The RET gene and its associated diseases
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
1995

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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The human protein kinase RET

Transfection studies using DNA from human T cell lymphoma led to the isolation of a transforming gene, designated RET (REarranged during Transfection), which consisted of two sequences linked in vitro, due to cointegration during transfection (Takahashi & Cooper, 1985). Similar results were obtained when DNA from human colon cancer (RET-II) (Ishizaka et al., 1988) and stomach cancer (Kuneida et al., 1991) was used. The 3’ half of these transforming genes were identical, whereas the 5’ parts were different.

2.1 RET sequence and gene structure

Using the 3’ part of the rearranged RET genes as a probe, cDNAs of the RET gene could be isolated and their sequences could be determined (Takahashi et al., 1988; Takahashi et al., 1989; Itoh et al., 1992). As the reports published show only parts of the sequence, confusion about the correct numbering of nucleotides and amino acids has occurred. In Appendix 1 the respective sequences have been combined to represent the full length cDNA sequence of both isoforms. The derived amino acid sequence of RET, the RET gene protein product, is also given. The indicated numbering is used throughout this thesis.

Several approaches have been applied to determine the genomic structure. Kwok et al. (1993) used exon trapping. Ceccherini et al. (1993) sequenced cloned PCR products and cosmid subclones. The cDNA sequences turned out to be spread over 20 exons (Figure 1). Appendix 2 shows the intron-exon junctions found. Expression studies, however, showed that the gene is expressed in at least two different isoforms, coding for proteins of 1072 and 1114 amino acids, respectively. They differ in their last exon, which in the short form codes for 9 amino acids, in the long form for 51 amino acids. These isoforms are the result of alternative splicing involving the last two exons (Tahira et al., 1990). A recent report (Xing et al., 1994) showed that alternative splicing can also occur in intron 4, as demonstrated by the detection of two different splice forms, one with an insertion of 62 base pairs, the other with an insertion of 69 base pairs between the exons 4 and 5. These two isoform transcripts are present in lower amounts than the transcript without an insertion between exons 4 and 5. Whether these isoforms are also translated remains to be determined.

The RET gene was localized on chromosome 10 (Donghi et al., 1989; Ishizaka et al., 1989). Further genetic and physical mapping refined the location of the RET gene and its linked markers (Norum et al., 1990; Brook-Wilson et al., 1993; Gardner et al., 1993;
Figure 1. Map of the centromeric region of chromosome 10 giving the order of a number of loci including RET (Hofstra et al., in press), the subregion where the RET gene is located and a diagram of the intron-exon structure of RET (Ceccherini et al., 1993, Pasini et al., submitted).
Mole et al., 1993, Lairmore et al., 1993; Hofstra et al., in press). In a collaborative effort we cloned and physically characterized a 150 kb region around RET (Pasini et al., submitted). It could be demonstrated that the gene is spread over a minimum distance of 55 kb, in EcoRI fragments of 68 kb. The gene contains a putative CA repeat in intron 5 and is flanked by two other CA repeats (Pasini et al., submitted) (Figure 1).

2.2 RET protein structure

From the cDNA sequence it could be inferred that the RET gene product, RET, is a cell surface protein belonging to the family of protein kinases, more specifically to the receptor tyrosine kinases (Takahashi et al., 1985; Takahashi & Cooper, 1987). The extracellular domain of RET has no homology with other receptor tyrosine kinases (Takahashi et al., 1988; Takahashi et al., 1989).

Figure 2. Schematic representation of the RET protein.
It contains a cleavable signal sequence of 28 amino acids, as well as a conserved cysteine-rich region close to the cell membrane and a cadherin-like region more toward the amino terminus (Schneider, 1992; Iwamoto et al., 1993; Kuma et al, 1993). A single transmembrane domain is followed by an evolutionarily conserved tyrosine kinase domain (Takahashi et al., 1988) interrupted by an inter-tyrosine kinase region of 27 amino acids. Similarities have been found between the tyrosine kinase domains of RET and those of the subfamily of platelet-derived growth factor receptors (Hanks, 1988).

2.3 Expression of the RET gene

As already mentioned, the RET protein is expressed in two isoforms of 1072 and 1114 amino acids, differing from each other in their 9 and 51 carboxy-terminal amino acids, respectively, due to alternative splicing involving the last two exons of RET (Tahira et al., 1988). Upon Northern blot analysis, this causes five different bands representing transcript sizes of 7.0, 6.0, 4.6, 4.5 and 3.9 kb (Tahira et al., 1990). Expression studies of RET in normal adult rat tissue showed very low levels of expression in lung, heart, spleen, and small intestine, whereas high levels of RET were observed in brain, thymus, and testis (Tahira et al., 1988). In developing mice it was shown that RET is expressed during specific phases and in specific tissues. In the early stages of embryonic development RET was found expressed in the excretory system, and in the peripheral and central nervous systems (Pachnis et al., 1993; Avantaggiato et al., 1994; Schuchardt et al., 1994). In agreement with this analysis, homozygous knock-out mouse showed intestinal aganglionosis and renal agenesis (Schuchardt et al., 1994). Until now little is known about the expression of the RET gene in adult human tissues. Only in the thyroid a low expression of the RET gene was detected (Santoro et al., 1990), due to expression in some but not all C cells (Fabien et al., 1994). Studies of human neoplasia showed that RET expression is mainly limited to some solid tumor types which derive from migrating neural crest cells, such as neuroblastoma (Ikeda et al., 1990; Nagao et al., 1990; Tahira et al., 1991; Takahashi et al., 1991; Hofstra et al., submitted [Appendix 6]), medullary thyroid carcinoma and pheochromocytoma (Santoro et al., 1990; Itoh et al., 1992; Miya et al., 1992).
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2.4 Function of the RET protein

The RET protein, being a tyrosine kinase receptor for a yet unidentified ligand, is thought to be involved in the signal transduction required for proliferation, migration, differentiation, and survival of neural crest cells as well as for kidney organogenesis (Pachnis et al., 1994; Schuchardt et al., 1994). It is not clear whether the RET protein can also function as an adhesion protein (Takahashi et al., 1993).

Preliminary studies on the RET signal transduction pathway revealed that the RET intracellular domain is able to bind and phosphorylate SHC adaptor, PLC-gamma, and possibly RAS-GAP associated proteins, and suggest the existence of a RET-specific mitogenic pathway (Borrello et al., 1994; Santoro et al., 1994a).

Whether the different isoforms differ in function is presently unclear, although preliminary data suggests that the expression of the two isoforms could be tissue-specific (Pachnis et al., 1993). Furthermore, they differ in their ability to bind certain factors (e.g. the GRB2 adaptor) of the signal transduction pathway (Borrello et al., 1994).