



Published in final edited form as:

Science. 2009 August 21; 325(5943): 965. doi:10.1126/science.1174334.

Germline *DICER1* Mutations in Familial Pleuropulmonary Blastoma

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Pleuropulmonary blastoma (PPB) is a rare pediatric tumor of the lung that arises during fetal and infant lung development and is part of an inherited cancer syndrome (OMIM entry 601200). PPBs are composed of both epithelial and mesenchymal components. The early stage of disease features cystic expansion of the airspaces lined by benign-appearing epithelium. Mesenchymal cells susceptible to malignant transformation reside within the cyst walls and form a dense layer beneath the lining epithelium known as the cambium layer. Sarcomatous overgrowth by the mesenchymal cells growing as solid masses dictates clinical outcome for children with this tumor, however, not all cystic PPBs are destined to progress to sarcomas (1). The natural history of the tumor suggests a multi-step genetic pathogenesis (2).

Approximately 20% of children with PPB have a family history of pediatric neoplasia, most notably cystic nephroma of the kidney and rhabdomyosarcoma (3). We mapped the PPB locus to chromosome 14q with a family-based linkage study on four families with inherited predisposition to PPB (4) (Fig. S1, S2). Of 72 genes within the 7 Mb candidate region, *DICER1*, was an attractive candidate because of its known role in lung development. *DICER1* encodes a ~218 kDa RNase III endonuclease that is a key component of a highly conserved cellular pathway responsible for generation of small RNAs (miRNAs and siRNAs) (5). miRNAs negatively regulate gene expression and appear to play critical roles in stem cell maintenance, organogenesis, cell cycle progression, and oncogenesis. Conditional loss of *Dicer1* in the developing mouse lung results in cystic airway dilation, disruption of branching morphogenesis and mesenchymal expansion that resembles the early stage of PPB (6).

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Supporting Online Material

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Materials and Methods

Supplemental Text

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Heterozygous germline mutations in *DICER1* were identified by direct sequencing in affected members from each of 11 families (four included in the linkage study and seven additional families) (Fig. 1A, Fig. S3, Table S1). For ten of the 11 PPB families, the observed mutations result in proteins truncated proximal to *DICER1*'s two carboxy-terminal RNase III functional domains (Fig. 1B) and therefore are likely loss of function defects. The missense mutation (L1583R) detected in the 11th family (family C) involves an evolutionary conserved amino acid and the non-polar to polar change was neither a previously reported sequence variant (NCBI SNP database Build 128) nor was it detected in 360 cancer-free controls tested by Pyrosequencing.TM

Apart from PPB-associated tumors in a subset of family members, the majority of obligate carriers with *DICER1* mutations are phenotypically normal indicating that loss of one *DICER1* allele is compatible with normal development and insufficient for tumor formation. Consistent with this observation, mice haploinsufficient for *Dicer1* show no overt phenotypic abnormalities (7). *DICER1* immunohistochemistry of PPB tumors suggests expression from the wild-type allele is lost in tumor-associated epithelium in six of the seven families harboring PPBs with a residual epithelial cystic component, but is retained in the mesenchymal tumor cells (Fig. 1C, Fig. S5). The presence of inactivating missense mutations in mesenchymal tumor cells from all 10 families assayed is considered unlikely. The areas of absent staining in the tumoral epithelium were segmental in most cases but were clearly evident in areas overlying cambium layers. The genetic basis behind this altered expression in epithelium is currently unknown but the phenotype recapitulates that seen in the *Dicer1*^{-/-} conditional mouse model. Interestingly, the tumoral epithelial cells lacking *DICER1* protein expression were morphologically normally differentiated.

Based on our preliminary studies, we hypothesize that PPB is initiated through a novel mechanism of non-cell autonomous cancer initiation and speculate that loss of *DICER1* in the epithelium of the developing lung adversely impacts production of diffusible factors that regulate mesenchymal growth and predisposes these cells to additional genetic aberrations. If, or how, *DICER1* haploinsufficiency in the mesenchymal cells contributes to pathogenesis remains an open question. Given that hereditary PPB is associated with an increased risk for development of other more common malignancies, *DICER1*-dependent tumor suppressive mechanisms uncovered in PPB may also apply to other more common cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

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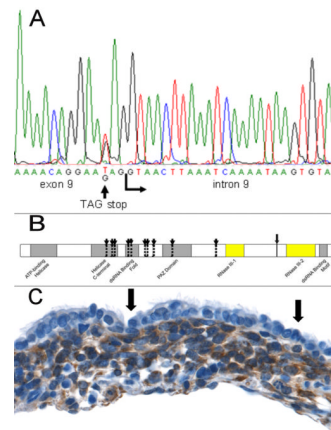


Figure 1. *DICER1* mutations and expression in PPB

(A) *DICER1* sequence alteration identified in one of the families in the linkage study. (B) Protein locations of *DICER1* mutations in 11 PPB families. Vertical dotted lines with arrows indicate the location of truncating or insertions/deletions in 10 families. Each of these occurs proximal to the RNase III domains. The larger arrow with the solid line marks the location of the missense mutation between the RNase III domains. (C) *DICER1* protein is absent in benign-appearing tumor-associated epithelium (arrows) and is present in the mesenchymal tumor cells forming the cambium layer underneath (anti-*DICER1* with brown chromagen and hematoxylin counterstain; original magnification x400).