

**Prostate Cancer Charitable Trust 2004 Forum:
a personal overview**

Sir Walter Bodmer FRS, PhD, FRCPATH, Hertford College, Oxford University, England*.

Basic Genetics

A somatic evolutionary perspective provides great insights into the cancer process. It is the determining genetic and epigenetic changes that define the nature of each specific cancer. Since the last forum in Oxford in 2002, there has been a major development in the knowledge of clear-cut genetic or epigenetic changes in prostate cancer. At previous meetings I have, each time, bemoaned the lack of such knowledge compared, for example to colorectal cancer. Now we are beginning to see interesting parallels and differences between prostate and other cancers, particularly due to prostate's initial androgen dependence which leads to selection, for example, for changes in the androgen receptor (AR).

Pier Paolo Pandolfi provided a clear demonstration of PTEN selection in the early stages of prostate cancer, emphasising in particular the importance of haplo-insufficiency. This has often, until recently, been overlooked. He showed that haplo-insufficiency for PTEN was selected for either by loss of heterozygosity (LOH) at 10q23, methylation silencing or disruptive mutations. The latter usually occur at lower frequencies and so are less likely to be found. This is analogous to the situation for the mismatch repair gene, hMLH1, in colorectal cancers.

Pandolfi also described elegant mouse models expressing different levels of PTEN activity. These interacted with p27 loss in crosses with p27 knock out mice. He also showed, surprisingly, that there was LOH for PML, the gene involved in leukaemias with the 15/17 chromosomal translocation, thus emphasising that there are lessons to be learnt from the leukaemias for the study of carcinomas.

Drugs can be targeted at down stream functions of known genetic and epigenetic changes, for example, RAD001 or mTOR. Appropriate mouse models are becoming increasingly necessary for the evaluation of the effectiveness of such targeted drugs.

Mouse Models

There has been a remarkable development of mouse models, based on the knowledge of somatic genetic and epigenetic changes known to occur in human cancers. The technology is now becoming much more sophisticated through the use of the "cre-lox" system for site specific expression. There is also the possibility of control of expression at defined tissues sites so that specific changes can be switched on in a tissue specific manner at chosen times of development, or at chosen ages.

Norman Greenberg has developed the extremely useful and pioneering TRAMP mouse model of prostate cancer, using the SV40 T antigen to drive the cancer. This has now enabled him and his colleagues to dissect the evolution of androgen unresponsiveness and to show that the AR mutations often involved occur in similar regions of the gene in mice and men. He has also shown a continuing role for the androgen receptor even after androgen unresponsiveness has developed.

Cory Abate-Shen has shown a key role for changes in early prostate cancer in the expression of the homeobox gene, NKX3.1. The specific role of this gene lies in the control of differentiation of prostatic epithelial cells. Early down regulation leads to an escape from differentiation, often by methylation. This is closely analogous to the role of the CDX1 homeobox gene in colorectal, and possibly other GI cancers. The CDX1 gene has a similar function to NKX3.1, but with respect to colonic epithelial differentiation. Similar changes in the expression of the appropriate homeobox genes may be a general phenomenon for carcinomas. Cory Abate-Shen has developed elegant

mouse models to show the combined effects of NKX3.1 loss with PTEN and p27 losses on the development of prostate carcinomas.

Attachment and Metastasis

Filippo Giancotti emphasised the key role of integrins in controlling attachment of epithelial cells to extracellular matrix. It is a well established idea, promoted by Mina Bissell and others, that early tumours need to "loosen" their attachment to extracellular matrix to enable them to escape from differentiation restraints on growth. When epithelial cells become less, or completely detached they are subject to an increased rate of apoptosis. This, in turn, leads to increasing pressure for selection for anti-apoptotic changes. These early ideas suggested some form of signalling via attachment through integrins to the extra cellular matrix, and this has now been shown to be the case. It is, thus, now clear that signalling via integrin attachment is important in growth control.

Giancotti provided an elegant demonstration of the role of Integrin β , interaction with EGFR, leading to stimulation of growth in an ordered fashion, and migration. After an early reduction in attachment, metastasis may require return of attachment in a new location possibly through different integrins, coupled with unregulated growth, depending on the site of metastasis.

The key event to be selected for in metastasis is, I believe, not connected with extravasation, migration and related functions. Epithelial cells in any case have those capacities. The key to my mind to metastasis is the evolution of the ability to grow in distant sites, namely autocrine growth. This explains the selection, for example, for constitutive EGFR activity and its correlation with androgen independence. Androgens are unlikely to be available in metastatic sites. There may, of course, be interactions between the requirements of metastasised cells and their newly associated stroma. However, there is bound to be continual pressure to select for greater and greater growth independence.

In order to make mouse models more relevant to the human situation, the goal is to put as many of the changes known to occur and be selected for somatically in Human cancers into the mouse germ line. This should (a) drive the mouse process in the human direction and (b) minimise the number of extra steps needed to get a cancer. This is necessary because the mouse is so much shorter lived and smaller, and so there is not the same time or cell number available for the development of cancers as there is in humans. Spontaneous mouse tumours without such manipulations may be analogous to human childhood tumours, with key events having to take place early *in utero* to enable enough opportunity for the evolution of a tumour to take place. This is especially relevant to the ability to get metastasis, which must occur before the mouse dies from the effect of a primary tumour as in the case of the MIN mouse, where it is the multiple adenomas causing constriction in the GI tract which eventually kill the mouse. Obtaining spontaneous metastasising tumours driven along human pathways must be the key to the construction of proper mouse preclinical models. Xenografts are, in my view, appalling models. They have none of the real features of a tumour as it actually occurs in the whole organism. They are, in fact, hardly better than cell lines *in vitro*, especially if enough of the lines are well characterised with respect to genetic and epigenetic changes so that it becomes possible to correlate the effectiveness of a drug with the tumour genotype. Only the role of the stroma is missing with cell lines *in vitro*, but that also is very abnormal in Xenograft models. Using the appropriate genetic manipulation, it is possible to create mouse models with the appropriate genotype for testing for drug/genotype correlations.

Normal and tumour stem cells

David Hudson emphasised the fact that it is the tumour stem cell that must be the target of therapy, and that this may have quite different properties from the tissue stem cell. This latter is so often, I believe wrongly, assumed to be the origin of tumours, rather than the next layer of

proliferating cells found in essentially all epithelial tissues. The initiating genetic change may occur in a stem cell but, often, if not mostly, be expressed in this next layer. Hudson proposed that the characterisation of the prostate cancer tumour stem cell might often be through differentiation patterns of changes in cytokeratin expression. This recognition enhances the ability to grow out and identify prostate tumour stem cells.

Norman Maitland showed that he could enrich for the tumour stem cell population by selecting, in primary cultures of tumour tissue, for low expression of CD44 and high expression of $\alpha 1\beta 2$ integrin and CD133 (another lesson from leukaemias). He showed that this selection gave rise to cells with an impressive improvement in cloning efficiency and so making it possible to accumulate sufficient cells *in vitro* to be able to characterise early changes and obtain mRNA expression profiles.

Though it is the tumour stem cell that must be targeted, the differentiated cells will mostly carry the same genetic and epigenetic changes, since there is no time for selection for different changes during the limited period of proliferation before differentiation. Therefore, if genetic or epigenetic changes are characterised in the bulk tumour, then targeting these genetic changes with drugs will nearly always necessarily target genetic or epigenetic changes in the tumour stem cell. Caution is needed really only when targeting downstream activities of a primary change, to ensure that these are relevant to the tumour stem cell.

There is a need to characterise the genetic signature of prostate tumours at different stages, both to predict the future evolution of a tumour and to determine which targeted therapy is appropriate for a given tumour. This is especially important for the prostate because of the trauma associated with unnecessary early treatment based for example, only on PSA levels. This is quite different from the situation for colorectal cancer where removal of all polyps seen by colonoscopy or sigmoidoscopy can be done with minimal invasiveness. In that case it matters much less if only a minority of positively screened patients would go on to have troublesome tumours. It is known for colorectal cancers that the vast majority arise from a polyp, and so removal of all polyps essentially eliminates the risk of a subsequent cancer. Would this be true if one could remove all PINs? Can the potentially evil nature of a PIN be identified, or is there too much chance in the somatic evolutionary process to be able to do this? We must hope the latter is not the case.

Gerald Cunha emphasised, using his elegant reconstruction model in the mouse, the enormous importance of tumour/stromal interactions, especially at early stages of tumour evolution. There always exists the possibility of induction of stromal expression changes to the advantage of the tumour. This could even lead to specific epigenetic or field changes induced, for example, by cytokines or chemokines. It is therefore clearly always important to assess the stromal environment, both in the primary site and, also in metastatic sites. This is simply a restatement of the old idea of "seed and soil", strongly promoted by Leslie Foulds,

Tumour profiling and expression array analysis

There are many approaches to profiling tumours with the aim of finding which molecular genetic signature gives the right clues as to how to treat, especially for early detected disease.

Howard Scher gave examples of some new biologically based potential treatments and targets for treatment including Iressa, RAD 001, and the Hsp90 binding compound 17-AAG. Is there a place for velcade in the treatment of Prostate cancer?

William George showed how it is possible to dissect the androgen response using micro-array expression comparisons of different stages of tumour progression. This implied a need for very careful micro-dissection of the material from which RNA preparations were made. It is also necessary to use stringent criteria for judging the significance of different levels of expression in order to sort out the wood from the trees and also not to miss potentially key small level changes.

Normal tissue contamination is a major problem for expression analysis from tumour material. Contaminating normal tissue may lead to expression changes that reflect changes induced in the surrounding stroma rather than in the tumour itself. This can, for example, give rise to lymphocyte products induced by the inflammatory response due to the presence of a tumour, but which are not products of the tumour.

Phil Febbo gave examples of further micro-array expression analyses, and of training programmes used to identify key genes with changed expression levels. The real goal, however, must surely be to use these analyses to identify potential specific primary genetic or epigenetic changes in tumours, or their immediate relevant downstream consequences. These will then be confirmed and assessed using other analytical approaches, such as quantitative RT-PCR and direct mutation and methylation analysis of the identified candidate genes.

Colin Cooper presented intriguing data on E2F3 transcription factor over expression in prostate cancers. His data emphasised the problem of sorting out what is the primary event. The E2F3 over expression could, for example, be a secondary response to a primary genetic or epigenetic change in a different, so far, unrecognised gene. This is a very general problem when looking at genes with expression level changes as potential drug targets, especially when the focus is on higher level changes. Many of the changes in expression involving the primary events may be modest, as exemplified by PTEN haplo-insufficiency. These are likely most to be missed by current approaches to micro-array expression analysis. Many significant changes, on the other hand, may be bystander effects, such as the increases in expression of the cancer testis antigens or CEA. These may provide useful clues to underlying primary changes, but are very unlikely themselves to be of any use for drug targeting, except for use as immune targets. Then, however, escape mutants which have no detrimental effect on the outgrowth of the tumour may readily be selected for following immunotherapy.

Clinical Studies

Freddy Hamdy outlined the plans for an elegant trial to decide between Active Monitoring and active treatment. "Active monitoring" is now the preferred term rather than "watchful waiting". This follows from the interesting results of the evaluation of responses of men to explanations of the different possibilities for treatment or monitoring of prostate cancer, and their likely consequences. We, however, will have to wait till after 2011 for the results of this trial become available. Let us hope that before then there will have accumulated better knowledge than we have now of how to deal with Prostate cancer, and that this trial will then provide the material for evaluating the new knowledge.

Peter Scardino raised the same question about whether to treat or actively monitor, and suggested approaches to finding the answers to this key question. There appears now to be an interesting convergence of transatlantic views. He described the potential use of clinically based nomograms for making decisions about whether to treat. There was an interesting clue from the most significant effect, which was the finding of a tumour in a second biopsy. However, the molecular signatures are clearly most likely to be the ultimate main way forward.

Jack Cuzick outlined an extensive collaboration aimed at studying the natural history of prostate cancer, based on a follow up of 4000 patients under age 76, 2000 of whom were subject to watchful waiting and the other 2000 to hormone therapy. End points would be raised PSA, clinical recurrence, change in therapy or death from prostate cancer. Many potential prognostic factors will be assessed, including serum markers, some genetic variants and pathological evaluation. The hope is to identify prognostic factors that will guide the treatment from a relatively early stage.

Don Newling gave an interesting review of the evolution of non-steroidal anti-androgen drugs. There is striking variation in the side effect spectrum of different drugs. Does this mean that these drugs often have other unidentified targets? After all, the specificity of a drug is usually only assessed against a limited number of related targets. Any drug that binds something well, usually a protein, may be quite likely to bind another one of the 30,000 or so other proteins quite well too! And if it does, that other reaction, or even reactions, may also be important for understanding the activity of the drug. Just remember aspirin!

Wayne Tilley gave an extensive discussion of the mechanisms by which the AR escapes from androgen ablation. He emphasised, as did Norman Greenberg, the continuing role of the AR in metastatic prostate cancer. AR mutations exist which augment AR activity and which are connected with a novel AR interacting protein. There is also relevant genetic variation in AR in a poly-glutamine tract, and the frequency of such variants is different in different populations, including especially African Americans. This may be connected to some extent with their higher incidence of prostate cancer. Wayne Tilley, finally, suggested some novel drug approaches to targeting the AR in androgen resistant tumours.

New or developing therapeutic approaches

Anthony Chalmers described approaches to controlling the rate of brachytherapy. He also discussed the possible role of Poly ADP – Ribose in tumours, and the PARP1 enzyme as a potential drug target.

Mark Emberton discussed some newer developments of PDT.

Jonathon Simons discussed the possible role of HIF, hypoxia inducing factor, and its relatives as targets for drug development.

Gus Dalglish described his approach to immunotherapy with allogeneic cell lines, using a preparation produced by the company Onyvox. He emphasised the interesting potential role of allo major histocompatibility differences in enhancing the uptake by macrophages of tumour cells or debris from them. This allows self HLA presentation of tumour antigens. He used the old warhorse of BCG as an adjuvant. He emphasised that the major limitation of this approach to immunotherapy was the limited number of available prostate cancer cell lines, an old problem frequently raised at previous meetings.

Jedd Wolchok described a DNA based PSMA immunotherapy approach. (It is my view that such products should not be called “cancer vaccines”. They are DNA based drugs or immunogens for immunotherapy. The use of the word vaccine, I believe, gives the wrong impression both to clinicians and scientists not familiar with the field, and perhaps more importantly, to the general public.) He used, following classical immunological approaches, xeno differences to provide help in order to overcome tolerance to the human PSMA. Many developments of this approach to DNA based immunotherapy are possible. There are other, perhaps more effective ways to provide help, multiple antigens can be used to avoid escape variant selection and there is a wide range of targets to choose from. Immunotherapy remains promising. Antibody therapy was hardly even mentioned at this forum, though that is the first widely used and approved immunotherapy for other cancers. However, it is so far always used in combination with other treatments, especially conventional chemotherapy.

Jonathon Coxon and David Dearnaley both emphasised the intriguing potential role for treatment with bisphosphonates. Do they do more than help deal with bone lesions due to metastases? Can they target the tumour itself, or at least diminish the probability of bone metastases, as seems to be the case for Breast cancer. Early results for prostate are unfortunately not encouraging in this direction.

Howard Scher emphasised the potential value of classical chemotherapy using taxanes and Tom Tichler discussed 5FU.

Ken Pienta described the use of early autopsy material, and emphasised the importance of exploring all possible sources of material and optimal approaches to their use. In this context it is always important to remember the importance of both the genotype of the tumour and of the individual, and so to get enough material from both to do DNA analysis. Not so much material is now needed, given the development of efficient whole genome amplification techniques

Hans Lilja described unique material for evaluating the historical progression of prostate tumours in relation to PSA levels. This, however, raised the very difficult question of how to decide whether to take note of quite low PSA levels which could indicate further problems, but only in few people. These results emphasise the need for sensitive early characterisation of prostate cancers, and Hans Lilja described his use of RT-PCR on blood samples for PSA and related products,

Walter Bodmer described his laboratory's approach to the detection and characterisation of rare potential cancer cells in the blood. This involves using a combination of antibodies with immunofluorescence for identifying cancer cells. This is preceded by partial purification of mononuclear cells, including epithelial cells, followed by antibody and complement based removal of lymphocytes and monocytes to limit the number of slides that need to be scanned (using eventually a specially developed automated microscope by Ikonisys) for the identification of rare tumour cells.

Paul Tempest described a very sophisticated approach to serum peptide analysis using the most up to date mass spectrometry to identify serum markers of early cancer.

Itamar Willner described a very sensitive detection assay for telomerase using very novel nano-technology approaches. This technology may be quite generally applicable to any potentially useful analyte.

Prostate Cancer Genetics

Lisa Canon Albright gave an account of the Utah prostate cancer families and described her approach to identifying the intriguing Xq27-28 linkage. This involved a clever dissection of the family material into sub-families. The linkage remains compelling because of the suggestion of a testis cancer gene in same place. It has, however, been very hard to pin down. In the end, as with all such studies, it will come down to candidate gene testing using a combination of the knowledge of the chromosomal location and intelligent functional guesses, together with the hard slog of gene sequencing and searching for variants that plausibly match the familial pattern of occurrence.

Ros Eeles again emphasised the difficulties of pinning down the many possible places where prostate cancer genes lie. She showed, however, how good candidate guesses, namely BRCA2, and BRCA1, can come up trumps even if they do only explain just one small part of the overall inherited susceptibility.

I believe that is the way the search will develop in the future. The final result will come from the cumulative effects of many rare variants (but not so rare as individual deleterious mutations) at many different loci. These can only be found by the candidate gene approach and the hard slog of looking for DNA sequence variants, ultimately by DNA sequencing. That is what we are finding for colorectal cancer and its precursor adenomas.

Frank Chinegwundoh demonstrated elegantly the considerable risk difference in prostate cancer between UK Afro Caribbeans and Whites, reaffirming the similar US data. This to my mind is likely to be one of the few ethnic differences that has a mainly genetic basis, something I have said at several previous meetings. The difference could, for example, be explained by selection for testosterone levels in West Africa. It is hard otherwise to see how the difference could be due to several variants, as seems likely, unless it is due to selection for variants all pointing in the same direction. Hence, as suggested previously, the answer may be to look for variants in all the genes involved in testosterone metabolism.

Ken Muir described a collaborative UK case – control study of early onset prostate cancer. A preliminary analysis showed a family history to be the major relatively high relative risk factor to be identified. In the absence of family history, baldness, body shape and radiation history all showed significant associations, though with comparatively small relative risks. In the presence of family history some of the effects of these other factors became greater, suggesting environmental-genetic interactions. The main challenge that clearly remains is the identification of the genetic variants associated with an increased risk of prostate cancer.

Henrik Gronberg described a Swedish large-scale population based case- control study. He also described prostate cancer families on which genome wide linkage scans had been carried with some of the usual problems of inconclusive results. Some useful extra information was obtained by stratifying the families by which other cancers they contained. A candidate gene approach is feasible for the analysis of this material and may be the only way forward. Even clear cut Mendelian families ultimately depend for their analysis on the choice of candidate genes. Population based DNA marker case-control studies need to be large if they are to detect variants with a low relative risk or that are rare, and need VERY carefully selected controls.

Conclusion

My apologies to those participants whose favourite work is not mentioned. I take full responsibility for the choice of material and can only say that it was not possible to do justice to all, especially in the short talk on which this article is based and which had to be prepared during the course of the Forum.

This was as exciting a meeting, if not more so, than its predecessors. We participants, clinicians, laboratory scientists, patients and supporters always learn from each other. These meetings are a wonderful forum for the exchange of results and ideas, and for fostering collaboration. That is what has drawn me into prostate cancer research. The arrangements for the meeting were excellent and also, of course, the science. This is thanks to our MSKCC colleagues, to Ros Eeles, and to Shirley Claff and her miracles, but above all to Clive and Joy Bourne who have made all this possible.

*Sir Walter, formerly Director-General of the Imperial Cancer Research Fund, having been Director of Research from 1979-91, is Principal of Hertford College, Oxford. A Fellow of the Royal Society since 1974, he received his knighthood in 1986. In 1997, he was elected an Honorary Fellow of the Royal Statistical Society and is a Foreign Associate of the US National Academy of Sciences and a Foreign Honorary Member of the American Academy of Arts and Sciences. Sir Walter has been a trustee of the Prostate Cancer Research Foundation since its inception.