignancy, may permit activation of quiescent tumor and/or compromise the hosts control, i.e., immunosurveillance of tumor, thereby being permissive (78).

With the objective of looking into select aspects of these interactions in the microenvironment, but confronted with the challenge of the heterogeneous characteristics of prostate cancer, Hendrix et al. (8) utilized, as they have described, an integrated *in vitro* and *in vivo* strategy permitting development of a series of new Dunning R3327 Copenhagen rat cell lines. Ongoing studies of these cell lines are revealing expression of multiple molecular phenotypes by aggressive tumor cells and that their co-operation is necessary for the successful progression of prostate cancer (8).

Within the framework of the progression of prostate cancer, Wilson and Sinha (79) have, in their Chapter "Matrix Degradation in Prostate Cancer", provided a superbly detailed overview, with a specific focus on proteases, of the means by which prostate cancer cells proceed from the primary prostatic tumor promoting growth of the tumor and passage from one biological compartment to another to their subsequent colonization at distant sites. Inclusive of the diverse proteases permitting passage of malignant cells are the MMPs. In addition to their role in contributing to the cleavage of the basement membrane (BM) and extracellular matrix (ECM) proteins of prostate tissues, tumor-derived MMPs also react with host immune cells in the primary tumor facilitating the escape of tumors from immunosurveillance, e.g., by inducing proteolytic cleavage of IL-2Rα (a receptor essential for the proliferation of T-cells) they suppress the proliferative capability of cancer encountered T-cells (80). Also, increased numbers of immune system cells, i.e., macrophages, neutrophils, known to

contain MMPs, may under select environmental conditions contribute to passage of tumor cells by proteolysis of the BM (80).

Turning briefly to the serine proteases, and PSA, Wilson and Sinha (79) have provided a brief comment on the clinical significance of serum PSA, the subject of which has also been considered herein by Penson and Albertson (1). For further specifics of current PSA assays the interested reader is referred to a brief communication by one of us (81) laying out the culpability of these assays (82). With an appreciation of the functionality of PSA as a serine protease, continuing investigations of its important role in the normal prostate and its pathophysiology (82) are further significantly considered (82).

Also of importance in the tumor microenvironment, are the presence of tumor-associated macrophages (TAMs). Contrary to general opinion, there is considerable recent evidence that TAMs not only fail to kill tumor cells, but contribute to tumor progression through enhanced invasiveness of tumor cells by the secretion of factors such as cytokines and MMPs (83). TAMs further inhibit lymphocytic activity at the tumor site via the production of immunosuppressive macromolecules.

Identified in TAMs and elsewhere in the normal and pathologic prostate and secretions (84), the calcium-dependent family of enzymes – transglutaminases (TGases), which include the thrombin-independent and – dependent, tissue and Factor(F) XIII (found in plasma and cells) forms, respectively, are noteworthy (84). Intriguingly: i) tissue and plasma TGases modulate the activity of select parameters of immune responsiveness and ii) significantly increased concentrations of plasma TGase have been found in association with prostate cancer vs. normal and benign prostate (84). The close association of TAMs and plasma TGase suggest it is involved in the binding of host proteins to tumor cells forming a stabilized intratumoral fibrin that facilitates tumor matrix generation, angiogenesis and a barrier to mechanisms of host defense. The localization of plasma TGase to prostatic histiocytes expressing monocyte/macrophage differentiation markers, providing a means for TGase in the regulation of antigen presentation and induction of immune responses, portends to the permissive, if not direct, role of TGase in the hosts regulation of invasion and metastasis of tumor cells (84, 85)

Of further interest regarding the role of TGase in regulation of the invasiveness of prostate cancer are studies of its effect on S100 protein function.

Members of the S100 family of Ca^{2+}-binding proteins have been implicated in a variety of cellular processes, e.g., calcium signal transduction, cytoskeletal-membrane interactions, cellular growth and differentiation. Referred to as psoriasin from its original association with abnormally

differentiating keratinocytes in psoriasis (86), the gain or loss of S100 protein expression has been linked to various disease states, wherein, e.g., as cited by Maitland (7), S100A4 has been linked directly to metastatic disease.

Based on psoriasin as a candidate substrate for TGases (87), Davies et al. (88) observed that at the mRNA level, TGase-4 (prostate TGase) was strongly expressed in the low invasive CA-HPV-10 prostate cancer cell line and its substrate psoriasin was increased in TGase-4 knock-out cells, accompanied by increased immunocytochemical staining at regions of cell-cell contact. Requisite of further study, these observations suggest that through its effect on the cytoskeletal-membrane properties of psoriasin, TGase may modify the invasiveness of prostate cancer.

Enhanced invasiveness correlates with induction of NFκB and *c-Jun*-NH_2-kinase (JNK), wherein NFκB promotes migration of tumor cells by inducing the expression of the chemokine receptor CXCR4 (89).

In a somewhat over-simplification of a complex process, prostate cancer cells showing a propensity to metastasize to the skeleton express the CXCR4 chemokine receptor and are attracted by the CXCL12 (also known as stromal-derived factor-1) [SDF-1] chemokine ligand to secondary sites, where they form metastases (90). The binding of CXCR4/CXCL12 leads to the activation of multiple signaling pathways, e.g., PI3K/Akt, with differential secretion of various cytokines and MMPs, particular MMP-9, into the local environment and ensuing events. Migration studies show that antibodies to CXCR4 inhibit chemotaxis of metastatic cell lines of prostate cancer (90). Similarily, pharmacological inhibition of PI3 kinase and MAP kinase pathways abrogates CXCL12-induced MMP-9 expression (91). These observations suggest inhibition of the CXCR4/CXCL12 pathway may prove therapeutically beneficial. In fact, this approach has already been pursued in the instance of breast cancer (92).

Additionally, we note that some chemokines may enhance innate and specific host immunity against tumors, but at the same time other chemokines may contribute to escape from the immune system by recruiting Th_2 effectors and regulatory T cells.

A final point on this lengthy, but we do believe pertinent commentary on the microenvironment is recent attention to the importance of recognizing the "microenvironment of the circulation" (93). Referred to as the "third" microenvironment, with the primary tumor site being the "first" and the metastatic or secondary tumor site constituting the "second", the circulatory system, from the perspective of its importance to metastasis, has according to Loberg et al. (93) been "under appreciated." Analysis therein of circulating tumor cells and factors permitting their passage and survival, avoiding

destruction by among other factors, e.g., the immune system, may disclose means for the therapeutic targeting of tumor cell survival.

The foregoing observations emphasize the importance of studying the tumor microenvironment. These observations suggest the biological behaviour of malignant cells are intimately related to the surrounding milieu, which in itself may be mutagenic and an important source of genetic instability.

With recognition of the critical importance of the microenvironment and advent and continuing refinement of today's technology, it should be possible to integrate molecular profiles of gene expression in the tumor microenvironment with the histological features of the tumor. This integration will permit a much-needed modification of the current tumor grading systems, i.e., in the case of prostate cancer – the Gleason score from static histopathology. The Gleason score cannot, in guiding treatment, presently distinguish an indolent from an aggressive cancer in more than 66% of newly diagnosed cancers with Gleason scores of 5–7. The significance of this is exemplified by innumerable observations where, biopsy specimens from two patients with prostate cancer may be histologically identical, but one may remain indolent, while the other may be aggressive.

Elucidation and understanding of interactions between the tumor and the microenvironment will also provide new opportunities for adjunct and new methods of diagnosis and treatment.

5. MODELS OF METASTASIS

A model whereby factors such as hepatocyte growth factor (HGF), produced by stromal cells, may induce tumor cells to metastasize is one that may be of importance (94). As well as introducing the possibility that antagonists of factors such as HGF might be useful in preventing metastases, it also serves to refocus attention on the "normal" stroma surrounding a tumor, to ask the question as to whether there may be interventions that might affect the natural history of the disease. This might fall exclusively into the realms of prevention, itself an important and worthy goal, but there is also evidence of cross-talk between receptors such as *c-met*, and the epidermal growth factor receptor family. Such treatments might, therefore, apply to established disease and maybe even to established metastatic disease. The development of appropriate orthotopic models is absolutely crucial for the further exploration of this area and we continually need to refine these in order to render them as realistic a model as possible, and free from the artificialities that such an approach can all too often engender (64).

From this perspective Hoffman (64) has provided a valuable overview of the background and description of orthotopic metastatic animal models of prostate cancer and their application for the study of the progression and metastasis of prostate cancer. Included, is an extremely useful narrative on materials and methods of implantation and evaluation of tumor growth and metastasis.

Surgical orthotopic implantation of human tumor to an animal host provides a more realistic model of human cancer. When used in concert with the introduction (via transfection) of a green fluorescent protein gene to tumor cells, this enables metastasis to be visualized throughout the skeletal system and to other important organs. The approach permits important insight into mechanisms of prostate cancer metastasis. With this in mind, and from the perspective of treatment, Hoffman (64) has considered the application of selected gene and other types of therapy in the metastasis models presented.

There may, however, be quite specific mechanisms which drive proliferation in prostate cancer cells, e.g., ornythine decarboxylase (95) and tissue-specific mechanisms, if they exist, would further open up new therapeutic avenues.

6. IMPACT OF BONE METASTASIS

A natural extension of this line of argument is to consider the impact of bone as a specific site of metastases, on the development of new methods of diagnosis, prediction of metastasis and treatments. In this regard, we are reminded from the chapter by Clarke and Fleisch (65) that with the axial skeleton involved in 85% of patients dying from prostate cancer, bone metastasis is the hallmark of metastatic prostate.

With reference to new methods of diagnosis and prediction of metastasis, use of fluorodeoxyglucose as a tracer to detect abnormal metabolism in invaded tissues, single-photon emission CT and positron emission tomography have improved sensitivity (96). For early prediction of bone metastasis, two promising osteoblastic markers –P1NP and P1CP are suggested as particularly promising (96).

Bone metastasis factors which encourage prostate cancer cell growth in bone, such as transferin, might themselves be amenable to manipulation. In addition, the use of bisphosphonates as a means of switching off osteoclast-mediated bone destruction, is already well established, at least in clinical trials (65). The optimum use of such agents remains to be determined, and as with almost every other possible therapeutic, is likely to be most useful

in combination i.e., in multimodality therapy. There is also an issue about the timing of therapy. The published data on bisphosphonates in clinical use is heavily weighted towards patients with established metastatic disease. The Medical Research Council (MRC) PR04 Study which, was a double blind randomised trial of adjuvant clodronate in patients with non-metastatic cancer, was an attempt to see whether bisphosphonate treatment earlier in the natural history might be of benefit. The first results of this trial have shown no improvement in time to onset of symptomatic bone metastases, in stark contrast to similar trials in breast cancer and myeloma (97), and one wonders what the effect might have been with a more potent, newer generation bisphosphonate.

On the subject of the use of bisphosphonates in combination with other agents, systemic administration of zoledronate combined with STI571 (imatinib mesylate, Gleevec [an inhibitor of phosphorylation of the platelet-derived growth factor receptor]) and paclitaxel in experimental prostate cancer bone and lymph node metastasis, produced a significant decrease in tumor incidence and size accompanied by significant preservation of bone structure and a decrease in lymph node metastasis (98).

Although the efficacy of treatment with bisphosphonates in inhibiting bone resporption has been clearly demonstrated, several secondary and undesirable side-effects have also been noted ranging from, e.g., from the recent FDA safety warning on Aredia (pamindronate disodium) and Zometa (zoledronic acid) injection after cases of osteonecrosis, particularly of the jaw (http://www.fda.gov/medwatch/SAFETY/2004/safety04.htm#zometa) to nephrotoxicity. Within this context, alternate treatments to bisphosphonates based on knowledge of osteoclast biology have been proposed. These include strategies based on cytokines, peptidomimetics and inhibitors of specific signaling pathways.

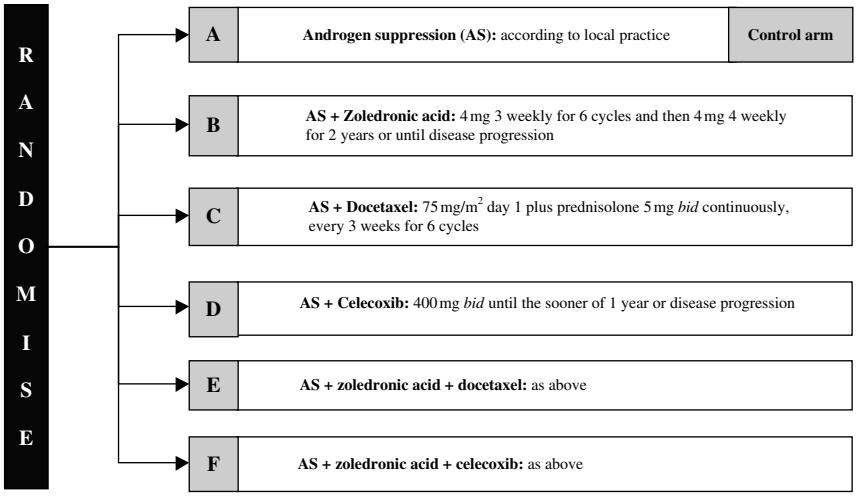
Before leaving these comments on bisphosphonates, we should mention recently reviewed preclinical studies have shown bisphosphonates exhibit antitumor activity (99).

7. OTHER BIOLOGICAL AGENTS

Other biological agents are also finding their way into clinical trials. The importance of prostaglandin synthesis in the development of prostate cancer has been highlighted earlier in this book. Whether or not inhibitors of COX-2 might be useful therapeutically is an open question (95). In this regard, recent concerns of COX-2 inhibitors have arisen due to their association with an increased risk of mortality from cardiovascular complications (100).

In the interim, studies in human prostate cancer cells, i.e., LNCaP and PC3, and PC3 xenografts in nude mice have shown celecoxib (Celebrix) not only targets COX-2, but reduces levels of cyclin D1 (impacting on the progression of cells from G_1 to S) and caused approximately a 50% decrease in proliferation and microvascular density (101). Although still an investigational product in prostate cancer, given confirmation and extension of the foregoing results, one may have to eventually balance the toxicity issues with celecoxib with those of chemotherapy.

This and other questions, are being addressed in the ongoing MRC "STAMPEDE" (Systemic Therapy in Advancing or Metastatic Prostate Cancer: Evaluation of Drug Efficacy) study, which is a 5 arm study randomising patients beginning hormone therapy to either standard hormone therapy alone, or to hormone therapy plus docetaxal, zoledronic acid, a COX-2 inhibitor, to a combination of chemotherapy and bisphosphonate, and to a combination of COX-2 plus bisphosphonate (Figure 1). This study is aiming to recruit 3000 patients in the next 6 years, and is currently in its pilot phase of patient recruitment (Figure 1).



AS = Androgen suppression as in Arm A

Figure 1. Schema of the Medical Research Council STAMPEDE Trial. Patients beginning long-term hormone therapy, for either metastatic or non-metastatic prostate cancer, are randomised as above. Following a pilot phase to establish feasibility and safety, the trial will proceed to a first analysis with failure-free survival as the primary endpoint. Arms not showing a benefit will then be dropped, and the trial will proceed to a second phase, using the successful arms, with overall survival as the primary endpoint.

In concert with the known capabilities and limitations of chemo- and radiotherapy considered by Scullin et al. (102), various approaches have been undertaken toward augmenting their effects. Within the obvious limitations of a complete discussion here of all of these approaches, mention and a brief comment is made on selected potentially promising approaches.

Evidence of the repopulation of surviving tumor cells between courses of chemotherapy and an increase of their rate of proliferation has suggested the use of short acting agents selectively inhibiting tumor cells may be beneficial (103). Toward this end, Wu et al. (103) have shown the use of the rapamycin analogue CCI-779 given between courses of mitoxantrone or docetaxel increased growth delay of PC-3 xenografts.

Coupling cytotoxic agents to specific monoclonal antibodies to target tumor antigens has been applied in prostate cancer. Based on the expression of prostate-specific membrane antigen (PSMA) on the surface of prostate epithelial cells, monoclonal antibody to PSMA coupled to maytansinoid 1, a microtubule-depolymerizing compound, demonstrated antitumor activity in the CWR22 xenograft model of osteoblastic prostate cancer metastasis (104).

With further reference to the earlier mentioned use of siRNA-mediated gene silencing (55), siRNA silencing of p53 mutant PC-3 prostate cancer cells and the caffeine target, ataxia telangiectasia mutated (ATM) gene (a member of the phosphatidylinositol 3-kinase [PI3K] family of proteins, that activates DNA repair and cell cycle checkpoint pathways) selectively increased the sensitivity of PC-3 cells vs. normal cells to doxorubicin (105). Within the context of silencing select checkpoint pathways toward the selective killing of tumor cells, the use of an antisense RNA to ATM also increased the radiosentivitivity of prostate cancer cells (106).

8. RADIATION THERAPY AND BEYOND

Toward clarifying the ion beam-specific biological effects, comparison of the metastatic capabilities of tumor cells following irradiation with photon, proton and ion carbon beams has demonstrated preclinical evidence that particle radiation best suppresses metastatic potential of tumor cells (107), at least in this experimental model. Whether this observation has clinical application or not may require a renewed effort to develop further studies of proton beam therapy in prostate cancer (108).

Looking toward expanding the use of radiation therapy to metastasis, several investigators have applied radioimmunotherapy. Here a radiolabeled antibody specific for a component of the primary tumor and metastatic cells is used to deliver radiation to the target. Radiolabeled antibody to

PSMA, represents one popularized approach (109). Most recently, Zhao et al. (110) have utilized an antibody to tomoregulin, a transmembrane protein selectively expressed in the brain, prostate and prostate cancer (primary tumor and metastatic tissues, i.e., in lymph nodes and bone), but not in other normal tissues or a wide range of tumors of other major organs, to deliver radiation to inhibit the growth of LNCaP xenografts in nude mice in the absence of any overt toxicity. As noted, the presence of tomoregulin protein in metastases portends that tomoregulin is a potentially excellent target for radioimmunotherapy.

In an attempt to target occult metastatic disease (presumptively suggested by many) as an explanation for biochemical failure, which occurs in 30–40% of patients treated by surgery or radiation for localized prostate cancer, Gulley et al. (111) demonstrated in a randomized Phase II Clinical Trial that a poxviral vaccine encoding PSA induced a PSA-specific T-cell response when combined with definitive external beam radiation therapy in patients with localized prostate cancer. The trial has demonstrated the feasibility of combination therapy, its effect on the immune system and that radiotherapy to the prostate is not broadly immunosuppressive. However, even though the authors state the foregoing was the purpose of the study, there is no reference to the identification of the supposed occult metastasis in the patients and thus no demonstrated clinical effect of the antibodies. Furthermore, two patients who received vaccine plus radiation therapy and previously had lymph node positive disease developed metastatic disease (to the liver and adrenals, respectively) and went on to receive chemotherapy! So much for the proposed synergistic effect of the vaccine plus radiation and the beneficial clinical effect of the immune response, at least in these two patients.

9. NOVEL THERAPEUTIC APPROACHES

Scullin et al. (102) have further given consideration, albeit brief, to "Novel (therapeutic) approaches".

On the subject of anti-angiogenesis agents, it is noted bevacizumab (Avastin), currently used in combination with taxane-based therapies is the recombinant humanized version of the murine antihuman vascular endothelial growth factor (VEGF) monoclonal antibody A4.6.1 referred to by Scullin et al. (102) in the studies by Melnyk et al. (112).

With further reference to VEGF, a *caveat* perhaps to regulation of angiogenesis in the prostate is appreciation that there are at least four isoforms of VEGF, i.e., A, B, C and D, each with different physiological

roles and receptor affinities (113). Therefore, a greater understanding of the differential role of VEGF receptors and whether therapies designed to target these specific molecules will prove efficacious. Presently, decreased VEGFR1 and increased VEGFR2 are associated with the transition from a differentiated cancer to more poorly differentiated state (114). Equally, different angiogenic mechanisms may be differentially expressed at various stages of tumor progression.

With reference to variation in angiogenic mechanisms in general and in accord with various stages of disease, there are in addition to VEGF, as recently reviewed by Lissbrant et al. (115), a variety of blood flow and angiogenesis regulatory factors. These include among others, fibroblast growth factor, transforming growth factor-beta1 (TGF-β1) and endoglin –a receptor for TGF-β1 on endothelial cells. A particularly interesting angiogenesis factor associated with a metastatic phenotype is pigment epithelium-derived factor (PEDF [116]). Independent studies by Halin et al. (116) and Filleur et al. (117), to which the interested reader is referred, provide evidence that decreased expression of PEDF contributes to tumor progression possibly through increased tumor cell proliferation and angiogenesis.

Buoyed by the increased incidence of prostate cancer and number of patients who fail local therapy for "presumed to be" localized disease, presenting with a recurrence of their cancer, the number of therapeutic agents and clinical trials have increased significantly over the past decade. A search, e.g., of the Pharmaceutical Research and Manufacturers of America for New Medicines in Development for Prostate Cancer 2005 (http://www.phrama.org, 5 October 2005) disclosed 50+ drugs under development. From these and others, we mention in brief the following which appear within Scullin et al.'s (102) category of "Novel (therapeutic) approaches".

Identification of unique or metastasis specific signal transduction pathways in prostate cancer may provide insight into development of small molecule inhibitors with clinical utility. Therapeutic small molecules that inhibit components of such signal transduction pathways have the potential to specifically target metastatic cancer.

Studies suggesting the epidermal growth factor receptor (EGFR) signaling pathways may be involved in angiogenesis and invasion in prostate cancer prompted investigations of targeting it as a potential therapeutic approach.

Early preclinical studies of Cetuximab, an anti-EGFR monoclonal antibody, directed toward prevention of activation of the tyrosine kinase receptor in an orthotopic model of androgen-independent prostate cancer - PC-3M-LN4, demonstrated activity alone and in combination with paclitaxel

(118) and based on stabilization of PSA in a small group of patients with androgen-independent prostate cancer (119). However, subsequent studies of an EGFR anatagonist, Iressa (Gefitinib, ZD1839) in androgen-independent prostate cancer were marked by inconsistent PSA responses and early progression of disease (120). These mixed results await further clarification of the role of EGFR in prostate cancer.

Another growth factor implicated in the progression of prostate cancer is platelet-derived growth factor (PDGF) and its receptor (PDGF-R). Binding of PDGF to PDGF-R and its activation stimulates cell division, migration and angiogenesis. Activation of PDGF-R also has been shown to inhibit pathways leading to apoptosis. Observations that Gleevec (STI157, imatinib mesylate) a specific inhibitor of the oncogene Bcr-Abl associated with chronic myloid leukemia (121) also inhibits PDGF-R, prompted investigation of its effect on prostate cancer. Therein studies by Uehara et al. (122) of the effects of Gleevec alone or in combination with paclitaxel in the androgen-independent PC-3MM2 mouse model of bone metastasis in prostate cancer, disclosed Gleevec, particularly in combination with paclitaxel resulted in significant inhibition of tumor growth, increased apoptosis, decrease in lymph node metastases and bone lesions and preservation of bone structure. While suggestive that PDGF-R would be a promising therapeutic target in prostate cancer, clinical trials of Gleevec in combination with the bisphosphonate Zometa or alone have thus far shown no (123) or at best a limited response (124). A current study is looking at Gleevec in combination with docetaxel.

The use of antisense oligonucleotides have been used to target the antiapoptotic proteins Bcl-2 and clusterin.

Preclinical studies of models of prostate cancer have shown that antisense Bcl-2 inhibits expression of Bcl-2 and delays the transition from an androgen-dependent to androgen-independent growth (125). A Phase II trial of Genasense (G3139, Oblimersen) and docetaxel by the European Organization for Research and Treatment of Cancer is ongoing.

Observations of increased expression of clusterin in association with high Gleason scores and high levels in tumor cells surviving androgen ablation therapy, suggests follow up to an initial Phase 1 clinical trial in prostate cancer of the second-generation antisense drug OXG-011 (which compared to the 1st generation, has a longer half-life and fewer side effects) may be of importance. OXG-011 may, in the trend of the multi-drug approach, i.e., a "cocktail" of drugs vs. a stand-a-lone drug, prove efficacious not only in treating localized but in hormone-refractory disease (126).

Ansamycin antibiotics, often referred to as "antineoplastic antibiotics," can inhibit the function of heat shock protein 90 (hsp90), a chaperone

protein for select signaling proteins, such as AR, HER2 and Akt. This leads to destabilization and degradation of the complexes, which is frequently mediated by the ubiquitin-proteasome pathway. In response to appropriate signals, a chain of ubiquitin molecules covalently attaches to a protein that targets it for destruction by the proteasome which controls the regulated turnover of proteins (127). The potential use of ansamycin antibiotics, for which geldanamycin (GA) (128) is an example of one such agent for prostate cancer, has been evaluated in preclinical studies (129). In addition to degradation of the AR, Mabjeesh et al. (130) have shown GA induces degradation of hypoxia-inducible factor 1α (HIF1α). HIF1α plays an essential role in the adaptation of tumor cells to hypoxia and in promoting angiogenesis. Inhibition of HIF1α blocks tumor angiogenesis and tumor cell growth (130).

Perhaps also broadly within the category of "antineoplastic antibiotics", studies by Lokeshwar et al. (131) and ongoing studies by one of us (RJA) have demonstrated the potential efficacy of chemically-modified tetracyclines in inhibiting metastasis in preclinical models of prostate cancer.

Another promising approach involves inhibition of the ubiquitin-proteasome pathway. Preclinical studies of the proteasome inhibitor, bortezomib (PS-341, Velcade) in LNCaP cells show it blocks the AR signaling pathway, inhibits tumor growth and induces apoptosis (132). Phase II studies of bortezomib alone and in combination with docetaxel are in progress.

10. ANTIOXIDANTS, PHYTOCHEMICALS AND OTHER NATURAL PRODUCTS

In recent years an interest in complementary and alternative medicine (CAM) not considered part of conventional medicine, has gained considerable popularity to the extent that upwards of 60% of cancer patients use one form or another of substances found in nature such as vitamins and herbs. In taking a cursory look at approaches within the broad category of CAM, select dietary antioxidants and natural products are suggestive of having potentially varying degrees of efficacy for metastatic prostate cancer.

Dietary antioxidants as vitamin E, lycopene and selenium have primarily been evaluated as potential chemopreventative agents. However, reviewed by Venkateswaran et al. (133), independent studies have shown each to have effects ranging from induction of cell arrest to inhibition of tumor progression in preclinical models of prostate cancer, with lycopene having an effect by reducing the odds of patients with prostate cancer developing advanced and aggressive disease. Of particular interest are recent studies carried out by Venkateswaran et al. (133) in the *Lady* transgenic

model of prostate cancer of the mouse prostate. This is a less aggressive version of the original model, which in spontaneously developing metastatic prostate cancer, mimics progressive forms of human prostate cancer. Therein, vitamin E, selenium and lycopene in the diet in proportion to the human equivalent dramatically reduced the incidence of prostate tumors and increased disease free survival.

Recently, vitamin D_3 (cholecalciferol) alone (134) and in combination with the synthetic retinoid N-(4-hydroxyphenyl) retinamide (135) have been observed to inhibit growth and invasion and the expression of vimentin and MMP-2 associated with tumor progression, respectively, in human prostate cancer cells.

It is important to note that studies have shown that antioxidants may have negative effects by promoting or protecting cancer cells (136, 137) and/or reducing the oncologic effectiveness of cytotoxic therapies (138). Therefore, the use of antioxidants concurrently during chemo- and radiotherapy may be contraindicated.

Several phytochemicals, exemplified by genistein –a prominent isoflavonoid found in soy products, have been shown to possess substantial anticancer activities in prostate cancer, and clinical trials not only for prevention, but for treatment of prostate cancer and its metastases are ongoing (139).

Proceeding with genistein as an example, several mechanisms have been suggested for its effects. Genistein is an inhibitor of protein tyrosine kinases which play key roles in cell growth and apoptosis. Studies have demonstrated genistein can inhibit cancer cell growth, induce apoptosis and inhibit the activation of NFκB and Akt in prostate cancer. Cognizant genistein is a phytoestrogen, the relationship of the foregoing effects to its oestrogenic content, as well as its potential to induce adverse effects associated with the earlier prevalent use of DES, remain to be determined. The interested reader is referred to two recent excellent studies by Li et al. (140) and Huang et al. (141) on the role of genistein in the invasion and metastasis of prostate cancer.

Logical as it is, as long as the AR is functional irrespective of AR-dependent or –independent status, the growth of prostate cancer continues.

Potentially effective as genistein may be, pending the outcome of clinical trials (139), emodin –a natural compound extracted from the plant, *Rheum palmatum* (commonly known as Chinese rhubarb), has been reported by Cha et al. (142) to be more potent and to directly target the AR. Specifically, emodin induces degradation of the AR through the proteasome-mediated pathway by decreasing the association of AR and heat shock protein 90 (hsp90) (142). This results in inhibition of cell proliferation and tumor growth of prostate cancer cells with increased survival of PC-3 xenografts of

prostate cancer (142). Pending the outcome of clinical trials, emodin could be a novel and vastly needed therapeutic for directly targeting the AR.

The earlier *caveat* regarding the possible adverse effects given for the use of antioxidants applies equally well for the foregoing natural products.

11. IN TRANSITION

Despite advances in the detection of localized disease, there is no effective treatment for patients who develop recurrent disease following surgery or radiation therapy or for those with metastatic disease. And, while hormonal therapy may be temporarily palliative for patients with advanced disease, the progression to incurable hormone refractory prostate cancer is inevitable. Furthermore, with the: earlier diagnosis; questionable treatment of prostate cancer; substantial number of patients with recurrent disease and the earlier institution of hormonal therapy, patients are becoming hormone refractory earlier in their course of disease while still having a reasonable remaining life expectancy, but with no further effective therapeutic options available.

Therefore, although it may appear to the newly interested basic- and clinical-investigators of prostate cancer that there has been an improvement in the treatment of this disease, if there has, it has been marginal at best. By way of example, the present management of patients with advanced prostatic cancer exemplified by the recent studies with docetaxel based regimens, i.e., docetaxel + prednisone (143) or docetaxel + estramustine (144) have been met with laudable enthusiasm. However, they unfortunately do not result in curing patients and the survival benefit has been on the order of 2–3 months compared with standard therapy, modest at best.

While it may too severe of a statement, the effective treatment of prostate cancer within the current accepted "standard of care," and overall survival for metastatic disease have not significantly changed since the introduction of hormonal therapy by Huggins et al. (145). Therefore, the urgent need to develop more effective treatments with the appreciation that prostate cancer requires localized as well as systemic therapy. The development and implementation of "novel therapeutic" approaches, some of which have already been considered, as well as those to follow, have recently emerged and/or are in development.

12. IMMUNOTHERAPY

Waxing and wanning since the time of Ehrlich's "magic bullet" (146) and the introduction of "immunosurveillance" by Burnet (147), the concept of immunotherapy (including vaccination) based on the exquisite sensitivity

and specificity of the immune response presents a formidable means for the destruction of localized tumor and metastases. However, a deeper appreciation and understanding of how immunologic responsiveness may play a fundamental role in prostate cancer is slowly becoming a reality.

With one of us (RJA) perhaps the first to introduce and review possible immunotherapeutic approaches for prostate cancer (148), it is particularly gratifying to see the recent and continued development of the use gene therapy and other vehicles to generate a systemic immunologic response as a therapeutic strategy for this disease.

Considered in a recent overview of viral gene therapy by Hawkins et al. (149), oncolytic viruses continue to receive widespread attention as novel therapeutic agents for the treatment of malignancies, including prostate cancer. *Herpes simplex* virus thymidine kinase (HSV—tk) has been one of the most widely used "suicide genes" to date, particularly because of its "bystander effect." In fact, the initial therapeutic strategy described for metastatic prostatic cancer herein by Satoh et al. (32) employed a direct cytotoxic approach using the HSV-tk gene combined with the prodrug ganciclovir (GCV). This strategy was further modified and expanded, as described by Satoh et al. (32), to include additional immunomodulatory gene therapeutic approaches.

In looking at other approaches, Kaminski et al. (150) and Markiewicz and Kast (151), have provided excellent reviews of immunotherapy for prostate cancer. Lest we be redundant, further and albeit brief comment here, will be reserved in the main, to what are viewed as potentially noteworthy approaches for metastatic prostate cancer not included in the referenced reviews.

Using active specific immunization, major histocompatibility complex (MHC) Class I and non-restricted CD8+ cytotoxic T lymphocytes (CTL) to PSA and specific residues of PSA have been demonstrated *in vitro* by a number of investigators (152, 153). In an extension of their initial study (152), Perambakam et al. (153) induced PSA peptide-specific CTL from two patients with hormone-refractory prostate cancer (Stage D_3). The T cells obtained from these patients were of a Tc2 (Type 2 $CD8^+$ T cells) as opposed to a Tc1 (Type 1 $CD8^+$ T cells), cytokine profile response, i.e., primarily IL-4 rather than IFNγ.

In an extension of an earlier study demonstrating PSA-specific CTL responses *in vitro* to monocyte-derived dendritic cells (DC) transfected with PSA mRNA (154), Hesiser et al. (155) have reported PSA-specific CTL responses in a Phase 1 study in 13 patients with metastatic prostate cancer (Stages D_1-D_3). Therein, the immune response was associated with a: i) significant decrease in PSA velocity and ii) transient clearing of circulating tumor cells in the peripheral blood of some patients. Albeit limited to 13

patients, an important concern is whether the immune response demonstrated using PSA RNA-transfected DC as a "surrogate target" in cytotoxic assays, will show lysis of autologous tumor cells? Given resolution of the foregoing, of particular significance for the further application of this approach is that in contrast to peptide-based vaccines, which are limited in use to select patient subsets based on their MHC Class I type, DC transfected with RNA-encoded antigens permits stimulation of PSA-specific CTL from all prostate cancer patients.

Mentioned earlier, the MAGE family of genes have been detected in disseminated tumor cells in the blood and bone marrow of patients with prostate cancer (20). As MAGE antigens can induce autologous CTL *in vivo*, it seems appropriate to redirect attention to them within the context of immunotherapy. The determination of MAGE expression patterns and their selected activation may play a role in immunosurveillance and provide a venue for immunotherapy.

A novel form of active immunization, which in addition to inducing a specific immune response, destroys the primary tumor, is known as "cryoimmunotherapy." (156). Likened to an autoimmune response and associated immunopathology, the response following cryosurgery (cryoablation) is characterized by the development of local and systemic tumor-specific immunity. The systemic immunity is critical to the destruction of tumor cells beyond the freezing site, i.e., metastases. This approach is particularly attractive because of the specificity of the immune response to destroy malignant cells while sparing, for the most part, normal tissue. Furthermore, the immune response may leave behind a long-term memory serving to protect the patient from subsequent disease. To our knowledge, there is presently no treatment regimen for cancer that can claim such specificity of memory.

Axiomatic to the success of cryoimmunotherapy is the necessity to augment its tumoricidal effectiveness. The use of immunomodulators (156, 157) in concert with re-attention to changes in the microcirculation following freezing permitting improved delivery of select chemotherapeutic agents (158), have provided initial observations toward maximizing the synergistic effects of cryosurgery, immunological responsiveness and chemotherapy in patients with metastatic prostatic cancer (159). Whether this success in concert with the use of cryosurgery for the treatment of bone tumors (recently reviewed by Veth et al. [160]) irrespective of its immunological aspects (for which there is limited and conflicting observations [156]) can be directly applied to the treatment of bone metastasis in prostate cancer remains to be determined. Of note in this regard, is the recent report of the

successful use of percutaneous cryosurgery for treatment of metastatic bone lesions and the resulting reduction in pain (161).

Albeit appealing, with early reports of remission of metastases (162), cryoimmunotherapy has, at best, received limited clinical application. This has been due, in part to: i) earlier reports of enhancement (progression) of metastases following cryosurgery of atypical (highly specialized) experimentally-induced tumors not comparable to other animal or human tumors which portended to an unfavorable clinical outcome (see Ablin [163] for further discussion) and ii) the need for technological improvements in cryosurgery itself and thereafter the absence of longterm follow up.

Observations that there is a gradual decrease in the presence of HLA molecules with the progression of prostate cancer, suggests that active immunotherapy may have its limitations. A reasonable alternative is passive immunotherapy via adoptive transfer. Here tumor specific antibodies or immunocytes, e.g., T cells, are adoptively transferred into the recipient. Two approaches using adoptive transfer of immunocytes are noteworthy.

The *first,* is essentially an extension of the *in situ* adenoviral-vector immunomodulatory gene therapy approaches considered herein by Satoh et al. (32). Here, adoptive transfer of splenocytes from an orthotopic mouse model of prostate cancer (178-2BMA) treated with adenoviral-vector-mediated interleukin 12 (AdIL-12) gene therapy or AdIL-12 in combination with the costimulatory gene B7-1 (AdlL-12/B7) resulted in significant suppression of tumor growth and spontaneous lung metastases, respectively, and improved survival of newly generated orthotopic tumors and pre-established metastases (164).

In consideration of a *second* approach of adoptive transfer, Pinthus et al. (165) have directed attention that a major limitation in its use for immunotherapy in cancer is the inefficient homing of the transferred immunocytes to their target, i.e., the site of metastasis. A pivotal factor according to Pinthus et al. (166) is mediated by the interaction between tissue-secreted chemokines and their corresponding receptor on the membrane of the transferred immunocytes, e.g., T cells. With this in mind, Pinthus et al. (166) have advocated the use of the "T body" ("chimeric-immune receptor") approach. This approach (166) ". . . combines effector functions of T and natural killer cells with the ability of antibodies to recognize a pre-selected antigen with high specificity and without MHC Class restriction." Stated another way, "The chimeric receptor (CR) combines the antitumor specificity of antibodies with the ability to activate lymphocytes" (167). The ability for immunological activity without MHC Class restriction is of particular significance as it enables elimination of tumor cells that have lost cell surface HLA expression.

Applying the principles of the "T body" approach, Pinthus et al. (165) initially demonstrated that direct intratumoral administration of erbB2 (HER-2) specific, CR-bearing human lymphocytes in xenograft models of prostate cancer (CWR22 and WISH-PC14) in SCID mice resulted in significant retardation of tumor growth, decrease in PSA levels and prolonged survival. Against this backdrop, Pinthus et al. (166) subsequently established an *in vivo* system extending the therapeutic scope of T bodies to metastatic prostate cancer. Therein, they demonstrated that induction of the chemokine, SDF-1 with low dose radiation or cyclophosphamide plus IL-2, within the bone marrow enhanced the homing of erbB2-specific human T bodies resulting in eradication of the tumor cells.

These preclinical studies point to the further importance of the tumor microenvironment and suggest implementation of clinical trials of the application of the T body approach for metastatic prostate cancer in man.

Based on their novelty, the foregoing are but two approaches of adoptive immunotherapy. Compared with active immunization (vaccine) strategies, adoptive therapy may overcome, as mentioned above, some of constraints effecting the magnitude and avidity of a targeted response. Additionally, through the transfer, e.g., of CTL of defined specificity and reactivity for tumor, the cells can be expanded for infusion to the patient.

The foregoing has illustrated with selected approaches and examples the role immunotherapy may play in the treatment of patients with advanced prostate cancer. Inherent to these and/or other approaches, is the necessity to give due consideration to the:

- Endogenous immunosuppressive microenvironment of the prostate (78, 84, 156, 168)
- Expression of membrane-bound complement regulatory proteins (CD35, CD46, CD55 and CD59), which are linked to the cell membrane via a glycosylphosphatidylinositol (GPI) linkage (169).

Prostate cancer cells also utilize sialic acid residues and intracellular protein phosphorylation cascades to resist attack by complement (170)

It is therefore important in terms of immunotherapeutic strategies to consider the use of agents that either block or down-regulate the foregoing factors.

13. EMERGING THEMES

In drawing to the conclusion, we believe it appropriate to direct attention to what we will refer to as "emerging themes." These for the purpose of comment fall into two broad areas: i) Clinical Biomarkers and ii) Targeted

Therapeutics, on which we have by necessity limited our discussion to the most pertinent points.

Parallel to our perceived importance for the re-attention to biomarkers, there is a re-interest by-an-large by the cancer community, including and specifically for prostate cancer by National Cancer Institute (171), for their use in identifying and analyzing primary tumors and metastases. This re-interest is perhaps nowhere better exemplified than in prostate cancer where the gradual, but eventual realization of the less-than-definitive nature of the PSA test as a marker for prostate cancer (81) is ever so slowly becoming a reality. Concomitant with this re-interest in biomarkers and the realization of the role of the tumor microenvironment in contributing to invasion and metastasis, biomarkers may further provide a means by which to elucidate the interplay between tumor cells and their microenvironment.

Emerging clinical biomarkers, for which brief comment follows are: telomerase, "a death-from cancer signature" and "antibody signature."

Telomerase and "A Death-From Cancer Signature". Knowledge of telomerase as a potentially useful biomarker for early detection, prognosis and monitoring of residual disease in prostate cancer is not new (172). However, perhaps with other potentially useful markers, the oversell and literally "ad campaign" by the manufacturers and their urological consultants of the PSA test virtually, until recently, obscured rightful attention to other biomarkers for prostate cancer.

Several studies have generated enthusiasm for the use of telomerase as a prognostic indicator for metastatic prostatic cancer. In one approach, Botchkina et al. (173) have shown that quantitative real-time PCR may be used to measure telomerase expression levels in exfoliated epithelial cells from the urine of patients after digital rectal examination of the prostate. One-hundred per cent of the patients with prostate cancer showed high telomerase expression in the assay, while 90% of patients with benign prostatic hyperplasia (BPH) showed low or no telomerase expression. Intriguingly, 10% of patients with BPH showed high levels of telomerase expression, which might indicate BPH in the very early stages of transition to cancer, or the presence of occult foci of cancer.

Rather than a single biomarker like telomerase, detecting a set of biomarkers may also prove useful. Using micro-array profiling Glinsky et al. (174) recently showed that in 10 different types of cancer, including prostate, metastatic cells displayed a conserved BMI-1 oncogene-driven 11-gene signature expression pathway. BMI-1 is one of the genes in the polycomb group (mentioned earlier) that determines the proliferative potential of normal and leukemic stem cells. Overexpression of BMI-1 causes neoplastic transformation of lymphocytes. Reports of the expression of BMI-1 in

non-small lung and breast cancer have suggested an oncogenic role for BMI-1 beyond leukemia and perhaps in epithelial malignancies. In the 100 patients analyzed, expression of the BMI-1 11-gene signature expression pathway was a consistent powerful predictor of a short interval to disease recurrence, distant metastasis, and death after therapy, *ergo*, "a death-from cancer signature."

It is of significance to note that both of these examples of new putative biomarkers for prostatic metastatic cancer are also expressed in normal somatic stem cells. The idea that tissue stem cells may be involved in cancer development has been vigorously pursued in recent years (175), with experimental data accumulating that shows stem cell involvement in dozens of solid tissue cancers, including prostate cancer (176, 177, 178, 179, 180). Other groups have shown that the Wnt and Notch signaling pathways, which are known to be crucial in maintaining stem cell self-renewal capabilities, are also active in metastatic cancers – consistent with stem cells being involved in the origin of the cancers (181, 182). Recently, Schmelz et al. (183) have proposed a new candidate prostate stem cell population within the basal epithelium, which is positive for cytokeratin 6a (Ck6a+) expression and Collins et al (184) have identified and characterized a cancer stem cell population from human prostate tumors. Further findings of novel prostatic cancer biomarkers may continue to be simply stem cell biomarkers.

"Antibody Signature." The exquisite sensitivity and specificity of the immune response make it an ideal biomarker. In principle, a malignant neoplasm may be diagnosed immunologically by detecting: i) Circulating tumor antigens (markers) in blood, secretions and/or tissue fluids and ii) An immune response – humoral or cellular, of the host against tumor (185). In applying this principle, prostate tumor-associated antigens and an immune response, the latter demonstrating autoantibodies, in patients with prostate cancer have previously been reported some 25+ years ago (185). With a renewed interest in the immune response and the urgent necessity to identify more exacting markers for prostate cancer, Wang et al. (186) have reported the use of a phage-display library derived from prostate cancer tissues in a phage protein microarray in which they have identified what they refer to as "autoantibody signatures" in the serum of patients with prostate cancer. Of particular significance pending follow up and the ability of the "autoantibody signatures" to differentiate BPH from prostate cancer (surprisingly not assessed in the study) was its positivity in PSA ranges of 2.5–10.0 ng/ml, where the PSA test is particularly inaccurate.

Screening for an immune response to prostate-specific antigens using protein microarrays could lead to improved biomarkers of disease. Whether the "autoantibody signatures" defined by Wang et al. (186) can distinguish

between indolent and aggressive tumors, a distinction urgently needed, remains to be determined. Additionally, the "autoantibody signatures" may permit identification of select antigens to be utilized in immunotherapeutic approaches.

Targeted Therapeutics. If prostate cancer originates from abnormal stem cells, the implications for future therapy are far reaching as this small proportion of the cancer cell population, $<1\%$ of the total (187), may be driving the disease. It is also true that this group of cells are fundamentally resistant to most types of chemotherapy and radiotherapy. These existing therapies have been developed to work against the bulk of cancer cells in a tumor and in many cases they are successful. However, in most solid tumors such as prostate cancer, relapse often occurs after a relatively short interval. It is therefore important to take into consideration the notion that whilst conventional treatments may destroy a large number of cancer cells in a tumor, the cancer stem cells, present in potentially very small numbers, will often survive (188). Future therapies which selectively target cancer stem cells may be necessary. Indeed, studies have already begun which target a normal stem cell associated characteristic, the expression of telomerase, in order to treat metastatic prostate cancer (189), including hormone-refractory prostate cancer (190).

Studies of telomerase inhibitors, immunotherapy using telomerase as a tumor associated antigen, and telomerase promoter based gene therapy for cancer are all on-going. The extension of this idea is clear – cancer therapeutics which target stem cell-like biomarkers or phenotypes. The development of drugs which interfere in the BMI-1 pathway can be envisioned, along with drugs which stimulate stem cell differentiation pathways.

While more effective detection and treatment of prostate cancer, based on a stem cell view of cancer development, will be enormously valuable, new methods of preventing prostate cancer may follow from knowledge of the role of stem cells in cancer promotion. In a recent paper, He et al. (191) have proposed that the promotion of a benign tumor to malignant cancer could arise from fusing of local benign tumor cells with bone marrow derived stem cells. The fusion of the two cells gives rise to a hybrid cell with all of the phenotypic requisites of malignant cancer, including being poorly differentiated and telomerase positive. The model by He et al. (191), as well as another recent stem cell fusion cancer promotion model (192) suggests that stem cell fusion may be triggered by components of the inflammatory prostate microenvironment, in which case prevention of the development of prostate cancer could be effected by reducing chronic prostate inflammation (193).

14. CONCLUSION

The thrust of this volume has, rightly, been on adenocarcinoma of the prostate, the commonest histological type of prostate cancer, and the one which makes the disease the public health problem that it is. It should not, however, be forgotten that other histological variants also exist. The most noteworthy of these, small cell carcinoma of the prostate, is of neuroendocrine origin, and is characterized by a number of specific immunohistochemical and other markers (194). The interest in this rare subtype is, firstly, because of the presence of neuroendocrine cells in the prostatic epithelium, and the possible origin of such cells from prostatic epithelial cells may be some reflection of their behaviour, though they have their own distinct patterns of cell signaling and growth control (195). Like neuroendocrine tumors elsewhere, they are exquisitely chemo-sensitive, but there is a limit to how far conventional chemotherapy, e.g., with cisplatin and etoposide, can go in controlling the disease, with the addition of other drugs simply increasing treatment toxicity (196). The disease has a propensity for widespread dissemination and a poor prognosis in comparison with adenocarcinomas.

Last but not least, in consideration of metastasis of prostate cancer, we cannot omit brief mention to the female prostate. Within the wall of the urethra, recognition of the female prostate, first described and assigned the term by deGraaf in 1672, has been hindered by use of the historically acquired terminology "Skene's paraurethral glands and ducts", so named after Alexander Skene, who redescribed the female prostate in 1880 (197, 198).

With structural and functional parameters, including PSA and prostatic acid phosphatase (197, 198) and diseases, i.e., prostatitis, BPH, and cancer (albeit rare accounting for $<0.003\%$ of all female genital malignancies) of its male counterpart, metastasis of the female prostate have been observed (199).

Absence of knowledge and/or the vestigial concept of the female prostate, failure to distinguish carcinoma of the female urethra from that of the prostate and high frequency and long term persistence of undetected prostatitis in association with chronic inflammatory disease and the subsequent development of cancer, portends the frequency of cancer of the female prostate and metastasis may be greater than currently thought.

By the nature of the title of this closing Chapter "Distilling the Past, Envisioning the Future," the foregoing, inclusive of what we have referred to as "emerging themes," has endeavoured to capture the highlights of the topics covered and bring them into perspective and prospective relative

to the biology and treatment of metastasis of prostate cancer. If we have succeeded, this in great measure may be attributed to the excellent and timely contributions by each of the authors. With further references to the contributors, we make special mention of appreciation to the late Gaynor Davies, who passed away during production of this volume.

Never has there been a greater need for communication between scientists and clinicians as there is today in the field of prostate cancer. The difficulties, on both sides, are formidable, but if this book has played its part, even in a small way, then it will have been worthwhile.

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