The RET gene and its associated diseases

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Summary / Samenvatting

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This thesis starts with a brief description of protein kinases, a large family of proteins involved in cell proliferation and differentiation and also in a number of cancer types and hereditary diseases (chapter 1), and subsequently discusses in greater detail the receptor protein kinase RET (chapter 2) and its involvement in several diseases (chapter 3). Furthermore, our own data on the *RET* gene and its role in diseases as well as results obtained in collaborative efforts with other groups are presented in the appendices 2-7.

The RET protein is involved in the normal development and the neoplastic growth of neural crest lineages. The ligand of the receptor is as yet unidentified. During embryogenesis, *RET* expression is high in neuroectodermal tissues, suggesting a function of RET in the proliferation, the migration and the differentiation of these cell types. In adult tissues the gene is hardly expressed. Expression is high in several tumor types derived from neural crest cells (chapter 2).

Transfection studies with DNA from different tumors revealed focal proliferation due to the presence of different DNA sequences that, however, shared a common part called *RET*. The original *RET* gene turned out to be rearranged in such a way that the sequences coding for the extracellular part of its protein product were replaced by sequences from elsewhere, resulting in a rearranged protein with a steady tyrosine kinase activity. The same rearrangement occurs in papillary thyroid carcinoma (PTC) (chapter 3).

After the genes involved in multiple endocrine neoplasia types 2A (MEN 2A) and 2B (MEN 2B) had been mapped to the centromeric region of chromosome 10 by linkage analysis, mutations of *RET*, a gene present in this very region, appeared responsible for the development of MEN 2A. The establishment of the intron-exon junctions of the *RET* gene and the determination of the flanking intronic sequences in a collaborative effort with the group of professor Romeo (Genua, Italy), made it possible to design primers and to develop PCR conditions for SSCP analysis (Appendices 2 and 3). Using this mutation detection system (Appendix 3) we found that a single *RET* mutation is uniquely associated with MEN 2B (Chapter 3 and Appendix 5).

In some families, MEN 2A is also found associated with cutaneous lichen amyloidosis (CLA), a rare skin disorder. We screened two of these families for *RET* mutations to determine whether specific mutations are involved in these families. A Cys634→Arg mutation was found. Though the same codon was affected in an earlier described family, the mutation in that family was different. This makes it hard to suggest a correlation between MEN 2A associated with CLA and a specific *RET* mutation. Because of the association of CLA with MEN 2A, *RET* might also be involved in hereditary "CLA only". We, therefore, screened *RET* in three families with hereditary CLA, but did not find any mutation. We concluded that the CLA lesions found in MEN 2A patients and those found in inherited CLA without MEN 2A must be caused by different genes. (chapter 3 and appendix 7).

An estimated 25% of medullary thyroid carcinomas (MTC) appear in the context of inherited disease (MEN 2 syndromes and familial MTC). For pheochromocytoma, the percentage of cases being part of an inherited neoplastic syndrome (MEN 2A, von Hippel Lindau,

neurofibromatosis I) is similar to that in MTC. The large majority of MTC and pheochromocytoma, however, consists of sporadic cases. We analyzed *RET* in sporadic MTC and *RET* and the von Hippel Lindau gene (*VHL*) in pheochromocytoma for the presence of mutations. In sporadic MTC and in sporadic pheochromocytoma *RET* mutations appeared to account for only a proportion of cases. The same could be concluded for the *VHL* gene in pheochromocytoma (chapter 3 and appendix 5).

Besides the gene for the neoplastic syndromes MEN 2A and MEN 2B, the gene for Hirschsprung disease could also be mapped by linkage analysis to the same small region of chromosome 10. Using the *RET* mutation detection system described in appendix 3, the Romeo group was one of two research groups who demonstrated that HSCR was also associated with *RET* mutations. The mutations appeared to be scattered all over the gene (chapter 3).

Publications in the recent literature on *RET* explain how these different diseases can be caused by one single gene. These are discussed in chapter 3. Mutations causing MEN 2A and MEN 2B activate the protein product, whereas mutations for HSCR result in a loss of function of the translated protein (chapter 3).

Based on the involvement of *RET* in the development of neural crest-derived tissues and on the association of *RET* mutations with neurocristopathies such as the MEN 2 syndromes and HSCR, a search for *RET* mutations in other neurocristopathies seems justified. Neuroblastoma occasionally occurs in diseases associated with abnormal neurocrest differentiation, e.g. Hirschsprung disease. Furthermore, neuroblastomas express *RET*. We therefore scanned the entire *RET* gene in 16 neuroblastoma cell lines and in a neuroblastoma patient belonging to a family in which different neurocristopathies occurred, including Hirschsprung disease and ganglioneuroma. We did not find any *RET* mutation. Therefore, expression of *RET* in neuroblastoma might just reflect the differentiation status of the tumor cells, rather than indicating an involvement in the tumorigenesis of neuroblastoma (chapter 3 and appendix 7).

We may conclude that *RET*, as a gene in which different mutations lead to different diseases, is a good example of allelic heterogeneity. On the other hand, in some diseases *RET* plays a role in only a proportion of the cases and other, yet unidentified, genes account for the remaining cases. Therefore, *RET* is also a good example of non-allelic heterogeneity.