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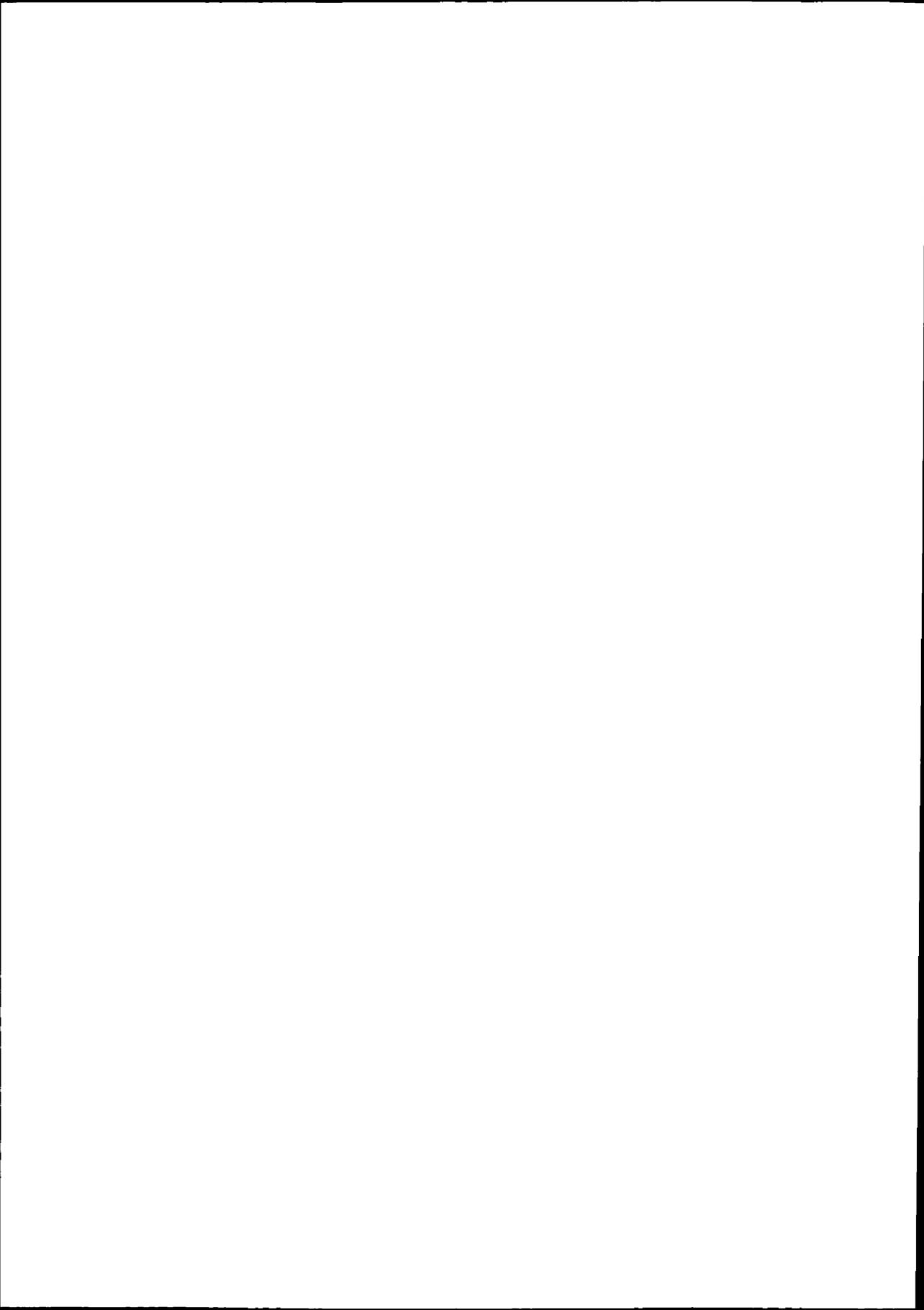
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# **Chapter 5**

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# **LOH of *PTPRJ* occurs early in Colorectal Cancer and is associated with Chromosomal Loss of 18q12-21**

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**Genome-wide comparative genomic hybridization (CGH) indicated that chromosomal aberrations in colorectal adenomas and carcinomas are not randomly distributed but occur in specific clusters. Recently, the gene *Ptprj* (protein tyrosine phosphatase receptor type J) was identified as the candidate gene for the mouse colon cancer susceptibility locus *Sccl*. Its human homologue *PTPRJ* is frequently deleted in several cancer types, including colorectal cancer. To elucidate the role *PTPRJ* loss in different stages of colorectal cancer and in the cancer pathways, we expanded the previous CGH results with novel loss of heterozygosity (LOH) data on *PTPRJ*. We observed a strong association between LOH of *PTPRJ* and loss of chromosomal region 18q12-21 ( $P = 0.009$ ). This finding is specific for progressed colorectal adenomas, suggesting that a synergistic interaction between LOH of *PTPRJ* and loss of chromosome 18q12-21 may be involved in the development of a more progressed form of adenomas.**

**Keywords:** *PTPRJ*, Colorectal cancer, LOH, CGH and 18q12-21.

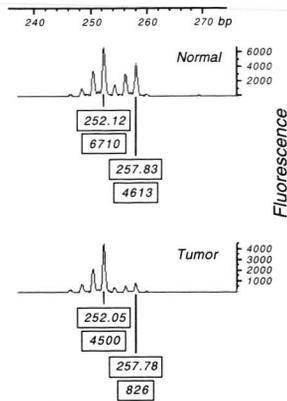
## **Introduction**

The accumulation of chromosomal imbalances such as losses and gains are common events in the progression of colorectal tumors (1). In a previous study, genome-wide analysis of such chromosomal aberrations in colorectal adenomas and carcinomas by comparative genomic hybridization (CGH) revealed seven chromosomal aberrations (i.e. loss of chromosomes 8p, 15q, 17p and 18q, and gain of chromosomes 8q, 13q and 20q) that are specifically associated with adenoma to carcinoma progression (2). Moreover, hierarchical cluster analysis of these results showed that these chromosomal changes were not randomly distributed, but occurred in more or less specific combinations, resulting in 4 clusters of colorectal adenomas and 2 clusters of carcinomas. Adenoma-cluster 1 showed no abnormalities. In the other adenomas three distinct combinations of genetic aberrations were identified: 17p12-13 loss and K-ras mutation (adenoma-cluster 2), 8q23-qter and 13q14-31 gain (adenoma-cluster 3), and 18q12-21 loss and 20q13 gain (adenoma-cluster 4), respectively. In the carcinomas two distinct patterns of chromosomal instability emerged. Carcinoma-cluster 1 showed losses of 11q, 12q, 17p, 17q, 18q and 21q and gains of 8q and 13q, whereas carcinoma-cluster 2 exhibited gains of 7p+q, 8q, 13q and 20q, and loss of 18q. The carcinoma-clusters showed great similarity with two of the adenoma-clusters, namely the chromosomal abnormalities observed in carcinoma-cluster 1 and 2 matched with those detected in adenoma-cluster 2 and 4, respectively. Adenoma-cluster 3 appeared to be an intermediate group. These data suggested that independent chromosomal instability pathways lead to colorectal cancer progression (2).

In the present paper we compared the CGH results with loss of heterozygosity (LOH) of the gene *PTPRJ* (protein tyrosine phosphatase receptor type J). *PTPRJ* is located on human chromosome band 11p11. Recently, it was demonstrated that one copy of the *PTPRJ* gene is deleted in a large percentage of human sporadic colorectal, lung and breast carcinomas (3). In the mouse, *Ptprj* was positionally cloned as the candidate gene (3) for the mouse colon cancer susceptibility locus, *Sccl* (4). In rats, it was reported that expression of *Ptprj* suppresses the malignant phenotype in transformed rat thyroid cells (5). All these findings implicate a role for *PTPRJ* in cancer. Here we further evaluate the occurrence of *PTPRJ* loss in human colorectal cancer by examining its correlation with other specific chromosomal imbalances.

## **Results & Discussion**

Four microsatellite markers along the gene *PTPRJ* on chromosome 11p11 were used to compare the alleles of normal and tumor DNA of 82 informative samples, consisting of 47 colorectal adenomas and 35 colorectal carcinomas. DNA copy number losses at the region of *PTPRJ* were not detected by CGH, probably because there is only a small region deleted (3) that is beyond the detection sensitivity of CGH technique. In 47% (22 of the 47) of the adenomas we detected LOH of *PTPRJ* (figure 1). LOH of *PTPRJ*



**Fig. 1** *PTPRJ* loss in human colorectal cancer. Fluorescent electropherograms for microsatellite marker *D11S4117*. The normal (top) and tumor (bottom) counterparts of one patient are indicated. The boxes under the peaks indicate the length of the allele in bp (top) and the fluorescence (bottom)

ranged from 25% in adenoma-cluster 1 to 67% in adenoma-cluster 4 (Table 1), but this trend did not reach statistical significance, probably due to the small tumor numbers per cluster. As the clusters 3 and 4 contained predominantly progressed adenomas (73%, 19 of the 26 contain a carcinoma part) the apparently more frequent LOH of *PTPRJ* in these two clusters suggests that *PTPRJ* deletion in their adenoma part may be related to the development of a progressed adenoma stage. LOH of *PTPRJ* was observed more frequently in the carcinomas (71%, 25 of the 35) than in the adenomas ( $P = 0.052$ ). No difference in the presence of LOH was detected between the two carcinoma-clusters (Table 1).

**Table 1** LOH of *PTPRJ* stratified by cluster.

Clusters	Adenoma				Carcinoma	
	1	2	3	4	5	6
LOH of <i>PTPRJ</i>	25% (1/4)	35% (6/17)	45% (5/11)	67% (10/15)	68% (13/19)	75% (12/16)

**Table 2** Correlation between LOH of *PTPRJ* and loss of chromosome 18q12-21 in colorectal adenomas ( $n=47$ ) and carcinomas ( $n=35$ ).

<i>PTPRJ</i>	Chromosome 18q12-21	
	No loss	Loss
No LOH	25	10
LOH	18	29

$P = 0.009$

We investigated possible correlations between LOH of *PTPRJ* and other specific cancer associated chromosomal aberrations. In the 82 tumor samples, LOH of *PTPRJ* correlated strongly with the loss of chromosomal region 18q12-21 ( $P = 0.009$ , Table 2), but not with any of the other cancer

## ***PTPRJ* LOH and 18q loss**

associated chromosomal aberrations. After stratification for tumor type we still observed a significant association between LOH of *PTPRJ* and loss of 18q12-21 in the 47 adenomas ( $P = 0.004$ , Table 3).

Subsequently, we tested the association between LOH of *PTPRJ* and loss of 18q12-21 for the different adenoma-clusters. Since only 47 adenomas were available for screening, we could not evaluate each cluster separately. Therefore, we divided the adenomas in two groups, namely adenoma-cluster 1 plus 2 containing 21 tumors (no or only few chromosomal aberrations) and adenoma-cluster 3 plus 4 consisting of 26 tumors (many chromosomal aberrations). Interestingly, in adenoma-clusters 1 and 2 seven of the adenomas exhibited LOH of *PTPRJ* and only one adenoma showed loss of chromosome 18q12-21 suggesting that LOH of *PTPRJ* occurs earlier in colorectal tumor progression than loss of chromosome 18q12-21. Obviously, there was no significant association, as only one adenoma had lost *PTPRJ* as well as chromosome 18q12-21. The correlation between LOH of *PTPRJ* and loss of 18q12-21 is highly suggestive in the adenoma-clusters 3 and 4 ( $P = 0.064$ , Table 4). Loss of *PTPRJ* (58%, 15 of the 26) as well as loss of chromosome 18q12-21 (62%, 16 of the 26) also mainly occurred in these two clusters of adenomas. Furthermore, since these two adenoma-clusters contain significantly more progressed adenomas than adenoma-clusters 1 and 2 ( $P < 0.0001$ ; 19 of the 26 versus 4 of the 21), the data suggest that the interaction between LOH of *PTPRJ* and loss of 18q12-21 is specific for progressed adenomas ( $P = 0.078$  for progressed adenomas, data not shown). In the model by Vogelstein and colleagues (8) the loss of chromosome 18q is also considered as an event in later adenoma progression.

**Table 3** Correlation between LOH of *PTPRJ* and loss of chromosome 18q12-21 in colorectal adenomas.

<i>PTPRJ</i>	Chromosome 18q12-21	
	No loss	Loss
No LOH	21	4
LOH	9	13

$P = 0.004$

**Table 4** Correlation between LOH of *PTPRJ* and loss of chromosome 18q12-21 in adenoma-cluster 3 and 4.

<i>PTPRJ</i>	Chromosome 18q12-21	
	No loss	Loss
No LOH	7	4
LOH	3	12

$P = 0.064$

In contrast to the adenomas, no association between LOH of *PTPRJ* and loss of 18q12-21 was detected in the 35 carcinomas (including carcinoma parts of progressed adenomas). Sixteen carcinomas exhibited loss of *PTPRJ*

and 18q12-21, 9 carcinomas showed only LOH of *PTPRJ*, 6 carcinomas lost chromosome 18q12-21 but not *PTPRJ* and 4 carcinomas retained *PTPRJ* as well as 18q12-21. The absence of the statistical correlation in carcinomas is due to the high frequency of both LOH of *PTPRJ* (71%, 25 of the 35) and loss of 18q12-21 (62%, 22 of the 35).

In summary, we describe a significant association between LOH of *PTPRJ* and loss of chromosome 18q12-21 in progressed colorectal adenomas. A more precise determination of the smallest possible overlap of the lost segments of chromosome 18q12-21 is required to define the gene(s) possibly interacting with *PTPRJ*. The genes *SMAD2* and *SMAD4* (9) could be considered, as their loss disrupts the TGF $\beta$  signaling cascade that inhibits growth and induces apoptosis. Why both chromosomal imbalances tend to accumulate in the same tumor needs to be further investigated. However, these two events may be critical steps for the development of colorectal cancer. We show here that LOH of *PTPRJ* is an early event in colorectal cancer, whereas loss of chromosome 18q12-21 is a later event (8). In this case, loss of chromosome 18q12-21 in colorectal adenomas that already lost *PTPRJ* might more effectively contribute to the development of a more progressed form of adenomas.

## Materials & Methods

### *Tumor Samples and DNA Extraction.*

Eighty-two sporadic non-familial colorectal tumors, consisting of 47 adenomas (24 non-progressed adenomas, i.e. simple adenomas without a focus of cancer, and 23 progressed adenomas, i.e. adenomas with a focus of cancer of which the adenoma part was analyzed) and 35 carcinomas (23 carcinoma parts of progressed adenomas and 12 simple carcinomas), as well as the corresponding non-tumorous tissue were obtained from the archives of the Department of Pathology VU University Medical Center, Amsterdam, the Netherlands. Histopathological examination is performed by one observer [G.M.] on H&E sections. For each tissue sample, DNA was extracted from microdissected paraffin material (7). For the extraction of tumor DNA the most tumor-rich areas were dissected allowing a maximum of 20% non-tumor cell contamination (6, 7).

### *LOH Analysis.*

A panel of 4 microsatellite markers mapping along the gene *PTPRJ* on chromosomal segment 11p11 was selected (*D11S4117*, *D11S1784* and *D11S4183* located in intron 1, and *D11S1350*, in intron 21 of *PTPRJ*, Research Genetics) (3). Oligonucleotide primers of microsatellites were labeled with 6-FAM fluorescent dye. PCR amplification was performed in a volume of 20  $\mu$ l including 5xPCR buffer (20mM KCL, 10mM Tris-HCL, 0.01% gelatin, 1.5mM MgCl<sub>2</sub>, and 200 $\mu$ M of each dNTP), 1 unit of Taq DNA polymerase and 5 pmol\_20pmol of each primer. PCR cycles consisted of 1 cycle at 94°C for 3 min followed by 35 cycles of 30s at 94°C, 1 min at 56°C–62°C, and 1 min at 72°C. One  $\mu$ l of PCR products was added to 10  $\mu$ l formamide and 0.5  $\mu$ l ROX 350 size standard (PE Applied Biosystems). Products were then run on a 3700 Automated Sequencer and analyzed using Genescan Analysis®2.1 and Genotyper®2 software. A difference of 30% or more in the intensity ratio of the two alleles in tumor DNA compared to DNA from normal tissue has been considered as evidence for LOH. A difference between 20-30% has been considered as ambiguous LOH.

### *Statistical Analysis.*

Two-by-two tables were analyzed using the  $\chi^2$  test. *P* values were Bonferroni corrected for multiple testing. *P* values < 0.05 were regarded significant.

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