Characterisation of 3p deletions in lung cancer

Kok, Klaas

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SUMMARY

CHARACTERISATION OF 3p DELETIONS IN LUNG CANCER

The importance of tumour suppressor genes in the development of solid tumours, both hereditary and nonhereditary, is well documented, and a number of such genes have been identified in the past decade. For positional cloning of a tumour suppressor gene the usual starting-point is the determination of the shortest region of overlap for consistently occurring deletions in a certain type of tumour. In those cases where the tumour also occurs in a hereditary form, linkage analysis can be used as an alternative or supplementary approach to provide equivalent data.

In lung tumours, allelic loss has been consistently and with a very high frequency detected for the chromosomal region 3p21. The observations from the literature are being reviewed in Chapter 1 of this thesis. Our own data are presented in Chapter 2. Since we found allelic loss for the locus D3F15S2 in all informative cases of all types of lung cancer we could study, this suggested to us that a candidate tumour suppressor gene might map at a close distance to this locus. We conducted a search for genes in its vicinity, making use of a cosmid library constructed from a human-hamster hybrid cell line which contained a small part of 3p21 including D3F15S2, as its only chromosome 3 material. This strategy, discussed in more detail in Chapter 3, led to the identification of a gene that is now called UBE1L. Although one of the UBE1L alleles turned out to be always retained in the lungcancer-derived cell lines that we could analyze, Northern analysis of these cell lines failed to detect any expression of this in normal tissues - including lung- ubiquitously expressed gene. Only upon a highly sensitive RT-PCR analysis (Chapter 4), some residual expression, but more than an order of magnitude lower than expected, could be detected. Such a consistently dramatically decreased expression in lung cancer has thusfar not been described for any other gene in 3p21. Chapter 5 describes the sequence analysis of the UBE1L cDNA. The high degree of identity of its product with the human ubiquitin activating enzyme suggests that the UBE1L gene product might be involved in some ubiquitin pathway. The ubiquitin pathway has been implicated in a number of fundamental cellular processes including selective degradation of abnormal and short-living proteins, cell cycle progression, and DNA repair. The genomic structure of the UBE1L gene is presented in Chapter 6. The dramatically decreased expression in lung cancer-derived cell lines could not be attributed to mutations in the coding regions of the gene. It did correlate with an altered chromatin structure in the presumed promoter region. It might be that the UBE1L-gene looses its function in tumour cells due to some regulatory block of expression.

A strong indication for the presence in 3p21 of a tumour suppressor gene which is inactivated in tumours by loss or mutation of both its alleles, comes from transfection

experiments as described in the review of the literature in Chapter 1. In line with such a mechanism of inactivation Chapter 7 describes the characterisation of a homozygous deletion which we detected in a small cell lung cancer-cell line. The deletion maps in 3p21, between D3F15S2 and ZnF16 and just centromeric to the UBE1L gene. Since the complete loss of many genes may be lethal to cells, homozygous deletions tend to be relatively small. In this case the size of the deletion was determined to be about 440 kb. The region which is homozygously deleted is also part of a larger region homozygously deleted from another lung cancer-derived cell line recently described in the literature. Moreover, it is contained within a 2 Mb DNA segment that was able to suppress the tumorigenicity of a mouse fibrosarcoma cell line. Together these data strongly suggest that a tumour suppressor gene is located within these 440 kb of DNA.