



Editorial – referring to the article published on pp. 290–301 of this issue

## Bladder Carcinogenesis, in Search of Clues and Genes

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The era of genetic research has opened with challenging techniques for analysing the genomes of living organisms on a large scale, and, at the same time, on the minutest level. Gene arrays allow us to simultaneously determine the expression of all human genes on a chip, which enables scientists to compare tissues or even micro-dissected clusters of cells for differences in their genetic composition and activity. In cancer research, we are looking for changes that make cells susceptible to developing into cancer, and that determine the biological aggression of those malignancies. The strategies involve comparisons between the various stages and histological grades of the clinical disease, and between normal and diseased cells. The selection and isolation of tumour cells might be pivotal for this gene discovery effort, since contaminating normal supportive stroma or surrounding cells might obscure differences in gene expression in tumour cells. However, the subject is more complicated, as the supportive tissue of a tumour might also exhibit an abnormal expression profile that contributes to the carcinogenesis and continuation of tumours.

The strategy described in the article by Doherty et al. [1] in this journal is fairly new, and has to do with the clinical observation that bladder tumours are found more often in the trigonum than in other regions of the bladder or upper urinary tract. As such, the idea of comparing the genetic composition of tissues associated with tumour incidences in various areas of the same organ was addressed earlier for the different zones of the prostate [2,3].

It is an exciting idea, and, as we see, delivers information that might be useful for indicating metabolic processes in the cells of the trigonum that are responsible for initiating or promoting cancer. The discovery procedure and data interpretation, however, are not easy tasks to perform.

In a traditional gene array experiment, the expression of thousands of genes (by means of their mRNA) is measured in cells of interest and compared to expression in another set of cells, often from a normal population. Depending on the cut-off level or statistical evaluation for expression differences, a few hundred genes are identified as up- or down-regulated within the cells of interest compared to the other set of cells. Specific characteristics of the differentially expressed genes can be retrieved in a series of scientific databases. These include gene function, part of a particular pathway, tissue expression, cellular component, role in cancer progression, and many more. Retrieving and mining all this information are extremely laborious tasks, even with all databases in electronic form and the availability of software packages that can mine and visualise high-throughput data [4]. With so much information at hand, the main question can then be addressed: From the hundreds of candidates, which genes are responsible for the preferential occurrence of bladder cancer in the trigonum? Based on the level of differential expression and gene characteristics, a top-few-gene list can be generated. In the study by Doherty et al. [1], differential expression of Cdc25B, TK1, PKM, PDGFra, and Seladin-1 (DHCR24) was checked by RT-PCR and further

discussed. All these genes have been associated with cancer development or progression, and Cdc25B, TK1, and PDGFra are abnormally expressed in bladder cancer. However, their roles in the predisposition of carcinogenesis in the ureteric orifice of the bladder are not understood, and further experimentation beyond expression analyses needs to be instigated.

For transitional cell carcinoma, genetic alterations have been analysed in established tumours by Kim and Quan, although often on a limited number of cancers [5]. This study follows the general concept that bladder cancer develops in a step-wise manner and proceeds through two distinct genetic pathways that are responsible for generating different cancer morphologies: that of recurrent superficial bladder cancer (often low grade), and that of an ultimately muscle-invasive tumour (which is often high grade). During every step, genetic, structural, and numerical chromosomal alterations accumulate and contribute to the biological progression of the disease.

The origin of the aberrations in these pathways is thought to be multifactorial. The results of inherited changes combined with coincidental environmental factors lead to the activation or silencing of genes, and therefore to abnormal metabolic processes. Relatively small steps may have important results. For example, methylation of promoter regions of genes is a well-known process of inactivating gene promoters, and therefore of silencing genes. The methyl groups can influence protein-DNA interactions without altering the DNA sequence or base pairing. Aberrant methylation has been observed in very early changes of the urothelium, even before neoplastic lesions were seen. The frequency of these aberrations increased with disease progression. The polymorphic variations in the DNA sequence appear to be related most closely to the heritability of bladder cancer. In the end, genes will mutate. Such genetic changes can be protective and stimulatory for the gradual decline towards invasive cancer. The FGFR-3 (receptor for fibroblast growth factor) mutation indicates a favourable clinical development of superficial bladder cancer, without recurrences [6].

All this genetic activity leads the urologist towards new molecular tools for diagnosis and therapeutics. These tools are obviously needed for diagnosis because of the cyclic alterations of staging and grading classification that try to incorporate the outcome of bladder cancers into a system that is of sufficient prognostic value for the treatment decisions that need to be made early to improve the patients' outcomes. At this time, there is too much

interobserver variation between pathologists; thus, we still cannot predict which patient will have an innocent disease (sometimes of lifetime duration) and who needs an early justifiable aggressive treatment approach for a likely life-threatening tumour. Cluster analyses of microarray expression data can already classify benign and muscle-invasive cancers with close correlation to pathological staging [7,8]. Cytogenetic studies have identified many structural and numerical chromosomal changes in bladder transitional cell carcinomas. Chromosomal abnormalities in number (hyperdiploidy and aneuploidy), size, or configuration, correlate with an increased risk of tumour recurrence and cancer progression. These changes in bladder urinary cytogenetics need to be sharpened by the more specific molecular tools that have been developed recently [9]. Microsatellite analysis indicates early molecular changes in the urothelial cells that are not visible by cystoscopy, but are forerunners of macroscopic cancers. At the same time, such information can be used for prognosis and treatment decisions.

Further therapeutic translation of the discovery of genetic alterations might be expected. For example the epigenetic silencing of tumour suppressor genes might be corrected by DNA methylation inhibitors that at least partly change the adverse biological events of increased growth rate and deleted DNA repair in cancers by restoring these gene functions onto a cell. Already the observation that most bladder cancer recurrences are monoclonal (genetically from one original lesion) has led to the practise of applying early instillation therapy after transurethral resection [10].

Urologists must increase collections of biomaterials with a long clinical follow-up, even when they are not directly involved in basic research. And hardly anybody else can do this except urologists, as they still manage urothelial disease. The increasing capacities of available DNA collections, coupled with the rapid development of high-throughput genotyping technologies, are expected to vastly accelerate the research on bladder cancer susceptibility, diagnostics, and therapeutics.

So what is next? With regard to the number of human genes (about 30,000) and the methods to identify changes and differences, the genetic analysis is less complex than proteomic assessment. The intracellular pool of proteins that is responsible for the cellular metabolism might contain 100,000 different proteins, not including the conformational changes over time of a single molecule. This discovery process of identification and interaction has only just started.

## References

- [1] Doherty SC, McKeown SR, Lopez JA, Walsh IK, Mckelvey-Martin VJ. Gene expression in normal urothelium depends on location within the bladder: a possible link to bladder carcinogenesis. *Eur Urol* 2006;50:290–301.
- [2] Stamey TA, Caldwell MC, Fan Z, et al. Genetic profiling of Gleason grade 4/5 prostate cancer: which is the best prostatic control tissue? *J Urol* 2003;170:2263–8.
- [3] van der Heul-Nieuwenhuijsen L, Dits N, Veldhoven A et al. Gene expression profiling of the prostate zones. Abstract AACR Basic, Translational, and Clinical Advances in Prostate Cancer Conference Proceedings, November 2004.
- [4] Veldhoven A, de Lange D, Smid M, de Jager V, Kors JA, Jenster G. Storing, linking, and mining microarray databases using SRS. *BMC Bioinformatics* 2005;27:192.
- [5] Kim WJ, Quan C. Genetic and epigenetic aspects of bladder cancer. *J Cell Biochem* 2005;95:24–33.
- [6] van Rhijn BW, van der Kwast TH, Vis AN, et al. GFR3 and P53 characterize alternative genetic pathways in the pathogenesis of urothelial cell carcinoma. *Cancer Res* 2004;64:1911–4.
- [7] Dyrskjot L, Zieger K, Kruhoffer M, et al. A molecular signature in superficial bladder carcinoma predicts clinical outcome. *Clin Cancer Res* 2005;11:4029–36.
- [8] Wild PJ, Herr A, Wissmann C, et al. Gene expression profiling of progressive papillary noninvasive carcinomas of the urinary bladder. *Clin Cancer Res* 2005;11: 4415–29.
- [9] van Rhijn BW, Vis AN, van der Kwast TH, et al. Molecular grading of urothelial cell carcinoma with fibroblast growth factor receptor 3 and MIB-1 is superior to pathologic grade for the prediction of clinical outcome. *J Clin Oncol* 2003;21:1912–21.
- [10] Junker K, Boerner D, Schulze W, Utting M, Schubert J, Werner W. Analysis of genetic alterations in normal bladder urothelium. *Urology* 2003;62:1134–8.