In search of genes responsible for the development of renal cell carcinoma

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SUMMARY

This thesis describes the search for genes whose functional elimination would be responsible for the development of renal cell carcinoma (RCC). In Chapter 1 an overview is given of the different forms of RCC. RCC can occur sporadically or in a hereditary form, either as pure familial RCC or as one of the tumours which are characteristic for the von Hippel-Lindau syndrome (VHL). In all three forms cytogenetic studies indicate a specific loss of a part of the short arm of chromosome 3 (3p). In a family with pure familial RCC a constitutional translocation (3;8)(p14.2;q24) seems to predispose to RCC. Linkage analysis in families affected with VHL localized the gene for this syndrome in 3p25-p26. Epidemiological studies have indicated that a model, according to which a tumour develops as a consequence of the functional loss of both alleles of a tumour suppressor gene, may be applicable to RCC. Such a tumour suppressor gene should be located on 3p, the chromosome region which appears to play a role in the predisposition to hereditary RCC and which most often is found deleted in the tumours.

Chapter 2 describes the study of loss of heterozygosity on 3p in sporadic RCC to define the region in which to look for a tumour suppressor gene. We found deletions in 50% of the tumours and defined the region 3p21-p24 as the region common to all deletions. Since this chromosome segment does not include the location of the VHL gene, nor the 3p breakpoint of the aforementioned translocation predisposing to hereditary RCC it is concluded that different genes must be involved in the development of sporadic RCC, pure familial RCC, and VHL. For some genes located in 3p21-p24 a tumour suppressor function has already been considered, because of decreased levels or total absence of the mRNAs of these genes in certain tumour types. We found that in RCC tumours and tumour cell lines the mRNA levels for two of these genes, D8 and APEH, did not deviate from what we found in normal kidney cells. Therefore, we do not consider these genes to be likely candidate tumour suppressor genes in RCC.

Chapter 3 focuses on the chromosome region 3p14. We aimed at cloning the region around the 3p breakpoint in the previously mentioned t(3;8) associated

with hereditary RCC, to see whether this translocation has affected some gene which therefore could be responsible for hereditary RCC. Three widely used 3p probes were localized with respect to the breakpoint by in situ hybridization. A probe from the locus D3S3 failed to hybridize to either of the two translocation chromosomes. We considered this as an indication that D3S3 might be close to the breakpoint. Therefore, we constructed a long-range restriction map around D3S3. This became only possible after we treated cells from which we wanted to isolate DNA with a demethylating agent because the region around D3S3 appeared to be heavily methylated. However, there was no indication for the presence of the t(3;8) breakpoint within the approx. 1 Mb covered by this map. Probes that were more close to the breakpoint were isolated from a 3p14 specific microdissection library. Unique sequence clones from this library were used for long-range restriction mapping. The t(3;8) breakpoint was defined between two groups of clones, one distal to the breakpoint in 3p14.2 and one proximal in 3p14.1-p14.2. In the 3p14.1-p14.2 region a long-range restriction map covering 4.6 Mb was constructed. However, we found no indications that the breakpoint is located within these 4.6 Mb.

In Chapter 4 the use of somatic cell genetic criteria in the classification of renal cell tumours is discussed. It appeared that in our material loss of heterozygosity on 3p was only found in tumours that histopathologically could be classified as clear cell tumours. This may indicate that only clear cell tumours arise as a consequence of loss of a gene on 3p, or that 3p rearrangements in non-clear cell tumours are more subtle, and therefore not detectable with the set of probes we used. Chromosome studies in tumours belonging to different histopathological subtypes revealed that different subtypes of renal cell tumours are characterized by certain combinations of chromosome abnormalities. Moreover, there seems to be a relation between the occurrence of a certain combination of chromosome abnormalities and tumour progression in specific types of RCC.