Thyroid defects due to Pax8 gene mutations

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Introduction

Congenital hypothyroidism (CH) occurs in 1 in 3000–4000 newborns. Early diagnosis and therapy are absolutely critical because, when untreated, hypothyroid subjects develop severe and irreversible mental retardation. Thyroid dysgenesis (agenesis, ectopic location or hypoplasia) is the most common cause of CH. Tissue-specific transcription factors play a major role in organogenesis and cell differentiation. Therefore, defects of thyroid-specific transcription factors may be responsible for thyroid dysgenesis. Three transcription factors whose expression is restricted to the thyroid follicular cell (TFC) and few other cell types have been identified so far: the thyroid transcription factors-1 and -2 (TTF-1 and TTF-2) and Pax8 (1–2). In addition to the developing and mature thyroid gland, TTF-1 is expressed in lung and in several regions of developing brain (3), TTF-2 in the Rathke's pouch (2) and Pax8 in developing kidney (4). Recent data, obtained in mice by the gene inactivation technique, have demonstrated the importance of TTF-1, TTF-2 and Pax8 in thyroid organogenesis (5–7) (Fig. 1). As expected, the search for mutations of genes coding for TTF-1, TTF-2 and Pax8 in subjects with thyroid dysgenesis has revealed that defects in these factors are responsible for several cases of CH (8–11).

TTF-1 gene inactivation in mice, in addition to severe defects in lung and forebrain, gives rise to absence of the thyroid gland, indicating a critical role of this factor in early events of organogenesis (5). The lung defects are not compatible with life; this is probably why no CH due to TTF-1 gene mutations has been found in humans (8). Recently, however, a subject in which a heterozygous deletion of TTF-1 gene was associated with respiratory failure and raised serum thyrotropin (TSH) concentration was described (9).

TTF-2 gene inactivation has revealed that this factor is absolutely required for the downward migration of the thyroid gland primordium which occurs from 9.5 to 15.5 days post-conception (p.c.), as well as for palate closure (6). Newborn mice show either ectopy or absence of the thyroid gland, associated with cleft palate. In accord, a family in which thyroid agenesis, cleft palate and choanal atresia are associated with homozygous TTF-2 gene mutation has been recently described (10).

Pax8 gene mutations elicit also thyroid defects in both mice and humans. The reported data, however, reveal a complex picture suggestive for future development in the field, therefore deserving a detailed comment.

Pax8 gene mutations

Pax8 is a member of a family of transcriptional regulators that control differentiation of several cell types (12). In the mature TFC, Pax8 regulates the expression of thyroglobulin (Tg) and thyroperoxidase (TPO) genes (13). The role that this factor plays in thyroid development has been recently demonstrated by gene targeting (7). In fact, Pax8 knock-out mice show a smaller thyroid gland than control animals, with a complete absence of follicular structures. Immunohistochemistry demonstrates that the thyroid of Pax8 null mice is composed almost completely of calcitonin-producing cells. In these mutants, though a TTF-1-expressing thyroid diverticulum is detected up to 11.5 days p.c., no TFC are present at later stages. Therefore, the absence of Pax8 is still compatible with very early stages of thyroid development (appearance of the thyroid diverticulum from endodermal cells of the primitive pharynx), but precludes further differentiation events leading to the mature TFC.

These findings, however, only partially fit with data from thyroid diseases in humans (11). Pax8-inactivating mutations have been found in two sporadic cases and in one family affected with CH. The observed mutations consist of either missense or nonsense nucleotide changes, abolishing the capability for specific DNA recognition. Thyroid hypoplasia was present in all patients bearing the mutation, while heterogeneity was detected in terms of serum TSH, thyroxine and Tg concentrations. Such a variable expressivity (the extent to which a genetic defect is expressed in a single subject) is not related to the type of mutation, since it is present also among subjects of the same family. The heterogeneity of phenotypes is a common feature for diseases due to Pax gene mutations. In fact, mutations of the Pax2 gene elicit different degrees of renal and ocular abnormalities (14), the phenotypes of Waardenburg syndrome (due to Pax3 mutations) are highly variable even within families (15), and mutations of Pax6 can variably cause aniridia, Peters’ anomaly (congenital corneal opacity) or simple cataracts in heterozygotes without any obvious correlate with the molecular change (16).
Though Pax8 gene inactivation gives rise to thyroid abnormalities in both mouse and human, a striking difference between the two species has also been noticed. The thyroid abnormalities in humans are always detected in the heterozygous state, whereas, in mice, only homozygous individuals show the thyroid defects. It could be likely that humans homozygous for Pax8-inactivating mutations may display a complete lack of TFC, similar to that observed in knock-out mice. Heterozygous mice do not display a clear abnormal phenotype. They are viable and fertile and show only more frequently elevated serum TSH concentrations (7). Therefore, heterozygous mutant mice are normal or may develop only mild hypothyroidism, whereas heterozygous humans display clear thyroid abnormalities (11). Why does the heterozygosity of Pax8 mutations elicit thyroid defects in humans but not in mice?

Several hypotheses can be put forward: (i) differences between mice and men; (ii) possibility of dominant-negative effects; and (iii) haploinsufficiency coupled to difference in genetic backgrounds.

Figure 1 Schematic view of thyroid development in mice and major effects of inactivation of thyroid-specific transcriptional factor genes. The effects of TTF-1, TTF-2 and Pax8 gene inactivation are described in references 5–7.
Mice and humans can differ in some aspect of development. For example, human subjects with the Waardenburg syndrome suffer from deafness, while heterozygous Splotch mice (carrying a Pax3-inactivating mutation) lack this defect. A difference between mice and humans could be related to the mono- or biallelic expression of the gene. Observations exist indicating that mice and humans diverge in mechanisms leading to expression of several genes. The insulin-like growth factor-II receptor gene has been studied in detail; it shows monoallelic expression in mice but biallelic in the human (17). Monoallelic expression of Pax5 in the mouse has been recently reported (18). Heterozygous subjects for a monoallelically expressed gene would have a higher possibility of displaying an abnormal phenotype than in the case of biallelic expression. Thus, it would be of interest to test whether Pax8 is expressed in TFC from one or two alleles.

(ii) In humans the mutant allele could exert a dominant-negative effect. In fact, the mutant allele of the Pax8 knock-out mouse is not able to generate any protein product. The mutated human alleles, in contrast, encode for full-length or truncated proteins which lack DNA-binding activity. These abnormal proteins could interact with products of the normal Pax8 allele, inactivating its function. However, dominant-negative effects have never been described for Pax proteins. In addition, one of the human Pax8 mutations consists of a premature stop codon that causes the synthesis of a very short protein. In this case, therefore, the existence of dominant-negative effects exerted by the mutant protein should be extremely unlikely.

(iii) The thyroid abnormalities detected in heterozygous Pax8 mutants could be due to a quantitative phenomenon called haploinsufficiency— the amount of Pax8 protein generated by a single normal allele is not sufficient to support the normal developmental program. From an evolutive point of view, this can occur in genes whose amount of protein product is so critical that new alleles with a higher degree of functionality (for example overproducing the protein) have a low fitness. Consistently, for the Pax6 gene, it has been demonstrated that overproduction of protein due to increasing gene dosage elicits eye abnormalities (19).

Several lines of evidence indicate that phenotypic effects of haploinsufficiency have a variable expressivity, and therefore could be very sensitive to the genetic background (20–24). Based on these considerations, the difference between mice and humans could be explained by hypothesizing that the genetic background of the mouse strains used for the Pax8 gene knock-out does not allow a strong expressivity of a single null allele. In contrast, the human study, focusing on patients with thyroid dysgenesis, is subject to an important bias – only cases with strong expressivity of the eventual Pax8 mutation have been investigated. The correctness of this hypothesis can be tested in both mice and humans. In mice, by investigating the effect of the Pax8 null mutation in different genetic backgrounds; in humans, by searching for Pax8 mutations in hypothyroid subjects without thyroid dysgenesis. Therefore, the prediction is that some cases of familial hypothyroidism with dominant inheritance, but without overt defects of thyroid development, would be due to Pax8 gene mutations. In accord, families with dominantly inherited hypothyroidism have been described (25, 26). Due to the variable expressivity, the penetrance (the frequency of expression of a mutated gene) of the genetic defect may be low. This would be compatible with a possible role for Pax-8 mutations not only in familial, but also in sporadic cases of hypothyroidism.

Finally, a different possibility does exist. It has been recently pointed out that Pax proteins could be contenders in a competition with other transcriptional regulators to occupy the same DNA sites (27). Pax8 provides a clear example of this phenomenon. At the level of both Tg and TPO promoters the Pax8 DNA site overlaps with that of TTF-1 and the binding of the two proteins is mutually exclusive (13). Competition between Pax proteins and other transcriptional regulators for common sites may easily explain the haploinsufficiency; a relatively small reduction of Pax protein levels would greatly perturb the binding equilibrium with the competitors. If competition explains the haploinsufficiency of Pax mutants, then the phenotype depends not just on the Pax gene but also on the competitor. Therefore, the identification of competitors of Pax proteins could reveal new genes responsible for CH. In fact, a large proportion of CH cases is still waiting for a molecular explanation.


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