Chromosomal Instability in Bladder and Colon Cancer

Studies involving microsatellites

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This thesis is based on four papers in English on chromosomal instability in bladder and colon cancer. The aim has been to characterize microsatellite alterations in tumors and urine from patients with transitional cell carcinoma (TCC), in urinary sediments from patients with benign prostatic hyperplasia (BPH) with and without cystitis, patients belonging to families with hereditary non-polyposis colon cancer (HNPCC), and patients suspected of belonging to HNPCC families. Various molecular biological methods were applied.

It is well known that germline mutations in the DNA mismatch repair genes *MSH2*, *MLH1*, *PMS1*, *PMS2* and others are associated with HNPCC. These mutations give rise to microsatellite instability, which gives rise to further mutations in tumor suppressor genes and oncogenes, resulting in tumor progression. Microsatellites are repeat DNA sequences spread through the human genome. A change of any length due to either insertion or deletion of repeating units is defined as microsatellite instability. This was registered when novel bands occurred, or when band shifts occurred, with a retained band pattern, in the tumor DNA compared to normal. Microsatellite alterations are also detected as loss of heterozygosity (LOH). Microsatellite alterations such as instability and LOH occur frequently in bladder cancer. LOH of chromosome *9p* is an early event in the development of bladder cancer, LOH of *17p (p53)* and others is involved in the progression to invasive cancer. Furthermore, it has been observed that microsatellite alterations in urine from bladder cancer patients match those in the urine. Microsatellite instability has also been observed in inflammatory conditions in organs like the colon and pancreas. However, no reports are available on microsatellite alterations in inflammatory conditions in the bladder epithelium, *e.g.* cystitis.

In the first paper silver staining, autoradiography and a fluorescence technique (ABI Prism 377 Sequencer) were compared for the detection of microsat-

ellite alterations in tumors from patients with suspected HNPCC, and tumors from patients with bladder cancer. The fluorescence method was the most sensitive. Furthermore, it appeared possible to avoid radioactivity using silver staining as an alternative. The results obtained varied when comparing silver staining with autoradiography and the fluorescence technique, but were similar when comparing autoradiography and the fluorescence technique. The interpretation of a single locus varied comparing silver staining with autoradiography and the fluorescence technique. However, the classification of the tumors based on several microsatellite loci was always identical.

In the second paper, microsatellite alterations were examined in tumors from young TCC patients with bladder cancer. The number of novel bands occurring was significantly higher in low-stage tumors than in high-stage tumors ($p<0.05$). LOH was most frequent in *9p* markers in low-stage tumors. In high-stage tumors a group of markers, *2p, 17p (p53), 9q, 5q* and *10p*, showed LOH most frequently. Interestingly, patients with a disease course longer than 1 year showed significantly more frequent microsatellite instability compared with patients with a disease course shorter than 1 year ($p<0.048$). Microsatellite markers located at *MSH2* and *MLH1* showed LOH in several cases.

Microsatellite alterations in urinary sediments from patients with TCC, and with BPH with or without cystitis, are examined in the third paper. The frequency of microsatellite instability and LOH was relatively high in markers at chromosomes 8 and 14 in urine from patients with TCC, or BPH plus cystitis. Controls with BPH also exhibited alterations. Novel bands appeared to be associated with TCC, and band shifts with cystitis. The occurrence of novel bands was significantly higher in tumors from patients with TCC than in the corresponding urine ($p<0.008$). Novel bands in urine occurred significantly more frequently in urine from TCC patients than in BPH patients with or without cystitis ($p<0.001$). Furthermore, the alterations found in urine from patients with TCC did not always reflect those in the tumor.

An approach to identify mismatch deficiencies in tumors from patients with HNPCC or suspected HNPCC by various screening methods is described in the fourth paper. Two antibody-based methods (immunohistochemical staining and Western blot analysis) were compared with more conventional methods (microsatellite analysis and sequencing). The concordance between various methods ranged from 43% (all four methods) to 100% (Western blot analysis and microsatellite analysis). Immunohistochemical staining and microsatellite analysis had almost the same concordance with respect to sequencing, 86% and

80% respectively, indicating that these methods are suitable for HNPCC screening.

In conclusion, this PhD thesis has shown that the fluorescence-based method for the detection of microsatellite alterations is the most sensitive. It was also shown that immunohistochemical staining should be used in combination with microsatellite analysis to pre-screen for HNPCC before sequencing the *MSH2* and *MLH1* mismatch repair genes in patients with suspected HNPCC. Furthermore, it was shown that microsatellite instability in tumors from young patients with TCC is very pronounced, and that alterations in urine do not always reflect those in the tumor. Finally, it was shown that microsatellite alterations in urine are indicators of not only malignancy, but also inflammatory conditions.