Abstract. Expression profiling and proteomics have the potential to transform the management of prostate cancer, identifying new markers for screening, diagnosis, prognosis, monitoring and targets for therapy. Expression profiling has revealed that the majority of prostate cancers contain fusion genes resulting in the upregulation of ETS family transcription factors. New diagnostic markers to replace PSA are being actively sought using a variety of proteomic platforms. Nevertheless, no single molecular marker has yet been discovered that is any more reliable for predicting outcome than histopathological grading. In the future, small custom-built chips will be used to detect a small panel of RNA or protein markers to answer specific questions concerning patient management for each type of cancer.

Clinical Applications of Expression Profiling and Proteomics in Prostate Cancer

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Prostate cancer is the most frequently diagnosed cancer in men, with about a one in eleven lifetime risk of developing the disease and about 3% of men overall dying as a result of it (1). Although prostate cancer is a lethal disease, more men die with it than of it. In autopsy series, the incidence of undiagnosed prostate cancer is even higher, with an incidence of about 30% by age 50, 70% by age 70 and 90% by age 90, so the majority of elderly men have prostate cancer by histopathological criteria (2).

In common with most other solid types of cancer, clinically localized prostate cancer can be cured with radical surgery or radiotherapy. Because it is a relatively slow growing disease, if the man's life expectancy is less than 10 years, it may be reasonable to follow clinically localized disease with active surveillance (3). In contrast, once it has spread outside the prostate, there is no curative treatment available. Hormone therapy will delay the disease for a median period of 12-18 months, but once the cancer has become hormone resistant, the usual fairly rapid outcome is death with widespread bone metastases (4).

There are many potential clinical applications for expression profiling and proteomics in early prostate cancer, partly because of the limitations of prostate specific antigen (PSA) as a diagnostic marker. PSA is produced in large quantities by the epithelial cells of the prostate and by their derivatives, the prostate cancer cells. PSA leaks into the circulation and consequently the serum level of PSA is used to monitor the progress of prostate cancer during treatment. In this situation, it is a classic tumour biomarker with high sensitivity and specificity. However, the problem is that is also used to screen men for prostate cancer, and in the diagnostic situation it has poor sensitivity and specificity (4).

The normal cut-off for serum levels of PSA is 4 ng/ml, so any man presenting to their doctor with a PSA of above 4 ng/ml is likely to require a per rectal needle biopsy. But only about a quarter of men with serum levels of PSA between 4 and 10 ng/ml have cancer. Up to 50% of men presenting with prostate cancer have PSA levels within the normal range. So one potential application of proteomics is to find a tumour marker that is more sensitive and specific for prostate cancer diagnosis than PSA.

The second issue in prostate cancer is that although early disease can be cured with radical surgery or radiotherapy, the side-effects are quite severe. Apart from the normal risks of surgery and radiotherapy, these treatments usually leave the man impotent and incontinent, with urinary incontinence after surgery and urinary and rectal incontinence following radiotherapy. Continence usually returns, but potency usually does not. The side-effects may be acceptable if the cancer is cured, but following radical surgery or radical radiotherapy about 20-30% of men relapse with local recurrence or metastatic disease. Hence these men have all the side-effects of radical therapy with...
few of the benefits. Therefore, the second potential application of expression profiling is the discovery of markers that distinguish clinically localized from metastatic disease.

The third issue in prostate cancer is that although radical surgery or radical radiotherapy cures around 75% of men, many of these men would not have developed symptoms from the disease. There is only one study so far that has randomised men between active surveillance and radical surgery (5). After a median follow-up period of 10 years, 30/347 men treated by radical prostatectomy died of prostate cancer compared to 56/348 in the active surveillance group. These figures indicate that 10 years after diagnosis, radical prostatectomy had saved the life of approximately one in 20 men. Bearing in mind the side-effects of radical prostatectomy, the question individual men have to face is whether they wish to go through the trauma and side-effects of radical surgery for a 5% better chance of being alive at 10 years. The third potential application for expression profiling is to select those patients with early prostate cancer who will benefit from radical treatment.

The fourth application relates to late prostate cancer, disease that has already spread outside the prostate. Most men respond to hormone therapy, either medical or surgical castration, but the duration of response is short, a median of 12-18 months, after which there is little effective treatment. Hormone therapy was pioneered in the 1940s by Huggins (6) and there has been little improvement in the survival of men with metastatic prostate cancer since that time. The fourth potential clinical application of expression profiling and proteomics is new targets for therapy.

The applications of expression profiling and proteomics are similar in most types of cancer. We need better classification tools for subclassifying different types of cancer – a molecular pathology classification. We need more accurate markers for diagnosis, markers that distinguish clinically localized from metastatic disease, more accurate markers of prognosis and more accurate predictive markers of response to treatment. We also need new targets for the effective treatment of metastatic disease.

Expression Profiling

One of the first papers describing the global expression profile of cancer cells was concerned with prostate cancer (7). The study compared cancer tissue from clinically localized and metastatic cancer with normal and benign prostate tissue. Hierarchical clustering distinguished four groups - cancer cell lines, clinically localized prostate cancer, metastatic prostate cancer and normal or benign tissue. The chips used had about 10000 spots, of which about 5000 were known genes, whereas today those figures could be trebled. Nevertheless, the bioinformatic processing revealed a large number of candidate biomarkers.

Expression profiling is only the starting point for the discovery of new biomarkers, as many of the candidate genes identified either prove not to be overexpressed or down-regulated, or if they do, have no clinical value. However, one of the major attributes of the Dhanasekaran study was the inclusion of a tissue microarray of samples from over 700 prostate tumours with clinical follow-up, not only providing confirmation of the gene expression analysis, but also giving an insight into the potential clinical value (7). One of the genes that was identified was hepsin, a cell surface serine protease that is expressed in clinically localized and metastatic prostate cancer. A range of expression was seen in the cancer cores on the tissue microarray and it was found that low levels confer a worse prognosis.

Because of the wealth of data generated by expression profiling, completely different sets of candidate genes can be identified depending on the bioinformatic tool used to analyse the data. Arul Chinnaiyan’s group re-analysed the data published in 2001 in Nature using a different algorithm (8). Significance analysis of microarrays (SAM) yielded a different list of genes and another paper in Nature: 55 genes were up-regulated and 480 genes were down-regulated when they compared clinically localized with metastatic disease. The most up-regulated gene was EZH2, a gene not mentioned in the earlier paper. EZH2 is a polycomb group protein, one of a group of proteins that modifies transcription and protein expression by inhibiting histone deacetylase. Using tissue microarrays, it was shown that EZH2 is a key gene in the progression of prostate cancer, with low expression in normal tissue, moderate expression in clinically localized prostate cancer and high expression in metastatic prostate cancer. EZH2 is of prognostic significance, with cancer patients showing high levels of expression relapsing earlier and more frequently than those with low levels of expression following radical prostatectomy (8).

Another clinical application is the use of molecular profiling to classify types of cancer and separate them into distinct groups. Again Arul Chinnaiyan’s group in Michigan used yet another bioinformatic tool called cancer outlier profile analysis to look for recurrent chromosomal translocations (9). These genetic changes are characteristic of the leukemias, but until this study no frequently occurring chromosomal translocations had been found in solid cancer.

By applying a cancer outlier algorithm to two data sets, it was found that in both sets either ETV1 or ERG were highly up-regulated in a subset of the cancer and that ETV1 and ERG up-regulation were mutually exclusive. As ETV1 and ERG are members of the ETS family of transcription
Proteomics

Whilst molecular profiling is revealing new biomarkers of potential clinical significance, the working end of the human genome is the protein. Gene expression profiling is limited by the fact that gene expression does not necessarily translate into protein expression. Consequently most people would prefer to have the protein as the biomarker for cancer, whether it is in serum, urine or the tissue itself, rather than RNA expression (11). However, proteomics is not as far advanced as expression profiling and is considerably more complicated.

There are many different proteomic platforms, including the classical 2D gels, peptide arrays and antibody arrays. However, most of the clinical applications so far have used SELDI - surface enhanced laser desorption ionization, because of its relative simplicity and relatively high throughput. In prostate and many other types of cancer, SELDI has been used to look for biomarkers in serum, urine and in tissue extracts (12).

One of the advantages of SELDI is that it provides a choice of array surfaces to which sets of proteins can bind, and by modifying the length and type of wash, the proteins detected can be further refined. Once the SELDI chip has been exposed to the biological fluid and washed to remove unbound proteins, it is exposed to a laser to ionise the proteins and protein fragments. The proteins are then detected by mass spectrometry, resulting in a series of peaks reflecting the size of the protein or peptide fragment and its relative abundance, but not the identity of the protein. It is hoped that some of these peak differences will be sufficiently specific and sensitive to provide clinical application (11, 12). Ideally the protein producing the peak would be identified and a more sensitive and specific application (11, 12). Ideally the protein producing the peak can be further refined. Once the SELDI chip has been exposed to the biological fluid and washed to remove unbound proteins, it is exposed to a laser to ionise the proteins and protein fragments. The proteins are then detected by mass spectrometry, resulting in a series of peaks reflecting the size of the protein or peptide fragment and its relative abundance, but not the identity of the protein. It is hoped that some of these peak differences will be sufficiently specific and sensitive to provide clinical application (11, 12). Ideally the protein producing the peak would be identified and a more sensitive and specific method used for quantification.

SELDI has attracted some criticism (13). There have been issues over reproducibility due to variations in sample collection and storage affecting protein stability. One major limitation is that SELDI tends only to detect high MW proteins present in very high levels in serum, such as acute phase proteins, which we already know are poor tumour markers. Nevertheless, despite its limitations, many groups are optimistic that SELDI will yield new markers and, even if it does not, there are a variety of other proteomic platforms that are more sensitive and specific and can, and probably will, yield clinically useful biomarkers.

Conclusion

No biomarkers detected by expression profiling or proteomics are yet in routine use to answer clinical questions. Moreover, it is unlikely that any single biomarker by itself will improve on what is already available (mainly staging and grading) and so it is probable eventually that each clinical question will be addressed by a small battery of markers. The battery of markers will not be detected by global profiling. Rather, there will be small defined and relatively cheap platforms designed to answer specific clinical questions. If global profiling lives up to its promise, the defined platforms could transform clinical practice in the areas of diagnosis, monitoring and treatment of cancer.

References


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