INVITED EDITORIAL

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Molecular alterations in bladder cancer

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Abstract In recent years, significant information has been accumulated on the molecular alterations that take place during development of transitional cell carcinoma (TCC). A number of studies aimed at defining loss of heterozygosity have shown a general chromosomal instability in TCC with loss of parts of chromosome 9 at early stages of papillomas, and of chromosomes 11, 13, 3, 4, 8, 17 and 18 during further development of the tumor. Oncogenes are activated, exemplified by mutations in the ras gene family and overexpression of the c-erbB-2 gene, in a minor fraction of tumors. Alterations of tumor suppressors (involved in control of the cell cycle, DNA quality control and activation of apoptosis) seem to be frequently involved. Among these p53 has a key role, and one p53 allele is frequently lost in TCC followed by mutation of the remaining allele. These alterations are correlated with survival, disease progression, invasion and recurrence. Also frequently lost are the cell cycle control genes p16 and p15. The predictive value of this has not yet been determined. Studies of glycosylation genes have shown downregulation of the ABO gene, followed by loss of ABO blood group structures and accumulation of the Lewis cell adhesion molecules in high grade tumors. Functional proteome analysis has furthermore identified biomarkers that are correlated with grade and stage. Molecular models for TCC development can now be built, and clinical testing of these is urgently needed.

Keywords Bladder cancer · Molecular alterations · Tumorsuppressor genes · Glycosylation · Microsatellites

Introduction

Multistage carcinogenesis is regarded as a consequence of the accumulation of somatic genetic alterations, which include activation of cellular proto-oncogenes, and inactivation of tumor suppressor genes. Tumor suppressor genes are a family of genes that encode proteins that have different cell biological functions, only sharing one condition in common, the suppression of tumor growth. As an example, some of these genes are involved in "quality control" procedures in the cell, as they control the quality of the DNA strands. If the DNA strands harbour incorrectly placed nucleic acids or other abnormalities, the cell is not allowed to divide, and may eventually be directed to programmed cell death. One way of inactivating a tumor suppressor gene is by deleting that part of the DNA strand that harbours the gene, or part of it.

Chromosomal deletions seem to be very common in cancer, and span from the deletion of small DNA fragments to loss of chromosomal arms or even entire chromosomes [7]. The deletions occurring in a given epithelial cancer seem to be non-random, and some seem even to be specific for a given cancer, whereas others are more generally present in cancers. In bladder transitional cell carcinomas (TCCs) chromosome 9 is subjected to deletions [22, 35], often very early in tumor development [22, 79], a finding also observed in other common cancers such as carcinoma of the lung [63], skin [80], ovary [88], and kidney [15]. A candidate tumor suppressor gene CDKN2;p16 was recently identified in the 9p21 region [13, 14, 24, 35, 46]. This gene encodes a protein involved in control of the cell cycle [45].

Various techniques have been used to identify non-random deletions. In the early 1980s cytogenetic analysis was used to identify monosomy, interstitial deletions, and isochromosomes with loss of genetic material at specific sites. In the 1990s molecular techniques have been developed (restriction fragment length polymorphism, polymerase chain reaction, fragment analysis)
that are able to identify very subtle deletions of DNA strands. These techniques are based on highly developed panels of informative markers that cover the complete human genome [25]. With the development of the polymerase chain reaction (PCR) it is now possible to use tandem repeat DNA sequences (e.g., CACACACA) known as microsatellites, as markers, as these are scattered throughout the genome, and highly polymorphic. The latter makes it easy to select microsatellites that can be expected to be heterozygous in the majority of examined individuals. To illustrate this, consider an individual who has a microsatellite of 60 CA repeats in the short arm (9p) of one of his chromosome 9 alleles, and one of 70 CA repeats on the other 9p allele. If this individual develops a bladder cancer that deletes part of, or the whole short arm of 9, there is only one microsatellite of 60 or 70 CA repeats left. In a gel system the 60 and 70 CA repeats will show up as two bands, the single 60 or 70 CA repeat as one band. In this simple way a deletion has been mapped to the area on 9p where we a priori know that the microsatellite is located. The knowledge on the physical microsatellite location in the genome is one of the benefits of large international research programs [25].

Several studies based on RFLP and microsatellite techniques have identified the most frequent aberration in bladder TCCs to be deletions of part of, or one entire, chromosome 9 [35, 79]. As evident from Fig. 1 some data are available on various parts of chromosome 9, making it possible to map those regions of chromosome 9 which are most prone to be deleted in TCC. These regions are marked by a black line in Fig. 1, and spans an area on 9p23 in which p16 is located, and an area on 9q31–34.

**Correlation of chromosomal deletions with histopathological parameters**

It is generally believed that chromosome 9 very frequently shows deletions in bladder cancer, however, when it occurs during the development of bladder cancer is not known. Chromosome 9 deletions have been shown to be independent of tumor grade and stage in several papers [46, 53, 65], and to occur much more frequently in low-grade, low-stage tumors in other papers [22, 36]. These last authors used their finding to advocate a very early role of chromosome 9 deletions in bladder tumorigenesis. Unfortunately, the number of tumors examined in all the cited papers is small (2–5 tumors in each group) and the conclusions reached should be regarded as preliminary. One explanation for the differences could be that the loci studied on chromosome 9 are deleted at different grades and stages, as shown in [71] in which some loci like D9S17, ASS on chromosome 9q and IFNA on chromosome 9p are lost in low-grade, low-stage tumors, and loci like D9S28 and D9S54/D9S13 on chromosome 9g lost in high-grade, high-stage tumors. This finding is still based on a small number of tumors; however, it indicates that it is important to realize what small part of chromosome 9 that is under investigation.

A confounding parameter is the number of normal cells present in the tumor tissue examined, as these may mask deletions in the tumor if they are present in a significant number. Furthermore, the cut-off level, as well as the techniques used, varies much from paper to paper. In some papers, the complete deletion of chromosome 9—monosomy 9—is taken out of the data when deletions are shown, and those papers only report on partial deletions of chromosome 9. This is important as the number of tumors showing monosomy 9 is reported to be as frequent as 32% [46], 41% [85], 54% [12], and 59% [53]. In conclusion, it seems that chromosome 9 alterations occur early in bladder carcinogenesis, and are partly independent of stage and grade. Approximately half of bladder tumors have lost one chromosome 9, and the chromosome 9 remaining is suffering from deletions in both the p and q arms, in more than two of three tumors. The deleted area on 9p seems to be between D9S126 and D9S109 reflecting 9p21–23, and on 9q the area between D9S167 and D9S176, reflecting 9q21.1–22.3. Some more telomeric placed regions on 9q are also very frequently lost: they are placed around D9S154-D9S103, reflecting 9q32–33, and D9S60–66, reflecting 9q34.

Chromosomal changes, apart from chromosome 9, which occur early in bladder tumorigenesis seem to be difficult to define. Some papers describe findings in Ta grade I/II tumors of 11p[22, 32, 36] and 13q [22], deletions, but most deletions occur at a frequency of less than 20% in these superficial tumors. In high-grade, high-stage bladder tumors several deletions seem to occur systematically compared with low-grade, low-stage tumors: 3p21–25 (P < 0.004 [79]); 4p/D4S1608–404 (P < 0.005 [78]); 4q 33–34/D4S408–D4S426 (P < 0.002 [78]); 8p21.3 (P < 0.05 [47]); 18q21.3 (P < 0.02 [9]) and DCC (P < 0.05 [9]). Interestingly, deletions of chromosome 17p11–13, which is the location of tumor inhibitor p53, are also frequent in high-grade (P < 0.06 [70, 79]), high-stage (P < 0.0015 [79]) tumors, and may be related to vascular invasion [79].

**Potential diagnostic and predictive use of microsatellite alterations**

In normal biological conditions, DNA is very well conserved, and any error in the base sequence is immediately corrected by either the mismatch repair system [66] or the excision repair system [86]. However, in malignant transformed cells a general feature seems to be chromosomal instability with incorporation of mismatched bases giving rise to either missense, or nonsense mutations. The former results in the substitution of one amino acid for another which often leads to a defect in protein function, the latter results in either a truncated protein, as the translation may stop before the total