Exploring the endocrine manifestations of DICER1 mutations

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The discovery of each new cancer susceptibility gene answers one set of questions but poses many more. In this article, we outline a recent example: a new cancer syndrome caused by germline mutations in DICER1, responsible for microRNA processing. In particular, we discuss the endocrine manifestations of mutations in this crucial gene.

Setting the scene
DICER1 is an endoribonuclease that processes hairpin precursor microRNAs (miRNAs) into short, functional miRNAs (~23mers). Mature 5’ miRNAs and other components of the RNA-induced silencing complex (RISC) down-regulate or silence targeted miRNAs [1]. Recently, germline mutations in DICER1 have been identified in children and young adults who exhibit distinctive dysontogenic hyperplastic or overtly malignant conditions; the most frequent and severe of these is the childhood lung tumor pleuropulmonary blastoma (PPB) [1,2]. An emerging feature of this syndrome is its predilection for disease arising in endocrine organs such as the thyroid, pituitary, and ovaries. We spotlight here the multiple endocrine manifestations of the DICER1 syndrome.

DICER1: the endocrine connection
Germline DICER1 mutations have been identified in an expanding range of conditions, which are summarized in Figure 1 [1]. In 1974, Fraumeni and colleagues identified a likely genetic link between families with ovarian Sertoli–Leydig cell tumors (SLCT) and nodular thyroid hyperplasia. International PPB Registry researchers have since described numerous conditions related to the hallmark PPB (Figure 1) and in 2009, led by Hill, these investigators identified the responsible gene [2]. Pituitary blastoma, an entity recognized only in 2008 by the late Bernd Scheithauer, is a third important endocrine phenotype linked to DICER1 mutations [3]. Finally, cervical rhabdomyosarcoma, also mutation-related, further suggests endocrine malfunction given the presentation of abnormal menses [1].

Clinical presentations related to DICER1 mutations

Multinodular goiter
Multinodular goiter (MNG) is characterized by nodular overgrowth of the thyroid gland, usually accompanied by diffuse parenchymal hyperplasia. The biochemical and genetic mechanisms underlying of many subtypes of thyroid goiter have been elucidated, but little is known about genetic factors underlying non-toxic goiter. Germ-line DICER1 mutations have been identified in several kindred with both MNG and SLCT or with familial MNG [1]. Although the penetrance for thyroid disease in affected kindreds is generally well below 50%, penetrance for MNG in some pedigrees was remarkably high – particularly for non-truncating mutations that affected the PAZ domain of DICER1. Follicular and papillary thyroid carcinomas are occasionally found in affected kindred, but germline DICER1 mutations were not found in DNA from 80 sporadic differentiated thyroid cancer cases (with or without MNG), suggesting that DICER1 mutations are rare outside of distinctive clinical contexts (Figure 1). Currently, no data address the underlying biology of DICER1-related goiter.

Ovarian sex cord-stromal tumors
Ovarian sex cord-stromal tumors, especially SLCT, are among the more frequent manifestations of DICER1 mutations. SLCT in these kindred are often associated with evidence of androgen excess and typically present in young adult women (but do occur in young girls). DICER1 is clearly implicated in the regulation of ovulation and is upregulated in the human fallopian tube such that steroid hormones are regulated by DICER1 in a manner dependent upon the phase of the menstrual cycle [4]. The importance of DICER1 mutation in SLCT is emphasized by characteristic, non-random somatic mutations in the RNAseIIIb domain of DICER1 in non-epithelial ovarian tumors, including 23 of 46 analyzed cases of SLCT. Intriguingly, these highly specific mutations affect cleavage of the 5’ mature miRNA strand [5]. Germline DNA was not studied in most of these cases, but 4 SLCT patients also carried
germline DICER1 mutations, suggesting that ‘two hits’ in DICER1 favor tumorigenesis (see below).

**Pituitary blastoma**

Pituitary blastoma (PitB) is a rare, recently-described primitive neoplasm noted in kindred with disease related to a DICER1 mutation [3]. Among 10 known PitB cases under study, germline DICER1 mutations have been found in 3 of 3 cases analyzed and a fourth child also had PPB and cystic nephroma [unpublished data]. PitB occurs before 24 months of age and presents with marked Cushing’s syndrome and/or ophthalmoplegia and ptosis. Elevated plasma adrenocorticotropic hormone (ACTH) and cortisol as well as urinary cortisol excretion are typical, whereas other tropic hormones levels are variable. Pathology reveals an uncontrolled proliferation of ACTH-staining follicular cells at an early stage of embryological differentiation. Approximately half of children with PitB die from the tumor; survivors require hormonal supplementation.

**Mouse models**

Dicer is essential for embryonic stem cell differentiation, but its diverse role in the development of endocrine organs has been elegantly elucidated in conditional knockout murine studies using Cre-lox recombination technology with tissue specific promoters. Loss of Dicer1 function in Pitx2Cre/Dicer1flx/+ mice results in hypoplastic anterior pituitary lobes and growth retardation with decreased levels of prolactin and growth and thyroid stimulating hormones. In this model, corticotropin/pro-opiomelanocortin expression was unaffected, suggesting that corticotrope lineage was relatively normal. Loss of a specific Dicer-dependent miRNA, miR-26b, in affected mice was associated with altered relative expression of key transcription factors Lef-1 and Pit1 known to affect anterior pituitary differentiation and hormonogenesis [6]. The relevance of this model to human PitB is unknown, especially as the effect of germline DICER1 mutations seems to result in neoplasia rather than hypoplasia.

In the Dicer1-disabled anti-Mullerian hormone receptor type 2-directed Cre (Amhr2-Cre) mutant mice, female Amhr2-Cre/Dicer1flx/flx mice are sterile due to abnormalities of the reproductive tract, with reduced uterine size, development of paratubal cysts, and increased follicular atresia [7]. These data demonstrate a potent role for Dicer1 in endocrine organogenesis but, as with the pituitary studies, do not account for the neoplastic phenotype of constitutional DICER1 mutations observed in humans. There are no conditional Dicer1 knockout murine models of goiter. Overall, many animal models, often using conditional Dicer1 knockout, support the importance of Dicer1 in embryology and early development of many organs, including the tissues affected by DICER1-related disease in humans.

A recent study using the Amhr2-Cre/Dicer1flx/flx system discussed above showed that complete Dicer1 loss, combined with complete PTEN loss in the same Mullerian-
derived cells (double knockout), leads to high-grade serous carcinoma of the fallopian tube [8]. This challenges the previous view that complete loss of Dicer1 is lethal to tumor cells [9] (discussed below) and provides a model for how specific combinations of knocked-out tumor suppressor genes, in combination with DICER1, might lead to specific tumors.

DICER1 and tumor suppression in humans: typical or atypical?

Initial mouse models had indicated that Dicer1 acts as a haploinsufficient tumor suppressor gene [9], but the recent studies in humans do not suggest that this model can explain the effect of DICER1 loss in humans because in all cases where DICER1 sequencing has been completed, the first inactivating hit, whether germline or somatic, is accompanied by a second hit (in nearly all cases) in the RNaseIIIb domain of DICER1, most particularly at nucleotides encoding amino acids Asp1709, but also those encoding Glu1813 [5]. These highly specific somatic mutations could be null mutations, but it seems more plausible that they result in an altered miRNA landscape. Nevertheless, recent data using altered versions of human DICER1 show that the mutations at the highly conserved amino acid Asp1709 do indeed result in complete loss of 5′ mature miRNAs (which are transported by Argonaute and other proteins to their target miRNAs), whereas the 3′ mature miRNAs are only partially reduced in expression. Interestingly, mutations at conserved amino acids in the companion RNaseIIIa domain have the reverse effect [10]. So perhaps the classical two-hit model is not so far wide of the mark. Two events are indeed required: the first hit to DICER1 reduces by half the amount of wild-type DICER1 protein by completely inactivating one copy and the second hit then specifically knocks out production of 5′ mature miRNAs. Although DICER1 loss is not complete, there is a total loss of mature 5′ miRNAs in certain cell types and at certain times. This may lead to the rare ‘blastoma’-like tumors that are seen in families carrying inactivating mutations in DICER1. Where goiter fits into this story is of particular interest because such multinodular goiters are hyperplasias, not tumors. Whether the effect of decreased miRNAs on thyroid development is a local effect owing to loss or altered miRNA production (i.e., within the thyroid itself, such as occurs in defects of iodination) or is initiated by the distant and systemic effects of miRNA perturbation [such as increased thyroid stimulating hormone (TSH) leading to Graves’ disease] is currently unknown. A list of DICER1 sequence variants is available at the Leiden Open Variation Database (https://grenada.lumc.nl/LOVD2/mendelian_genes/home.php?select_db= DICER1).

The clinical context

Although non-endocrine tumors predominate in the DICER1 syndrome (Figure 1), a multiple endocrine neoplasia phenotype is associated with this autosomal dominant familial cancer syndrome. Presentation of an infant with Cushing’s disease, an adolescent or young adult with multinodular goiter, or a female with a sex steroid secreting tumor, especially in the context of a family history of pulmonary and other related disorders (Figure 1), should alert the endocrinologist to the possibility of the DICER1 syndrome. Notably, PPB can be a life-threatening disease, so early recognition and prompt molecular diagnosis could have clinical benefits beyond the endocrine sphere.

Next steps

Germline DICER1 mutations are associated with an unusual array of primarily early-onset neoplastic, hyperplastic, and dysontogenic phenotypes, with a predilection for the lung, the kidney, endocrine organs, and other rare sites (Figure 1). Characterisation of conditions associated with germline DICER1 mutations is well under way, and with the availability of next-generation sequencing rarer manifestations of the DICER1 syndrome will soon be discovered. The prevalence of DICER1 mutations in most of the associated disorders remains to be established. Thus, the focus now extends to understanding exactly how inactivating this crucial enzyme has such specific effects. In particular, understanding the significance for miRNA production of the precise positioning of different non-truncating germline and somatic DICER1 mutations will require further study. Moreover, what other genetic and epigenetic alterations accompany DICER1 mutations, and in what way to do they help to determine the observed phenotypes? Future studies investigating DICER1 dysfunction may lead to improved diagnostic methods and may provide insight into a new mechanism of disease based on perturbed miRNA metabolism, which could underpin an emerging field of miRNA therapeutics.

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References

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