Genomic and genetic characterization of the chromosomal region 13q14-q21

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SUMMARY AND CONCLUSIONS

This thesis describes the isolation of unique DNA sequences (probes) from the region q14-q21 of the human chromosome 13 and their application as genetic markers in the analysis of families with a hereditary eye tumour (retinoblastoma) and in families with a hereditary copper accumulation disorder (Wilson disease), as well as in a study of the etiology of bone cancer (osteosarcoma).

In the Introduction gene mapping is described as well as the applied "reverse genetics" approach, in which the genetic characterization of a certain disorder is started from knowledge of the chromosomal localization of the gene responsible for that disorder. Genetic aspects of the disorders mentioned above are also discussed in the Introduction. In chapter 2 the isolation of chromosome 13-specific DNA sequences and their sublocalization at certain regions of chromosome 13 is described. From genomic DNA isolated from a hybrid cell line, which contains two copies of chromosome 13 as their only human genetic material in addition to a complete Chinese hamster genome, a lambda DNA-library is constructed. From clones containing human and, therefore, chromosome 13-specific DNA unique sequences have been subcloned. Subsequently, these probes have been localized at certain bands of chromosome 13 by making use of hybrid cell lines containing a human chromosome 13 with different interstitial deletions. Two approaches have been followed: (1) by somatic cell hybridization the intact human chromosome 13 has been separated from the chromosome with the deletion, so that the absence of a given DNA sequence can be determined directly, indicating its location within the deletion; (2) by quantitative hybridization using a second probe from a different human chromosome as a reference it is possible to distinguish between the presence of one copy (within the deletion) or two copies (outside the deletion) of the DNA sequence in the DNA from the cell lines (chapter 2). We have also constructed radiation hybrids containing just a partial human chromosome 13 as their only human genetic material. Two of them, ICD, and ICA, contained pter-q14:, and pter-q13:, respectively. These hybrids provided an alternative localization panel allowing regional mapping of our probes. Two probes, pG14E1.9 and pG24E6.8, respectively, appeared to be localized at the chromosomal region 13q14.1-2 which also contains the retinoblastoma locus.

Using these and other chromosome 13-specific probes several restriction fragment length polymorphisms (RFLPs) have been identified. Their application as genetic markers in the diagnosis of hereditary retinoblastoma, an embryonal tumor of the retina with a dominant

mode of inheritance, is described in chapter 3. By combining the use of these flanking probes and probes within the retinoblastoma gene it is possible -as shown- to identify asymptomatic carriers of a mutant retinoblastoma allele in families with hereditary retinoblastoma.

The probes have also been used as genetic markers in a linkage analysis in Wilson disease (WD) families. Wilson disease is a copper accumulation disorder with an autosomal recessive mode of inheritance. In a two-point linkage analysis, pG18E2.1 (D13S12), localized at the chromosomal region 13q21, appeared to be closely linked to the Wilson disease (WND) locus (chapter 4). This points at a more distal localization of the WND locus than was originally assumed because of the observed close linkage to the esterase D (ESD) locus at 13q14. In an extended multipoint linkage analysis of a larger number of families the WND locus has been localized more exactly (chapter 4). In this study, which contributes to the genetic characterization of the chromosomal region 13q14-q21 and thereby will be of help to clone the WD gene, crossovers possibly located close to the WND locus have been identified in a number of families. These crossover sites are potential markers of the chromosomal region in which the WND locus must be contained.

In chapter 5 the application of the probes in the study of the role of the retinoblastoma gene in the genesis of osteosarcoma is described. This bone cancer is the most frequent second primary tumour in survivors of hereditary retinoblastoma and can present itself at the age of adolescence. By studying the loss of heterozygosity of chromosome 13-specific probes combined with a molecular analysis of the retinoblastoma gene in a series of osteosarcomas from patients without a previous history of retinoblastoma, a number of homozygous deletions and a homozygous duplication could be identified. In addition, it has been observed that loss of heterozygosity is at least as frequent for chromosome 17 as it is for chromosome 13. This may indicate that in addition to the retinoblastoma gene a tumour suppressor gene at chromosome 17, possibly the p53 gene, can have an alternative or additional role in the initiation of osteosarcoma.

The study described in this thesis contributes to a refined DNA diagnosis of hereditary retinoblastoma, to the identification of asymptomatic carriers of a mutant retinoblastoma allele, to an insight in the etiology of osteosarcoma, to a presymptomatic diagnosis of Wilson disease, and by the genetic characterization of the chromosomal region 13q14-q21 to the cloning of the gene responsible for this disease.