7. Summary

Physical mapping and genomic structure of the Karyopherin Alpha 2 (KPNA2) Gene-Analysis of the loss of heterozygosity (LOH) in the chromosomal region 17q23 and investigation of the general state of microsatellites in microdissected colorectal and mammary carcinomas of men

The aim of the investigations was to localize the Karyopherin Alpha 2 Gene by using various methods of physical mapping and to identify it´s gene structure by means of sequence analysis. Furthermore colorectal and mammary carcinomas with microsatellite markers from the region 17q23 as well as from other regions, which often show changes in tumors, were examined for the existence of heterozygosity loss.

By using several somatic cell hybrids we localized the KPNA2-Gene on the chromosome 17. Due to the support of the YAC-library and five cell hybrids with chromosomal fragments, derived from the region 17q, it was possible to achieve a more specific determination of the position on the long arm of the chromosome (17q23).

The construction of a PAC/BAC-Contig enabled us to verify the positional datas and to make further statements about the rough structure of the certain region, in which the gene is localized as well as about the exact setting of the gene among the neighbouring microsatellites. This lead to the observation that a processed pseudogene lies directly proximal to the KPNA2-Gene.

The investigations on the loss of heterozygosity took place by using the PCR- technique in microdissected tumors. We tested 40 colorectal carcinomas with 12 microsatellite markers and 30 mammary carcinomas with 7 microsatellite markers. The colorectal carcinomas revealed for the regions, that are defined by the markers D17S1813 and D17S1870 and directly border on the KPNA2-Gene, a relatively high rate of loss in comparison to other markers localized on the chromosome 17. It is imaginable that the investigated region is identical to the region of loss 17q22-23, that is described in the literature (LEGGETT et al. 1994).

The everage LOH-frequency of microsatellite markers that are localized on other chromosomes showed to be approximately by 17 % higher than that of markers on 17q. The examined mammary carcinomas in the region, that contained the regions D17S1813, D17S789 and D17S1870, had the highest LOH-figure, mentioning that the image of loss in total appears more steady.

This might indicate, that the deleted part comprises a larger area of the chromosome. In contrast to this, one assumes in colorectal carcinomas, that there is a gene localized in a relatively small region that has one of the highest LOH-rates and is involved in the formation and development of tumors. As the KPNA2-gene is situated directly next to this locus it is absolutely imaginable, that this is the canditate being searched for.

The sequencing of cloned DNA fragments and the examination of the genomic structure of the KPNA2-Gene yielded that the gene consists of 11 exons and has the size of 11000 base pairs, not considering the promotor. It is very conspicious that there are repetitive sequences in the introns 5, 7 and 9 (so called ALU- repetitions), that make the gene susceptible to internal deletions and duplications.

The discovery of the tumor suppressor gene OHO 31 in *Drosophila melanogaster* (TÖRÖK et al. 1995), which is involved in the import of proteins, allows the conclusion that a mechanism for the formation of cancer exists, which is also of significance for human beings.

It is possible that, due to the chromosomal position and the particularities in the sequence and the genomic structure, the KPNA2 Gene – like the OHO31Gene - also plays an important role in the formation and development of tumors. To prove this hypothesis, further investigations, that base on these results, will be necessary in the future.