CHAPTER 2

Genetic Background of MEN1:
From Genetic Homogeneity to Functional Diversity

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Abstract

Multiple Endocrine Neoplasia Type 1 corresponds to a monogenic predisposition syndrome inherited as a dominant trait that affects a variety of endocrine tissues, in particular parathyroids, endocrine pancreas and anterior pituitary. It is caused by mutations in the MEN1 tumor suppressor gene that inactivate menin, the MEN1 encoded protein. Menin is involved in cell cycle control and apoptosis through its participation in functional dynamics of chromatin and regulation of transcription. In addition, genetic investigations have implicated menin in the maintenance of genomic integrity. However, the role of menin does not—by far—end here. It plays (too) many roles in the control of cell life and normality, far beyond endocrine oncogenesis, making it unlikely that the function of menin can be deciphered only by genetic investigation. In this context, writing a chapter on the genetic background of MEN1 appears at the same time as a challenge and a paradox. A challenge as everything has been either already written on the topic or included in the present book. A paradox since genetics is simultaneously at the background and at the forefront of MEN1. Our attempts are thus more investigating new—as well as already open issues than delivering a catalog of MEN1 gene mutations.

Introduction: The History of a Rare Endocrine Genetic Disease

Initially, multiple endocrine neoplasia Type 1 (MEN1, OMIM 131100), then named multiple endocrine adenomatosis was described by Paul Wermer as a syndrome affecting the anterior pituitary, the parathyroids and the pancreatic islets in a family in which it was assumed to be "caused by a dominant autosomal gene with a high degree of penetrance". Larsson et al have compared constitutional and tumor tissue genotypes of MEN1-related insulinnomas and showed "that oncogenesis in these cases involves unmasking of a recessive mutation at (the MEN1) locus". Hence, it became clear that the mutations that cause MEN1 are recessive although the syndrome is inherited as an autosomal dominant trait, similarly to most cancer predisposition genes. Despite an extensive variability in the expression of MEN1 between and within MEN1 families, genetic linkage could not evidence any genetic heterogeneity. The one MEN1 gene was mapped in 1988 to the long arm of chromosome 11 (11q13) by family studies and found tightly linked the PYGM gene. It turned out that it is only 70 kb telomeric to this gene that MEN1 was identified by positional cloning ten years latter (Fig. 1).

The MEN1 gene, as it is known today, consists of 10 exons, spanning 9 kb of genomic sequence and encoding a nuclear protein of 610 amino acids (menin). Menin does not reveal homologies to any other known protein. Protein sequence conservation indicates that MEN1 is present throughout the animal world, from mollusks to Homo sapiens (Fig. 2). However, its functions might have

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evolved differently in human/mammals and other species. In fact, while it has been possible to generate homozygous mutations in Drosophila, the double knock-out of Men1 in the mouse is lethal early during embryogenesis. In addition, no homozygous mutation has ever been found in humans either in families where consanguinity was suspected or even in a family where two affected children had received a defective allele from both parents.

The menin protein and its multiple partners are detailed in other chapters of this book. From a genetic point of view, menins complex partnership network might open the way to multiple tumor pathways in which those partners are being involved. Various mutations could affect the genes driving those pathways, although probably not as bona fide MEN1 alternative causes since no genetic heterogeneity has been observed in MEN1 families. Nevertheless, those genes might contribute to a fine tuning of MEN1 expressivity (gene modifiers).

**On the Nature of the MEN1 Gene**

An interspecies genomic comparison in the immediate vicinity of the MEN1 gene has not revealed conserved features apart from the sequences encoding menin. In vertebrates a MAP kinase gene, MAP4K2, is located immediately 3' of MEN1 (Fig. 1). This genomic organization is not conserved in Drosophila. The intergenic region 5' of MEN1 is seemingly not conserved. However, it is covered by repetitive elements both in man and in mouse over large distances (≥12 kb, Figs. 2, 3). These features developed independently since the repetitive elements, SINE/Alu in man and SINE/B2 in the mouse, are paralogs. SINE B2 elements can contain RNA polymerase II promoters and repetitive elements may provide tissue-specific regulatory elements for promoter activity. Alu sequences have recently been shown as the most reliable hallmarks for housekeeping genes. Similarly, transcription factor binding sites have been found in Alu sequences that may be associated with early markers of development. In a genome-wide comparison, a correlation between the mouse B1 and human Alu densities within the corresponding upstream regions of orthologous genes has been observed, as it is the case for the MEN1 gene.

Northern blot analysis showed two RNA species (2.9 and 4.2 kb in size) in thymus and pancreas, two target tissues for MEN1. We therefore suspected that MEN1 might exist in more than one splice variant. By 3'-RACE only a single product was obtained, consistent with the theoretical polyadenylation site found in MEN1. 5' RACE gave a completely different picture. Different splice isoforms were detected, which hooked 6 alternative exons 1 (named a to f) to the first coding exon, exon 2 (Fig. 3). None of the possible exons 1 seems to alter the open reading frame. However, the possibility exists that they could interfere differently with the translation of menin and/or that they correspond to some sort of tissue specificity. Accordingly, nuclease protection assays revealed the splice forms were differently distributed in different cell lines (Lovisa Bylund, personal communication). For the shortest and apparently most abundant transcript containing e1b (as well as, probably e1a and e1c), the transcription initiation site was determined and was identical to the corresponding site in the mouse. For e1e and e1f, the 5' ends have not been determined, but would likely be located in the highly repetitive region. No probes could be developed to determine

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**Figure 1.** Genomic environment of the MEN1 gene in the human genome. The information schematized here has been recovered from: http://www.ensembl.org/Homo_sapiens/contigview?region=11&vc_start=64250000&vc_end=64370000.