Notch signaling and inherited disease syndromes

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The Notch signaling pathway is an evolutionarily conserved, intercellular signaling mechanism essential for proper embryonic development in organisms as diverse as insects, nematodes, echinoderms and mammals. Disruptions in conserved developmental pathways frequently result in inherited congenital anomalies in humans. Mutations in genes encoding Notch pathway components underlie three inherited human diseases: Alagille syndrome, spondylocostal dysostosis, and cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. Mouse models for these three diseases have been developed, and are leading to novel insights into the pathology of these diseases in humans.

INTRODUCTION

The Notch signaling pathway is an evolutionarily conserved, intercellular signaling mechanism essential for proper embryonic development in all metazoan organisms in the animal kingdom. This review will summarize studies demonstrating that perturbations in the Notch signaling pathway contribute to the pathogenesis of several inherited human diseases. Perturbations in Notch signaling also contribute to the formation of lymphoid malignancies (for a review see 1). Biochemical aspects of Notch signaling have been the subject of numerous excellent reviews (2–8), so only a brief summary of how the Notch pathway functions in mammals will be presented here.

NOTCH SIGNALING PATHWAY

Genes of the Notch family encode large single-pass transmembrane proteins (Fig. 1). In mammals, four Notch family receptors have been described: NOTCH1–NOTCH4. The extracellular domain of Notch family proteins contains a large number of tandemly repeated copies of an epidermal growth factor (EGF)-like motif. A Notch family receptor exists at the cell surface as a proteolytically cleaved product consisting of a large ectodomain and a membrane-tethered intracellular domain. These products remain associated at the cell surface as a heterodimer through non-covalent, calcium-dependent interactions. Notch receptors interact with membrane-bound ligands that, in mammals, are encoded by the Jagged (JAG1 and JAG2) and Delta-like (DLL1, DLL3 and DLL4) gene families. The Notch ligands are also single-pass transmembrane proteins that contain multiple EGF-like repeats in their extracellular domains.

The signal induced by ligand binding is transmitted intracellularly by a process involving proteolytic cleavage of the receptor and nuclear translocation of the intracellular domain of the Notch family protein. The receptor/ligand interaction induces two additional proteolytic cleavages that free the intracellular domain of the Notch receptor from the cell membrane. The cleaved fragment translocates to the nucleus due to the presence of nuclear localization signals located in the Notch intracellular domain. Once in the nucleus, the Notch intracellular domain forms a complex with the RBPSUH protein, a sequence-specific DNA binding protein (also known in mammals as CSL, CBF1 and RBP-J). In the absence of Notch signaling, the RBPSUH protein binds to specific DNA sequences in the regulatory elements of various target genes and represses transcription by recruiting histone deacetylases and other components of a corepressor complex. Nuclear translocation of the Notch intracellular domain displaces the histone deacetylase/corepressor complex from the RBPSUH protein, leading to the transcriptional activation of Notch target genes. For a more detailed view of the complexities of the Notch signaling pathway and for references to the primary literature, refer to previous reviews (2–8).

ALAGILLE SYNDROME

Alagille syndrome (AGS; OMIM 118450) is an autosomal dominant disorder characterized by developmental abnormalities of the liver, heart, eye, skeleton and, at lower penetrance, several other organs (9,10). AGS patients typically present with neonatal jaundice and cholestasis resulting from a paucity of intrahepatic bile ducts. Accompanying features of AGS include cardiac defects (including pulmonary artery stenosis and hypoplasia, pulmonic valve stenosis and tetralogy of Fallot), skeletal defects (primarily butterfly vertebrae), ophthalmological

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The receptor consisting of a large ectodomain and a membrane-tethered intracellular domain. The Notch receptor exists at the cell surface as a proteolytically cleaved product (NOTCH1 cell, shown in green) interact with Notch family transmembrane receptors Jagged (JAG1 and JAG2) and Delta-like (DLL1, DLL3, DLL4) families (upper activation of Notch target genes.

The cleaved fragment translocates to the nucleus (shown in blue) owing to the presence of nuclear localization signals located in the Notch intracellular domain. Once in the nucleus, the Notch intracellular domain forms a complex with the RBPSUH protein, displacing a histone deacetylase (HDAC)/corepressor (CoR) complex from the RBPSUH protein, leading to the transcriptional activation of Notch target genes.

abnormalities (primarily anterior chamber defects such as posterior embryotoxon), a characteristic facial appearance, renal and pancreatic abnormalities, and intracranial bleeding (9,11–14). Newly reported AGS phenotypes include craniosynostosis (15) and digit abnormalities (16).

Positional cloning studies revealed that AGS is caused by mutations in the Jagged (JAG1 and JAG2) and Delta-like (DLL1, DLL3, DLL4) families (upper cell, shown in green) interact with Notch family transmembrane receptors (NOTCH1–NOTCH4) on an adjacent cell (lower cell, shown in yellow). The Notch receptor exists at the cell surface as a proteolytically cleaved product consisting of a large ectodomain and a membrane-tethered intracellular domain. The receptor–ligand interaction induces two additional proteolytic cleavages that free the intracellular domain of the Notch receptor from the cell membrane.

Figure 1. Simplified overview of Notch signaling in mammals. Ligands of the Jagged (JAG1 and JAG2) and Delta-like (DLL1, DLL3, DLL4) families (upper cell, shown in green) interact with Notch family transmembrane receptors (NOTCH1–NOTCH4) on an adjacent cell (lower cell, shown in yellow). The Notch receptor exists at the cell surface as a proteolytically cleaved product consisting of a large ectodomain and a membrane-tethered intracellular domain. The receptor–ligand interaction induces two additional proteolytic cleavages that free the intracellular domain of the Notch receptor from the cell membrane. The cleaved fragment translocates to the nucleus (shown in blue) owing to the presence of nuclear localization signals located in the Notch intracellular domain. Once in the nucleus, the Notch intracellular domain forms a complex with the RBPSUH protein, displacing a histone deacetylase (HDAC)/corepressor (CoR) complex from the RBPSUH protein, leading to the transcriptional activation of Notch target genes.

mutations in the Jagged1 (JAG1) gene (17,18). An extensive survey of the types and frequency of JAG1 mutations in AGS patients revealed that 72% led to premature termination codons, 15% were splice site mutations and 13% were missense mutations (19). Three to seven percent of AGS patients have mutations (19). Three to seven percent of AGS patients have mutations (19). Three to seven percent of AGS patients have mutations (19). Three to seven percent of AGS patients have mutations (19).

JAG1 missense mutations that were studied in detail resulted in frequent near the amino-terminus of the protein. Two missense mutations that were studied in detail resulted in defective intracellular transport and processing of the mutant JAG1 protein. The mutant proteins were abnormally glycosylated and were not present on the cell surface, further supporting functional haploinsufficiency as an AGS disease mechanism (20).

AGS exhibits high penetrance but extremely variable expressivity, even within family members carrying identical JAG1 mutations (9–11). Possible explanations for this variable expressivity include the existence of either genetic or environmental (i.e. non-genetic) modifiers. Kamath and colleagues recently reported a clear example of non-genetic modification in a case of monozygotic twins with discordant AGS phenotypes (21). One twin had severe pulmonary atresia and mild liver disease, while the other twin had tetralogy of Fallot and severe cholestatic liver disease that required transplantation. The existence of genetic modifiers is supported by studies with mouse models described below.

Since the identification of JAG1 as the AGS gene, it has become apparent that the majority of JAG1 mutation carriers within a family do not meet the clinical criteria for diagnosis of AGS (10,22). JAG1 mutations can cause disease of only one or a few organ systems, without accompanying defects in the liver or most of the other tissues typically affected in AGS patients.

More than 90% of individuals with JAG1 mutations exhibit cardiovascular abnormalities, which may or may not be associated with anomalies in other organ systems (14). Examples include multigenenational pulmonary stenosis due to a JAG1 missense mutation, and a case of tetralogy of Fallot and hypoplastic pulmonary arteries due to JAG1 gene deletion (12). In another study, individuals in a large kindred segregating tetralogy of Fallot as an autosomal dominant trait were found to have a JAG1 missense mutation (13). Another kindred with a JAG1 missense mutation exhibited hearing loss, inner ear vestibular defects, congenital heart defects and posterior embryotoxon (23). Vestibular defects of the inner ear also have been demonstrated in two independent Jag1 missense mutant mice identified in large scale mutagenesis screens (24,25).

My laboratory has been characterizing mouse AGS models generated by gene targeting. Mice homozygous for a targeted null mutation of the Jag1 gene die in utero due to cardiovascular defects in the embryo and the yolk sac (26). Mice heterozygous for the Jag1 mutation, whose genotype mimics that of human AGS patients, proved to be a disappointing animal model for this disease. The Jag1+/− heterozygous mice exhibited anterior chamber eye defects, but did not exhibit other phenotypes associated with AGS in humans (26). However, mice doubly heterozygous for the Jag1 null allele and a Notch2 hypomorphic allele (27) reproduced most of the clinically relevant phenotypes observed in AGS patients. These mice (designated J1N2+/−) exhibited jaundice, growth retardation and bile duct, heart, eye and kidney abnormalities that were similar or identical to the abnormalities observed in AGS patients (28).

Mice homozygous for a targeted mutation of the Hey2 gene, which encodes a basic helix–loop–helix transcription factor that is a downstream Notch target gene (29,30), exhibited cardiac defects similar to those observed in J1N2+/− mice, suggesting that the Hey2 gene mediates Notch signaling in the developing heart (31,32).
AGS exhibits high penetrance but extremely variable expressivity. A possible explanation for this variable expressivity is the existence of genetic modifiers of the disease phenotype in the human population. Our mouse models demonstrate that the Notch2 gene acts as a genetic modifier to interact with a Jag1 mutation to create a more representative model for AGS (28). We hypothesize that similar genetic interactions may occur in human AGS patients, and that particular NOTCH2 alleles may influence the severity of AGS phenotypes. Preliminary results from my laboratory indicate that the Jag1 mutant can interact with mouse mutants in additional Notch pathway components to recreate phenotypes similar to those observed in AGS patients. Jag1 mutations have been found in only 70% of AGS patients (19). It is not clear at present whether the remaining patients have unidentified Jag1 mutations (perhaps in regulatory regions of the gene), or whether they have mutations in other genes. Our mouse models suggest that genes encoding other components of the Notch signaling pathway are candidates for additional AGS disease genes or modifiers.

SPONDYLOCOSTAL DYSTOSIS

In spondylocostal dysostosis (SD), vertebral segmentation defects are associated with rib anomalies. SD patients exhibit short trunk dwarfism due to multiple hemivertebrae accompanied by rib fusions and deletions, and both autosomal dominant and autosomal-recessive modes of inheritance have been reported (OMIM 122600) (33). One form of autosomal-recessive SD was mapped to chromosome 19q13.1 (34). Two of the identified mutations were predicted to cause protein truncations, while the third mutation caused a missense mutation in a highly conserved amino acid residue of the DLL3 protein (35). The phenotype exhibited by these patients is very similar to the phenotype exhibited by mice homozygous for the prudy mutation, a spontaneous mutation of theDll3 gene (36). The Dll3

mutant allele has a 4 bp deletion that results in a frameshift and premature protein truncation. Comparison of the phenotypes of embryos homozygous for the Dll3

mutant with embryos homozygous for a Dll3 targeted mutation indicated that both were Dll3 null alleles (37). The similarities between the Dll3 mutant mice and human SD patients suggest that the human SD mutant alleles also result in loss of Dll3 function.

The Notch signaling pathway plays a major role in regulating somite formation and in partitioning somites into anterior and posterior compartments (38). Notch pathway components appear to regulate, or constitute essential components of, a cell autonomous oscillator functioning in the presomitic mesoderm. This oscillator has been termed the somite clock (38). Analysis of marker gene expression inDll3 mutant mice indicates that progression of the somite clock is disrupted in these mutants (37,39). A similar mechanism probably underlies the vertebral defects observed in SD patients.

Autosomal recessive SD is a genetically heterogeneous condition. For example, one family segregating autosomal-recessive SD did not show evidence of linkage to 19q13, where the Dll3 gene is located (40). Studies of mouse models have shown that mice homozygous for a null mutation of the Lunatic fringe (Lfng) gene, which encodes a glycosyltransferase that modulates Notch signal transduction (41,42), exhibit axial truncation defects and rib fusions very similar to those observed in Dll3 mutant mice and in SD patients (39,43,44). This suggests that Lfng is another candidate gene for autosomal-recessive SD.

CADASIL

CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy; OMIM 125310) is an autosomal-dominant vascular disorder. Affected individuals exhibit a variety of symptoms, including migraine with aura, mood disorders, recurrent subcortical ischemic strokes, progressive cognitive decline, dementia and premature death (45–47). The vascular lesions underlying CADASIL are a non-atherosclerotic, non-amyloid arteriopathy affecting primarily small cerebral arteries, although the vascular defects are systemic and CADASIL can be diagnosed by ultrastructural or immunohistochemical examination of arterioles in skin biopsies (48–51). Electron microscopic studies revealed the degeneration and loss of vascular smooth muscle cells in CADASIL patients, and the accumulation of granular osmiophilic material within the smooth muscle cell basement membrane and the surrounding extracellular matrix (45–47).

CADASIL is caused by mutations in the NOTCH3 gene (52). All mutations associated with CADASIL result in a gain or loss of a cysteine residue in one of the 34 EGF-like repeats in the extracellular domain of the NOTCH3 protein. Most mutations are missense mutations that are clustered near the amino terminus of the protein (53). Some splice site mutations have also been described. However, these splicing mutations invariably cause in-frame deletions that result in the loss of cysteine residues (54,55). All EGF repeats contain six conserved cysteine residues that form three intradomain disulfide bonds (56). The types of mutations found in CADASIL patients lead to an odd number of cysteine residues in the affected EGF repeat. This fact, in addition to the absence of any examples of obviously inactivating mutations or deletions of the NOTCH3 gene of CADASIL patients, strongly suggests that these mutations do not create NOTCH3 null alleles. Transfection of rat NOTCH3 cDNA clones encoding CADASIL-like mutant proteins into cell lines demonstrated that the mutations did not affect cell surface expression or ligand binding ability of the mutant proteins (57). However, it is not known whether or how NOTCH3 signaling is altered by the CADASIL mutations.

Expression of the NOTCH3 protein in the vasculature is confined to arterial vascular smooth muscle cells in both humans and rodents (58–61). Joutel and colleagues made the important finding that the ectodomain of the NOTCH3 protein accumulates in the cerebral microvasculature of CADASIL patients (58). The NOTCH3 ectodomain accumulated at the cytoplasmic membrane of vascular smooth muscle cells, in close vicinity to but not within the granular osmiophilic material deposits. These data suggest that the CADASIL mutations impair the clearance of the NOTCH3 ectodomain from the cell surface (58).
Ruchoux and colleagues have developed a mouse CADASIL model by expressing a human NOTCH3 cDNA containing a common CADASIL mutation (Arg90Cys) in vascular smooth muscle cells (62). Transgenic mice expressing the mutant cDNA demonstrated age-dependent accumulation of the NOTCH3 ectodomain and of granular osmiophilic material in both cerebral and peripheral arterioles. Surprisingly, vascular defects were more severe in the tails of the transgenic mice than in the brain. In addition, the transgenic mice did not exhibit any evidence of damage to the brain parenchyma. Further analysis and development of this model should lead to insights into the pathogenesis of these diseases in humans.

REFERENCES


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