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Review

Possible roles of DLK1 in the Notch pathway during development and disease ☆

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ABSTRACT

The Delta-Notch pathway is an evolutionarily conserved signaling pathway which controls a broad range of developmental processes including cell fate determination, terminal differentiation and proliferation. In mammals, four Notch receptors (NOTCH1–4) and five activating canonical ligands (JAGGED1, JAGGED2, DLL1, DLL3 and DLL4) have been described. The precise function of noncanonical Notch ligands remains unclear. Delta-like 1 homolog (DLK1), the best studied noncanonical Notch ligand, has been shown to act as an inhibitor of Notch signaling *in vitro*, but its function *in vivo* is poorly understood. In this review we summarize Notch signaling during development and highlight recent studies in DLK1expression that reveal new insights into its function.

Introduction

The Delta-Notch pathway is an evolutionarily conserved signaling pathway which controls a broad range of developmental processes including cell fate determination, terminal differentiation and proliferation [4,5,9]. Notch receptors and ligands are transmembrane proteins that belong to the Epidermal Growth Factor (EGF)-like family of proteins. In mammals, four Notch receptors (NOTCH1–4) and five activating canonical ligands (JAGGED1, JAGGED2, DLL1, DLL3 and DLL4) have been described. After ligand binding, Notch receptors release their intracellular domain (NICD), which is cleaved by γ-secretase and then translocates to the nucleus. The NICD interacts with the DNA-binding transcriptional repressor C-repeat/DRE Binding Factor 1 (CBF1) also known as Recombination Binding Protein for immunoglobulin kappa J region (RBP-J) and converts it into a transcriptional activator that induces transcription of target genes (Fig. 1). In addition to the canonical ligands, noncanonical ligands can bind to Notch receptors, but the precise function of these ligands remains unclear. Delta-like 1 homolog (DLK1), the best studied noncanonical Notch ligand, has been shown to act as an inhibitor of Notch signaling in vitro [8,74], but its function in vivo is poorly understood. In this review we summarize Notch signaling during development and highlight recent studies in DLK1 expression that reveal new insights into its function.

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1. The Delta-Notch pathway

1.1. Notch receptors and ligands

The first *Notch* gene was cloned in 1983 and shown to encode a cell-surface receptor in *D. melanogaster* [6]. Functional analysis revealed that Notch is important for cell fate decisions during development of *D. melanogaster* [7]. Subsequently, two Notch genes (glp-1 and lin-12) were identified in *C. elegans*, whereas four Notch homologs (*Notch1–4*) were identified in vertebrates [101], probably as a result of duplication events. Phylogenetic analysis of vertebrate Notch genes suggested that *Notch1a* and *Notch1b* (in fish) resulted from a duplication near the teleost/mammalian divergence [52]. It was further shown that *Notch2* appeared in the first round of vertebrate duplication events and that vertebrate *Notch2* group is closely related to *Notch3* [101]. *Notch4* is found only in mammals and is possibly the result of a rapid divergence from *Notch3* [52].

Five canonical mammalian Notch ligands have been described, namely JAGGED1, JAGGED2, Delta-like 1 (DLL1), DLL3 and DLL4. Canonical Notch ligands are characterized by three related structural motifs: an N-terminal Delta-Serrate-LAG-2 (DSL) domain (a cryptic EGF-like repeat), specialized tandem EGF-repeats called the DOS domain and a variable number of EGF-like repeats (Fig. 2). Notch ligands can be further classified on the basis of the presence or absence of a cysteine-rich domain into the Jagged/Serrate or Delta-like group (Fig. 2). Both the DSL and the DOS domains are involved in receptor binding [21,51], but DLL3 and DLL4 are DSL-only ligands.

In addition to the canonical ligands, noncanonical ligands can bind to Notch receptors. The function of noncanonical ligands is still poorly understood, but soluble noncanonical ligands may act as dominant-

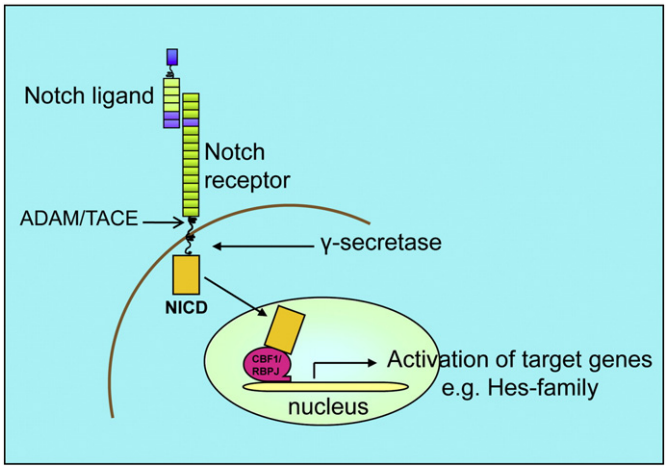

Fig. 1. Notch intracellular signaling. Schematic representation of the Notch signaling cascade. Binding of the Notch ligand to the membrane-bound Notch receptor leads to a sequence of proteolytic events resulting in cleavage of the Notch extracellular domain by the ADAM17 (also known as TACE) protease, followed by cleavage of the intracellular domain by γ-secretase [10]. The Notch intracellular domain (NICD) then translocates to the nucleus and binds to the DNA-binding transcriptional repressor CBF1/RBP-J. This binding converts CBF1 into a transcriptional activator, which leads to transcription of target genes. One of the best characterized Notch target genes is the Hairy and Enhancer of Split (HES) family of transcriptional repressors which encode for basic helix-loop-helix (bHLH) DNA-binding proteins [4,9].

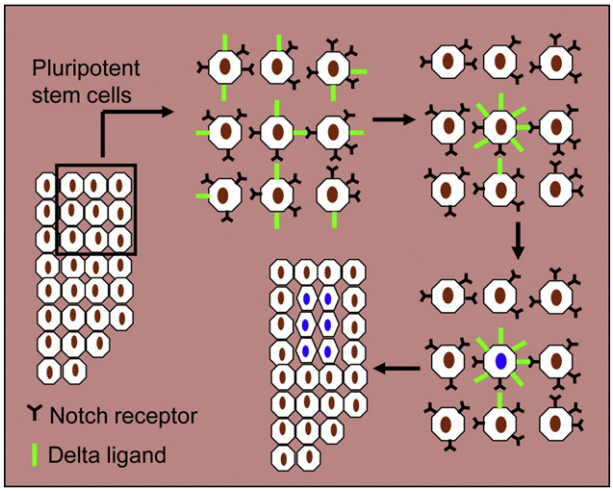

Fig. 3. Lateral inhibition. Schematic respresentation of the process of lateral inhibition by which pluripotent stem cells adopt alternative cell fates. First, an instructive signal leads to upregulation of the Delta ligand in one cell(the central cell in this scheme; doubling of Delta ligand expression). This leads to activation of Notch on the neighboring cell, which in turn results in downregulation of Delta in that same cell, which enhances the first signal. The divergence between the two cells can then lead to differentiation into alternative cell fates: the central cell in the scheme initially expresses two delta ligands and two Notch receptors interacting with their right and lower neighbor cells, which express 1 and 2 Delta ligands, respectively. After an instructive signal, Delta becomes upregulated in the central cell, depicted by 5 Delta ligands instead of 2, which then leads to downregulation of Delta ligands in its right and lower neighbor cell, which now express 0 and 1 Delta ligand.

negative proteins that block Notch signaling [21,51]. Delta-like 1 homolog (DLK1) is the best studied noncanonical Notch ligand. It resembles DLL ligands, but misses the DSL domain (Fig. 2) and was shown to inhibit NOTCH signaling as a DOS co-ligand [8,74].

One of the most prominent features of canonical Delta-Notch signaling is that the ligand-receptor association occurs only between neighboring cells. This feature becomes accentuated in the process of "lateral inhibition", which occurs when two initially identical progenitor cells adopt different cell fates due to upregulation of the Delta ligand in one cell. This activates the Notch receptor on the neighboring cell, which in turn results in down regulation of *Delta* expression in that same cell, enhancing the divergence between the two cells [47,57]. These cells can then adopt alternative cell fates (Fig. 3).

1.2. Importance of Delta-Notch signaling during development: loss of function studies

Knockout studies for each of the mammalian Notch receptors and ligands have been conducted in mice. Table 1 provides an overview of the resulting phenotypes of these Notch pathway knockouts [26,27,33,40,42,46,53,71,78,98,111].

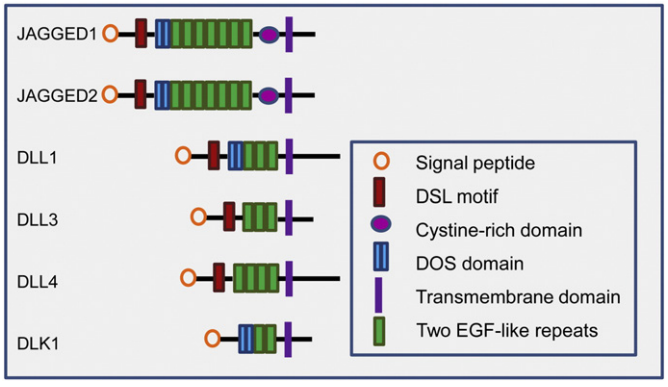

Fig. 2. Notch Ligands. Schematic representation of the structural organization of Notch ligands (adapted from Kopan et al. 2009 [51]). Classical Notch ligands contain the DSL (Delta-Serrate-Lag-2), DOS (Delta and OSM-11-like proteins) and EFG (Epidermal growth factor) motifs. DLL3 and DLL4 are considered DSL-only ligands. DLK1 is considered a DOS co-ligand [51].

1.2.1. NOTCH1

Homozygous disruption of the *Notch1* gene is fatal around embryonic day (ED)10, indicating that *Notch1* is essential for normal embryonic development. Morphological and histological analysis of homozygous *Notch1*-deficient embryos showed normal pattern formation through the first nine days of gestation. However, histological analysis revealed widespread cell death after this stage, which was attributed to disorganized and delayed somitogenesis [18,98]. To explore the role of NOTCH1 later in development, inducible *Notch1* knockout were made. Mice, in which *Notch* expression was deleted neonatally, were transiently growth retarded, severely deficient in thymocyte development and developed nodular hyperplasia in the liver [20,77]. Inactivation of *Notch1* in mouse skin resulted in epidermal and corneal hyperplasia, followed by the development of skin tumors [72]. Additionally, activating *NOTCH1* mutations are associated with human T-cell acute lymphoblastic leukemia (T-ALL) [28]. These findings implicate that in the adult stage NOTCH1 is still involved in regulation of cell growth including both tumor suppressor and oncogenic functions.

1.2.2. NOTCH2

Homozygous *Notch2*-deficient embryos show developmental retardation, widespread cell death and embryonic lethality before ED11.5, but have, in contrast to *Notch1* knockouts, normal somitogenesis [40]. Mice homozygous for a hypomorphic *Notch2* mutation show defects in development of the kidney, heart and eye vasculature [66]. The human Allagille syndrome is associated with mutations in both *NOTCH2* and *JAGGED1*, and is characterized by growth retardation, jaundice due to impairment of intrahepatic bile duct formation and defective development of skeleton, heart, eyes and kidneys [68,97]. Mice doubly heterozygous for a hypomorphic *Notch2* allele and a *Jagged1* null allele exhibit developmental abnormalities that resemble the human Alagille syndrome. Heterozygous *Notch2* mutants show no abnormalities, while heterozygous *Jagged1*-deficient mice exhibit

Table 1
Phenotypes of mice with targeted disruption of Notch pathway genes and DLK1.

Disrupted gene	Phenotype
Notch1 (Swiatek, 1994)	Embryonically lethal at ED10, widespread cell death, disturbed somitogenesis
Notch2 (Hamada, 1999)	Embryonically lethal at ED11.5, widespread cell death, normal somitogenesis
Notch3 (Domenga, 2004)	Viable and fertile, defects in postnatal maturation of vascular smooth muscle cells
Notch4 (Krebs, 2000)	Viable and fertile; in combination with Notch1 deficiency severe vascular defects
Jagged1 (Xue, 1999)	Embryonic lethal at ED10, defects in vascular remodeling of embryo and yolk sac
Jagged2 (Jiang, 1998)	Perinatally lethal, craniofacial defects, skeletal defects, impaired thymic differentiation
Dll1 (Hrabe, 1997)	Embryonically lethal at ED12, severe somite patterning defects, hyperplastic CNS
Dll3 (Dunwoodie, 2002)	Viable with severe axial skeletal defects
Dll4 (Gale, 2004)	Embryonic lethal from ED10.5, severe vascular remodeling defects in embryo and yolk sac
Dlk1 (Moon, 2002; Raghunandan, 2008; Waddell, 2010; Puertas-Avendano, 2011)	Increased perinatal lethality, growth retardation, rib deformations, increased adiposity Altered B-cell differentiation in spleen and bone narrow, reduced skeletal muscle mass, disturbed pituitary cell type development

limited eye defects without the other characteristic features of Alagille's syndrome [67]. Furthermore, mice with a perinatal, liver-specific complete elimination of *Notch2* (*Notch2*$^{fl/fl}$/*Alb-Cre*$^{tg/-}$) have a paucity of bile ducts and jaundice, demonstrating that Notch2 signaling is responsible for the liver phenotype in Alagille's syndrome [37]. Recently, we investigated the effects of early embryonic elimination of *Notch2* in *Notch2*$^{fl/fl}$/*Alfp-Cre*$^{tg/-}$ (*Notch2*-cKO) mice and showed that *Notch2* is indispensable for biliary differentiation in mice [Falix et al., *Notch2* is required for cholangiocyte differentiation, *Submitted*]. Neonatal *Notch2*-cKO mice were severely jaundiced with livers completely devoid of cytokeratin19-positive ductal structures. mRNA levels of transcription factors involved in biliary development, including *Hnf6*, *Foxa1*, *Foxa2*, *Hhex*, *Hnf1β*, *Cebpα* and *Sox9* were either permanently or transiently decreased in postnatal *Notch2*-cKO livers, indicating that during cholangiocyte differentiation, they lie downstream from *Notch2* [Falix et al., *Notch2* is required for cholangiocyte differentiation, *Submitted*]. The above findings imply that mutations in both *NOTCH2* and *JAGGED1* determine the severity of the phenotype of Alagille's syndrome.

1.2.3. NOTCH3 and NOTCH4

Notch3-null mice are viable and fertile without any apparent phenotypic abnormalities. However, adult *Notch3* knockout mice show obvious arterial defects due to abnormalities in differentiation and maturation of vascular smooth-muscle cells [26,53]. In agreement with a role for *Notch3* in vascular development, mutations in the EGF-repeats of the *NOTCH3* gene in humans cause the cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) syndrome, leading to early stroke and dementia [49]. Similar to *Notch3*, *Notch4*-null mice are viable and fertile [53]. Involvement of *Notch4* in vascular development is likely because its expression during embryonic development is restricted to vascular endothelial cells [53]. Furthermore, *Notch1*/*Notch4* double knockouts show a more severe phenotype than *Notch1* knockouts, with extensive defects in angiogenic vascular remodeling that affect the embryo, yolk sac and placenta at ED9.5 [53]. The aggravation of the phenotype of *Notch1* deficiency by *Notch4* deficiency suggests a partial functional redundancy of *Notch4* for *Notch1*.

1.2.4. Notch canonical ligand knockouts

Homozygous disruption of the Notch ligands results in severe developmental defects. *Jagged1*-null mice exhibit defects in vascular remodeling of the embryo and yolk sac and die at ED10 from extensive hemorrhage [111]. Mice homozygous for a *Jagged2* deletion die perinatally because of defects in craniofacial- and limb morphogenesis with cleft palate, fusion of the tongue with the palatal shelves, syndactyly (digit fusions) of the fore and hind limbs, and defective thymus development [46]. Homozygous inactivation of *Dll1* causes severe defects in somite patterning and a hyperplastic CNS. *Dll1*-deficient mice become hemorrhagic after ED10 and die around ED12 [42]. This implies that *Dll1* expression is a prerequisite for Notch receptors to function during somitogenesis and CNS development. In addition to DLL1, DLL3 is also involved in somitogenesis, but *Dll3*-null mice are viable despite severe axial skeletal defects, which probably result from delayed and irregular somite formation [21,27]. In agreement, mutations in the human *DLL3* gene are associated with spondylocostal dysplasia, that is, with similar vertebrocostal defects as seen in *Dll3*-deficient mice [13]. DLL3 differs structurally from the other canonical DSL ligands (Fig. 2) and is considered a Notch-signaling antagonist [21]. In agreement, *Dll3* expression in the presomitic mesoderm is unable to rescue the *Dll1*-deficient somite phenotype in mouse embryos [21]. *Dll4* deficiency causes severe vascular remodeling defects and embryonic lethality even in the heterozygous condition. The phenotype of *Dll4*$^{Lz/+}$ mice is reminiscent of that reported for the homozygous *Notch1*/*Notch4* double knockout, suggesting that DLL4 is a major physiologic ligand for these receptors and initiates their signaling during vascular development [33]. Interestingly, mice lacking *Jagged1* also exhibit a similar phenotype, which suggests an overlapping functional capacity for JAGGED1 and DLL4.

The comparison of the phenotype of Notch receptor- and Notch-ligand knockouts does not reveal extensively overlapping phenotypes, apart from the described DLL4 and NOTCH1/4 vascular phenotypes. In the case of fixed ligand-receptor-pairs, one would expect that the deficiency of either the ligand or the receptor to cause a similar rather than a different phenotype. The existence of non-overlapping phenotypes is, on the other hand, also compatible with particular ligand-receptor pairs, which, upon modification, affect a specific phenotype, but not another. Possibly, therefore, both conditions are met if only a limited number of permutations of ligands per receptor or *vice versa* are functional. Alternatively, the interactions between receptors and ligands could become unique by having additive effects (the *Notch1*/*Notch4* double knockout has the same phenotype as the *Dll4* knockout). The relatively mild phenotype of *Notch3* and *Notch4* knockout mice suggests functional redundancy with NOTCH1 and/or NOTCH2, but not *vice versa*.

2. DLK1, a noncanonical Notch ligand

2.1. Protein structure

Both the human *DLK1* and the murine *Dlk1* genes are maternally imprinted, paternally expressed genes on chromosome 14 and 12 in man and mouse, respectively [54].

Delta-like 1 homolog (DLK1), also known as Preadipocyte factor 1 (Pref-1) and Fetal antigen (FA1) [31,95], is an EGF-like membrane-bound protein. It contains six tandem EGF-like repeats, a juxtamembrane region with a TACE (ADAM17)-mediated cleavage site, a transmembrane domain, and a short intracellular tail [109]. TACE-mediated cleavage yields a soluble form of DLK1 with a molecular weight of 50 kDa [109]. Alternative DLK1 splicing products have been described in several mammalian species [1,23,69], which mostly

result from in-frame deletions of the juxtamembrane region and the sixth EGF repeat, resulting in membrane bound forms that sometimes lack the TACE-sensitive cleavage site. The biological activity of these splicing variants is yet not fully understood.

The structure and amino-acid sequence of the EGF repeats in DLK1 are closely related to those present in the canonical DLL ligands. However, DLK1 misses the conserved cryptic EGF repeat that is called the DSL domain, which is present at the N-terminus of all canonical Notch ligands (Fig. 1). For this reason, DLK1 is considered a DOS (co)ligand [21,50,51]. Despite of the absence of a DSL domain, interaction between DLK1 and the NOTCH1 receptor was shown in the yeast GAL4 two-hybrid system. In this model system, pairs 10/11 and 12/13 of the NOTCH1 EGF-like repeats interacted with DLK1 EGF repeats 1, 2, 5 and 6. [8]. NOTCH1 EGF-like repeats 11 and 12 are those reported to interact with the DOS domain of canonical ligands [30,79,92,93]. DLK1 behaved as a negative regulator of NOTCH1 signaling in mesenchymal cell lines [8,74]. Furthermore, overexpression of murine *Dlk1* in Drosophila altered the cellular distribution of *Notch* and inhibited the expression of *Notch* target genes [11]. Recently, a new protein, highly homologous to DLK1, named DLK2, has been discovered that also interacts with the NOTCH1 receptor and inhibits Notch signaling [73,89]. The inhibitory effect of DLK1 and DLK2 on Notch signaling may be mediated by competition of with canonical ligands of the DSL type for the binding site on the Notch receptor [25,74]. DLK1 was also shown to be involved in other signaling pathways, such as the ERK/MAPK pathway [48,87,110,114] and the FGF-signaling pathway [70]. In this review we will focus on its possible roles in the Notch pathway.

2.2. Expression during development

Dlk1 expression is widely distributed during mouse embryonic development, with high expression in placenta, liver, adipose tissue, skeletal muscle, lung, vertebrae, and the pituitary- and adrenal gland(s) [31,95,100,113]. In the adult, in contrast, expression becomes restricted to (neuro)endocrine tissues like the pituitary gland, adrenal glands, pancreas, monoaminergic neurons in the central nervous system, testes, prostate and ovaries [15,31,43,44,55,81,102,113]. The reported expression pattern, together with its involvement in the Notch pathway, suggests an important role for DLK1 during the maturation of several tissues. However, *Dlk*-null mice display a relatively mild phenotype, with increased perinatal lethality and growth retardation accompanied by accelerated adiposity and developmental defects in the eyelids, ribs and lungs, [71] as well as alterations in B-cell differentiation [78], and pituitary cell type development [75]. Furthermore, muscle-specific *Dlk1*-deletion resulted in disturbed muscle development and regeneration [108].

3. Role of DLK1 and Notch in different cellular systems

Despite of the reported inhibitory action of DLK1 on Notch signaling *in vitro* [8,11,74], DLK1 involvement in Notch signaling during development remains poorly understood. DLK1 is expressed in many embryonic tissues, in which active Notch signaling was also reported [17,35,36,38,39,90,103,104]. Postnatally, DLK1 expression has disappeared from most of these tissue, but is associated with pediatric malignancies, such as hepatoblastoma, neuroblastoma and nephroblastoma [32,58,63,64], and some adult malignancies, such as myelodysplastic syndrome, pituitary tumors, breast, colon and prostate carcinoma [1,15,76,88,112]. Therefore, we discuss DLK1 expression in relation to previously reported (interventions in) Notch signaling during development in the above mentioned tissues (Section 2.2) and provide a review of DLK1 and the Notch pathway in pediatric malignancies.

3.1. Adipogenesis

The best established function for DLK1/Pref1 is that of an inhibitor of adipogenesis, as it prevents the differentiation of preadipocytes into mature adipocytes [94–96,109]. DLK1 is highly expressed in murine preadipocytes, whereas its expression is completely abolished in mature adipocytes. The 3T3L1 cell line is a frequently used murine preadipocyte cell line to study the mechanism of adipocyte differentiation after hormonal induction [19,95]. When 3T3L1 cells are induced to differentiate into mature adipocytes, constitutive overexpression of soluble DLK1 prevents adipogenic differentiation by inhibiting the expression of the key transcriptional regulators of adipogenesis *Cebpα* and *Pparγ* [94,95]. A direct effect of DLK1 on adipogenesis is supported by the observed decrease in size of all adipose tissues, including brown fat, in transgenic mice with adipocyte-specific overexpression of *Dlk1* [56] and the increased adiposity and development of fatty liver, with increased expression of lipogenic enzymes *Fas* and *Scd1*, in mice lacking *Dlk1* [71].

It is well possible that soluble and membrane-bound DLK1 differ in function, as membrane-bound DLK1 is required for adipogenesis in the 3T3L1 cell line [34]. Furthermore, overexpression of full-length DLK1 (which generates both soluble and membrane-bound DLK1) significantly enhances adipogenic response in the mesenchymal stem cell line C3H10T1/2 [74]. Additionally, we recently showed that liver-specific overexpression of full-length *Dlk1* resulted in a sex- and diet-dependent increase of expression of *Notch1* and the downstream Notch target *Hes1*, as well as adipogenic (*Cebpα* and *Pparγ*) and lipogenic genes (*Fas* and *Scd*) in liver and adipose tissue. Dlk1-overexpressing female mice on a high-fat diet were most sensitive [*Falix* et al., Liver-specific overexpression of *Dlk*1 aggravates high fat diet-induced steatosis in mice, *Submitted*].

The precise role of the Notch pathway in adipogenesis remains controversial with reportedly both stimulatory and inhibitory roles for *Notch1* and *Hes1* during adipocyte differentiation [8,35,74,82,83]. The effect of DLK1 on the Notch pathway during adipogenesis has been studied *in vitro* in cell lines with (3T3L1 mouse preadipocyte cell line) or without (Balb/c14 mouse fibroblast cell line) endogenous DLK1 expression [8]. Increased *Dlk1* expression correlated with a decrease in *Notch1* expression and a concomitant decrease in levels of downstream target *Hes1* in both cell lines, and resulted in inhibition of adipogenesis in 3T3L1 cells [8]. Furthermore, in the mesenchymal stem cell line C3H10T1/2, DLK1 overexpression also resulted in Notch signaling inhibition [74]. These findings support the hypothesis that DLK1acts as a negative regulator of Notch signaling. Interestingly, constitutive *Notch1* expression leading to increased *Hes1* mRNA levels resulted in a decrease of *Dlk1* mRNA levels and prevented adipocyte differentiation in the 3T3L1 cell line [83]. Additionally, inhibition of *Notch1* expression prevented the potentiating effects of DLK1 on adipogenesis in C3H10T1/2 cells [74]. It was, therefore, proposed that "a proper balance of Notch signaling is critical for adipogenesis to proceed" and that "DLK1 might be a critical factor to control the proper level of Notch signaling for cells to undergo adipogenesis" [8]. Collectively, these findings indicate that DLK1 is not only an inhibitor of adipogenesis, but that its role in adipogenesis is dependent on the biological context.

3.2. Muscle development

The callipyge (CLPG) phenotype is an inherited skeletal muscle hypertrophy of sheep. The CLPG mutation occurs in a highly conserved motif between the imprinted *Dlk1* and noncoding *Gtl2* genes [22]. This mutation causes abnormally high postnatal *Dlk1* expression in affected muscles, without altering its imprinted status [16]. Normally, *Dlk1* expression in muscle is rapidly downregulated after birth in both sheep and mice [16,31], and becomes re-expressed during muscle injury and chronic myopathies [2,108]. Transgenic mice

expressing ovine *Dlk1* under control of the murine myosin light chain 3F promoter have high *Dlk1* expression in type myosin heavy-chain type IIB (MYH4) muscle fibers throughout pre- and postnatal development. Compared to controls, these mice also show increased relative muscle mass and average fiber diameter in both the foreleg and hind-leg muscles [22]. Deletion of *Dlk1* in the myogenic lineage resulted, on the other hand, in reduced skeletal muscle mass due to a reduction in the number of myofibers and *Myh4* gene expression and also impaired muscle regeneration. *Dlk1* knockout inhibited the expression of the muscle-determining transcription factor *MyoD*, and facilitated the self-renewal of activated satellite cells. Conversely, *Dlk1* over-expression inhibited the proliferation and enhanced differentiation of cultured myoblasts [108].These findings show that DLK1 participates in the regulation of muscle fiber growth during development and that postnatally persisting *Dlk1* expression in skeletal muscle contributes directly to the muscular hypertrophy observed in CLPG sheep.

Notch signaling inhibits myogenic differentiation by suppression of *MyoD* expression, which is critical for the proper expansion of muscle progenitors during development [12,90,104,105]. Mice carrying either a hypomorphic allele of the Notch ligand *Dll1* or a myocyte-specific deletion of the Notch downstream transcription factor *Cbf1* both display severe muscle hypotrophy due to uncontrolled premature differentiation of the muscle progenitor cell pool, with increased expression of myogenic regulatory factors MyoD and Myogenin and a reduced number of muscle progenitor cells [90,105]. Comparison of the DLK1 and Notch muscle phenotypes shows that DLK1 and NOTCH have opposite effects on myogenesis, which is compatible with the putatively inhibitory effect of DLK1 on Notch signaling.

3.3. Liver development

Dlk1 is one of the most abundant transcripts in mouse ED14.5 liver, while expression is rapidly downregulated thereafter to become undetectable just before birth [100]. DLK1-positive fetal liver cells show higher proliferative potential compared to the DLK1-negative cell population and are able to differentiate into both the hepatocyte and cholangiocyte lineages [100], which implies that DLK1-positive liver cells are still hepatoblasts. *Notch1*, *Notch2,* and *Jagged1* are expressed in prenatal liver, with a peak just prior to birth. Immediately after birth, their expression returns again to basal levels [61,99].

Notch2 and *Jagged1* are necessary for appropriate bile duct development [37,67] [Falix et al., *Notch2* is required for cholangiocyte differentiation, *Submitted*]. Recently, NOTCH1 was shown to stimulate both pre- and postnatal bile-duct proliferation [116]. Overexpression of the NICD in *in-vitro* differentiated DLK1-positive hepatoblasts resulted in downregulation of hepatocyte-marker genes (albumin, CPS, TAT) and, subsequently, in upregulation of cholangiocyte marker genes (CK7, CK19, HNF1ß, integrin ß4) [99]. Notch signaling may, therefore, confer the capacity to differentiate along the cholangiocyte lineage upon hepatoblasts [99]. The rapid downregulation of *Dlk1* expression just prior to birth, which coincides with the peak in *Notch1*, *Notch2* and *Jagged1* expression, is also compatible with induction of hepatoblast maturation along the cholangiocyte lineage due to activation of NOTCH1/2 receptor signaling. In agreement, we recently showed that *Notch2* is indispensable for cholangiocyte differentiation and that its absence does not affect *Dlk1* expression [Falix et al., *Notch2* is required for cholangiocyte differentiation, *Submitted*].

3.4. Branching morphogenesis in lung and pancreas

In the developing lung and pancreas, *Dlk1* expression is restricted to the distal growing epithelia and the surrounding mesenchyme [113]. In agreement, we recently demonstrated that DLK1 protein in the developing lung and pancreas is only found in the distal parts of the tubular trees and the surrounding mesenchyme, where branching morphogenesis takes place [Falix et al., DLK1 expression during mouse embryonic development provides more insights in its function, *Submitted*]. Branching morphogenesis is a characteristic process in developing tubular structures that is dependent on interactions between the growing epithelial bud and the surrounding mesenchyme [41]. Notch signaling does regulate branching morphogenesis in the developing lung [103]. Disruption of Notch signaling during the initial stages of murine lung development results in a dramatic expansion of the population of distal progenitors and in prevention of the formation of proximal airway structures [103]. Constitutive Notch signaling by contrast, prevents the differentiation of alveolar (distal) epithelium and induces the development of cysts at the location of the distal airways, which are composed of cells that express markers of proximal airway epithelium [38].

Analogous to lung development, disruption of Notch signaling during pancreatic development led to pancreatic hypoplasia due to depletion of pancreatic epithelial precursors [3,45], whereas constitutive overexpression of Notch signaling led to impaired branching of the pancreatic epithelium with formation of cyst-like structures without exocrine cells and repression of endocrine development [39], that is, suppression of differentiation of distal epithelial cells. These observations indicate that Notch signaling regulates branching and the establishment of a proximal–distal gradient in cell fate during gland formation in mammals, with probably a regulatory function for DLK1.

3.5. Pituitary gland development

DLK1 expression in developing mouse fetuses declines after ED16, including the pituitary gland, but this organ is one of the few that remains positive for DLK1 expression, also after birth [75,113]. During pituitary development in the mouse, between ED11 and ED14 the first pituitary-specific cell types are formed: thyrotroph and corticotroph cells. Thereafter, around ED15 somatotroph, gonadotroph and lactotroph cells are formed with completion of cell specification and differentiation on ED17 [91,107]. This time point coincides with the significant overall decrease in DLK1 expression, also in the pituitary gland. The temporal expression of canonical Notch pathway members in the pituitary is comparable to that of DLK1, with an overall decrease of both Notch receptor mRNAs and ligands in the late embryonal period [115]. Interference with Notch signaling results in premature differentiation of the early differentiating corticotropic lineage and inhibition of differentiation of the later differentiating somatotropic and gonadotropic lineages. In contrast, sustained Notch signaling in somato- and lactotropic precursors results in a reduction of the prevalence of these cell populations. These findings show that Notch signaling prevents conversion of the late-arising cell lineages to early-born cell lineages and that attenuation of Notch signaling later in pituitary development is required for proper terminal differentiation of the lineages [115]. The observed expression pattern of DLK1 in the developing pituitary suggests that DLK1 may be a player in the regulation of differentiation of pituitary cell types by modulating Notch signaling activity. In agreement with such a role, the pituitary of adult *Dlk1* knockout mice contains a significantly lower number of somatotrophs and a reduction in cell-specific gene expression in gonadotrophs compared to wild-type mice [75].

4. DLK1 and Notch in pediatric malignancies

Pediatric tumors like neuroblastoma, hepatoblastoma, and nephroblastoma (Wilms tumor) are believed to arise from cellular populations that have not completed the process of differentiation. In agreement, signal-transduction pathways involved in embryonic development, like the Wnt/β-catenin pathway, are frequently

upregulated in these tumors [14,62]. Recently, both DLK1 and the Notch pathway have also been associated with these pediatric malignancies [24,32,59,63,64,117].

4.1. Neuroblastoma

Neuroblastoma, an embryonic tumor originating from immature sympathetic neuroblast, displays a remarkable spectrum of clinical and biological behavior, ranging from spontaneous regression of metastases to rapid and fatal progression despite intensive therapy [58]. High expression of *DLK1* and the *NOTCH3* receptor was reported in subsets of neuroblastoma tumors and cell lines [59]. *DLK1* expression correlated perfectly with dopamine β-hydroxylase (*DBH*) expression, an enzyme which is normally highly expressed in the chromaffin cells of the adrenal medulla and converts dopamine to noradrenaline [59]. During early embryonic development, *DLK1* expression is detected throughout the adrenal gland, while later during development expression becomes restricted to the chromaffin cells, one of the few cell types that maintains postnatal *DLK1* expression [44,113]. Interestingly, the reported *DLK1* expression in neuroblastoma cell lines was inversely correlated to *NOTCH3* expression [59]. Therefore, it was suggested that overexpression of *NOTCH3* in neuroblastoma cell lines corresponds with early precursor stages, whereas overexpression of *DLK1* reflects differentiation arrest in a relatively late stage of the chromaffin lineage [59].

4.2. Hepatoblastoma

Hepatoblastoma, a malignant pediatric liver tumor, is believed to derive from hepatoblasts, because of the stem-cell like appearance of the hepatoblastoma cells [85,86,106]. Hepatoblastomas are characterized by a diversity of epithelial and often mesenchymal patterns of differentiation, with some epithelial variants that morphologically resemble embryonic or fetal hepatocytes [65,84]. Recently, increased expression of DLK1was found to be a consistent feature among hepatoblastomas [14,24,63,64]. *DLK1* was significantly elevated in all histological subtypes when compared to normal liver, sometimes even higher than in fetal liver [63]. In fact, we recently showed that serum DLK1 levels were significantly elevated in hepatoblastoma patients compared to age-matched controls, even in the youngest patients, in whom serum α-fetoprotein levels are often in the same range as the still elevated control levels [29].These findings make DLK1 a candidate serum marker to diagnose hepatoblastoma in the young infant age group.

NOTCH2 receptor expression was increased in 92% of hepatoblastomas compared to normal liver tissue. *HES1*, the best studied Notch downstream target, was also elevated in hepatoblastomas, especially in the pure fetal subtype [60,63]. These findings indicate that active Notch signaling occurs in hepatoblastoma tumors and might regulate tumor growth. The abrupt disappearance of DLK1 expression in late liver development, together with its re-appearance in hepatoblastoma, suggest a role for DLK1 in hepatoblastoma pathogenesis. However, we showed recently that transgenic mice with hepatocyte-specific overexpression of *Dlk1* do not develop liver tumors up to 1.5 years of age [Falix et al., Liver-specific overexpression of *Dlk1* aggravates high fat diet-induced steatosis in mice, *Submitted*]. These findings imply that increased Notch signaling, probably via the NOTCH2 receptor, is more likely to be involved in the pathogenesis of hepatoblastoma.

4.3. Wilms tumor

Nephroblastoma is a pediatric tumor of the kidney, also known as Wilms tumor. Loss of imprinting (LOI) of the reciprocally imprinted *H19*/*IGF2* domain is a common feature of Wilms tumor, where *H19* is a non-coding gene and *IFG2* an important regulator of fetal growth [32,80]. The *DLK1* gene is similarly arranged by formation of an imprinted domain with a noncoding gene called *GTL2* [32]. *DLK1* expression is absent in developing kidney, but interestingly, high *DLK1* expression was detected in 11 out of 30 Wilms tumors with prominent myogenic differentiation and blastemal components. The imprinting status of the *DLK1*/*GTL2* domain was shown to be retained [32]. Since DLK1 is associated with muscular growth and development [22], *DLK1* expression in Wilms tumor may only reflect the presence of myogenic differentiation in a significant proportion of the tumor cells (see Section 3.2).

5. Conclusion

In this review we summarized Notch signaling pathway during development and disease, and discussed the relation of the noncanonical Notch ligand DLK1 with Notch signaling in developing organs and pediatric malignancies. DLK1 is expressed in many organs prior to and during terminal differentiation of the parenchymal cells and becomes abolished thereafter. DLK1 seems to be involved in developmental processes, such as branching morphogenesis (lung, pancreas) and terminal differentiation (adipose tissue, muscle, liver, pituitary), with as common features among organs stimulation of growth and inhibition of differentiation. Based on its expression pattern during development and adipogenesis, and its effects upon experimental interventions, DLK1 appears to function as an inhibitory modulator of Notch signaling, either by competing with canonical ligands or by direct interaction with the NOTCH(1) receptor, or both.

References

[1] T. Altenberger, M. Bilban, M. Auer, E. Knosp, S. Wolfsberger, W. Gartner, I. Mineva, C. Zielinski, L. Wagner, A. Luger, Identification of DLK1 variants in pituitary- and neuroendocrine tumors, Biochem. Biophys. Res. Commun. 340 (2006) 995–1005.

[2] D.C. Andersen, S.J. Petersson, L.H. Jorgensen, P. Bollen, P.B. Jensen, B. Teisner, H.D. Schroeder, C.H. Jensen, Characterization of DLK1+ cells emerging during skeletal muscle remodeling in response to myositis, myopathies, and acute injury, Stem Cells 27 (2009) 898–908.

[3] A. Apelqvist, H. Li, L. Sommer, P. Beatus, D.J. Anderson, T. Honjo, A.M. Hrabe de, U. Lendahl, H. Edlund, Notch signalling controls pancreatic cell differentiation, Nature 400 (1999) 877–881.

[4] S. Artavanis-Tsakonas, K. Matsuno, M.E. Fortini, Notch signaling, Science 268 (1995) 225–232.

[5] S. Artavanis-Tsakonas, M.A. Muskavitch, Notch: the past, the present, and the future, Curr. Top. Dev. Biol. 92 (2010) 1–29.

[6] S. Artavanis-Tsakonas, M.A. Muskavitch, B. Yedvobnick, Molecular cloning of Notch, a locus affecting neurogenesis in *Drosophila melanogaster*, Proc. Natl. Acad. Sci. U. S. A. 80 (1983) 1977–1981.

[7] S. Artavanis-Tsakonas, P. Simpson, Choosing a cell fate: a view from the Notch locus, Trends Genet. 7 (1991) 403–408.

[8] V. Baladron, M.J. Ruiz-Hidalgo, M.L. Nueda, M.J. az-Guerra, J.J. Garcia-Ramirez, E. Bonvini, E. Gubina, J. Laborda, dlk acts as a negative regulator of Notch1 activation through interactions with specific EGF-like repeats, Exp. Cell Res. 303 (2005) 343–359.

[9] H. Boehmer von, Coming to grips with Notch, J. Exp. Med. 194 (2001) F43–F46.

[10] S.J. Bray, Notch signalling: a simple pathway becomes complex, Nat. Rev. Mol. Cell Biol. 7 (2006) 678–689.

[11] S.J. Bray, S. Takada, E. Harrison, S.C. Shen, A.C. Ferguson-Smith, The atypical mammalian ligand Delta-like homologue 1 (Dlk1) can regulate Notch signalling in Drosophila, BMC Dev. Biol. 8 (2008) 11.

[12] M.F. Buas, T. Kadesch, Regulation of skeletal myogenesis by Notch, Exp. Cell Res. 316 (2010) 3028–3033.

[13] M.P. Bulman, K. Kusumi, T.M. Frayling, C. McKeown, C. Garrett, E.S. Lander, R. Krumlauf, A.T. Hattersley, S. Ellard, P.D. Turnpenny, Mutations in the human delta homologue, DLL3, cause axial skeletal defects in spondylocostal dysostosis, Nat. Genet. 24 (2000) 438–441.

[14] S. Cairo, C. Armengol, R.A. De, Y. Wei, E. Thomas, C.A. Renard, A. Goga, A. Balakrishnan, M. Semeraro, L. Gresh, M. Pontoglio, H. Strick-Marchand, F. Levillayer, Y. Nouet, D. Rickman, F. Gauthier, S. Branchereau, L. Brugieres, V. Laithier, R. Bouvier, F. Boman, G. Basso, J.F. Michiels, P. Hofman, F. rbez-Gindre, H. Jouan, M.C. Rousselet-Chapeau, D. Berrebi, L. Marcellin, F. Plenat, D. Zachar, M. Joubert, J. Selves, D. Pasquier, P. Bioulac-Sage, M. Grotzer, M. Childs, M. Fabre, M.A. Buendia, Hepatic stem-like phenotype and interplay of Wnt/beta-catenin and Myc signaling in aggressive childhood liver cancer, Cancer Cell 14 (2008) 471–484.

[15] J.A. Ceder, L. Jansson, L. Helczynski, P.A. Abrahamsson, Delta-like 1 (Dlk-1), a novel marker of prostate basal and candidate epithelial stem cells, is

downregulated by notch signalling in intermediate/transit amplifying cells of the human prostate, Eur. Urol. 54 (2008) 1344–1353.
[16] C. Charlier, K. Segers, L. Karim, T. Shay, G. Gyapay, N. Cockett, M. Georges, The callipyge mutation enhances the expression of coregulated imprinted genes in cis without affecting their imprinting status, Nat. Genet. 27 (2001) 367–369.
[17] J. Chen, A. Crabbe, V. Van Duppen, H. Vankelecom, The notch signaling system is present in the postnatal pituitary: marked expression and regulatory activity in the newly discovered side population, Mol. Endocrinol. 20 (2006) 3293–3307.
[18] R.A. Conlon, A.G. Reaume, J. Rossant, Notch1 is required for the coordinate segmentation of somites, Development 121 (1995) 1533–1545.
[19] R.M. Cowherd, R.E. Lyle, R.E. McGehee Jr., Molecular regulation of adipocyte differentiation, Semin. Cell Dev. Biol. 10 (1999) 3–10.
[20] A. Croquelois, A. Blindenbacher, L. Terracciano, X. Wang, I. Langer, F. Radtke, M.H. Heim, Inducible inactivation of Notch1 causes nodular regenerative hyperplasia in mice, Hepatology 41 (2005) 487–496.
[21] B. D'Souza, A. Miyamoto, G. Weinmaster, The many facets of Notch ligands, Oncogene 27 (2008) 5148–5167.
[22] E. Davis, C.H. Jensen, H.D. Schroder, F. Farnir, T. Shay-Hadfield, A. Kliem, N. Cockett, M. Georges, C. Charlier, Ectopic expression of DLK1 protein in skeletal muscle of padumnal heterozygotes causes the callipyge phenotype, Curr. Biol. 14 (2004) 1858–1862.
[23] J.A. Deiuliis, B. Li, P.A. Lyvers-Peffer, S.J. Moeller, K. Lee, Alternative splicing of delta-like 1 homolog (DLK1) in the pig and human, Comp. Biochem. Physiol. B Biochem. Mol. Biol. 145 (2006) 50–59.
[24] K. Dezso, J. Halasz, H.C. Bisgaard, S. Paku, E. Turanyi, Z. Schaff, P. Nagy, Delta-like protein (DLK) is a novel immunohistochemical marker for human hepatoblastomas, Virchows Arch. 452 (2008) 443–448.
[25] I. Dikic, M.H. Schmidt, Notch: implications of endogenous inhibitors for therapy, Bioessays 32 (2010) 481–487.
[26] V. Domenga, P. Fardoux, P. Lacombe, M. Monet, J. Maciazek, L.T. Krebs, B. Klonjkowski, E. Berrou, M. Mericskay, Z. Li, E. Tournier-Lasserve, T. Gridley, A. Joutel, Notch3 is required for arterial identity and maturation of vascular smooth muscle cells, Genes Dev. 18 (2004) 2730–2735.
[27] S.L. Dunwoodie, M. Clements, D.B. Sparrow, X. Sa, R.A. Conlon, R.S. Beddington, Axial skeletal defects caused by mutation in the spondylocostal dysplasia/pudgy gene Dll3 are associated with disruption of the segmentation clock within the presomitic mesoderm, Development 129 (2002) 1795–1806.
[28] L.W. Ellisen, J. Bird, D.C. West, A.L. Soreng, T.C. Reynolds, S.D. Smith, J. Sklar, TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms, Cell 66 (1991) 649–661.
[29] F. Falix, D. Aronson, K. Hiralall, W. Lamers, J. Seppen, DLK1, a serum marker for hepatoblastoma in young infants, Pediatr. Blood Cancer (2011) doi:10.1002/pbc.24024. [Epub ahead of print].
[30] R.G. Fehon, P.J. Kooh, I. Rebay, C.L. Regan, T. Xu, M.A. Muskavitch, S. Artavanis-Tsakonas, Molecular interactions between the protein products of the neurogenic loci Notch and Delta, two EGF-homologous genes in Drosophila, Cell 61 (1990) 523–534.
[31] C. Floridon, C.H. Jensen, P. Thorsen, O. Nielsen, L. Sunde, J.G. Westergaard, S.G. Thomsen, B. Teisner, Does fetal antigen 1 (FA1) identify cells with regenerative, endocrine and neuroendocrine potentials? A study of FA1 in embryonic, fetal, and placental tissue and in maternal circulation, Differentiation 66 (2000) 49–59.
[32] R. Fukuzawa, R.W. Heathcott, I.M. Morison, A.E. Reeve, Imprinting, expression, and localisation of DLK1 in Wilms tumours, J. Clin. Pathol. 58 (2005) 145–150.
[33] N.W. Gale, M.G. Dominguez, I. Noguera, L. Pan, V. Hughes, D.M. Valenzuela, A.J. Murphy, N.C. Adams, H.C. Lin, J. Holash, G. Thurston, G.D. Yancopoulos, Haploinsufficiency of delta-like 4 ligand results in embryonic lethality due to major defects in arterial and vascular development, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 15949–15954.
[34] C. Garces, M.J. Ruiz-Hidalgo, E. Bonvini, J. Goldstein, J. Laborda, Adipocyte differentiation is modulated by secreted delta-like (dlk) variants and requires the expression of membrane-associated dlk, Differentiation 64 (1999) 103–114.
[35] C. Garces, M.J. Ruiz-Hidalgo, M.J. Font de, C. Park, L. Miele, J. Goldstein, E. Bonvini, A. Porras, J. Laborda, Notch-1 controls the expression of fatty acid-activated transcription factors and is required for adipogenesis, J. Biol. Chem. 272 (1997) 29729–29734.
[36] M. Gasperowicz, F. Otto, The notch signalling pathway in the development of the mouse placenta, Placenta 29 (2008) 651–659.
[37] F. Geisler, F. Nagl, P.K. Mazur, M. Lee, U. Zimber-Strobl, L.J. Strobl, F. Radtke, R.M. Schmid, J.T. Siveke, Liver-specific inactivation of Notch2, but not Notch1, compromises intrahepatic bile duct development in mice, Hepatology 48 (2008) 607–616.
[38] J.S. Guseh, S.A. Bores, B.Z. Stanger, Q. Zhou, W.J. Anderson, D.A. Melton, J. Rajagopal, Notch signaling promotes airway mucous metaplasia and inhibits alveolar development, Development 136 (2009) 1751–1759.
[39] J. Hald, J.P. Hjorth, M.S. German, O.D. Madsen, P. Serup, J. Jensen, Activated Notch1 prevents differentiation of pancreatic acinar cells and attenuate endocrine development, Dev. Biol. 260 (2003) 426–437.
[40] Y. Hamada, Y. Kadokawa, M. Okabe, M. Ikawa, J.R. Coleman, Y. Tsujimoto, Mutation in ankyrin repeats of the mouse Notch2 gene induces early embryonic lethality, Development 126 (1999) 3415–3424.
[41] A. Horowitz, M. Simons, Branching morphogenesis, Circ. Res. 103 (2008) 784–795.
[42] A.M. Hrabe de, J. McIntyre, A. Gossler, Maintenance of somite borders in mice requires the Delta homologue Dll1, Nature 386 (1997) 717–721.
[43] C.H. Jensen, M. Meyer, H.D. Schroder, A. Kliem, J. Zimmer, B. Teisner, Neurons in the monoaminergic nuclei of the rat and human central nervous system express FA1/dlk, Neuroreport 12 (2001) 3959–3963.
[44] C.H. Jensen, B. Teisner, P. Hojrup, H.B. Rasmussen, O.D. Madsen, B. Nielsen, K. Skjodt, Studies on the isolation, structural analysis and tissue localization of fetal antigen 1 and its relation to a human adrenal-specific cDNA, pG2, Hum. Reprod. 8 (1993) 635–641.
[45] J. Jensen, E.E. Pedersen, P. Galante, J. Hald, R.S. Heller, M. Ishibashi, R. Kageyama, F. Guillemot, P. Serup, O.D. Madsen, Control of endodermal endocrine development by Hes-1, Nat. Genet. 24 (2000) 36–44.
[46] R. Jiang, Y. Lan, H.D. Chapman, C. Shawber, C.R. Norton, D.V. Serreze, G. Weinmaster, T. Gridley, Defects in limb, craniofacial, and thymic development in Jagged2 mutant mice, Genes Dev. 12 (1998) 1046–1057.
[47] K. Katsube, K. Sakamoto, Notch in vertebrates—molecular aspects of the signal, Int. J. Dev. Biol. 49 (2005) 369–374.
[48] K.A. Kim, J.H. Kim, Y. Wang, H.S. Sul, Pref-1 (preadipocyte factor 1) activates the MEK/extracellular signal-regulated kinase pathway to inhibit adipocyte differentiation, Mol. Cell. Biol. 27 (2007) 2294–2308.
[49] T. Kitamoto, K. Takahashi, H. Takimoto, K. Tomizuka, M. Hayasaka, T. Tabira, K. Hanaoka, Functional redundancy of the Notch gene family during mouse embryogenesis: analysis of Notch gene expression in Notch3-deficient mice, Biochem. Biophys. Res. Commun. 331 (2005) 1154–1162.
[50] H. Komatsu, M.Y. Chao, J. Larkins-Ford, M.E. Corkins, G.A. Somers, T. Tucey, H.M. Dionne, J.Q. White, K. Wani, M. Boxem, A.C. Hart, OSM-11 facilitates LIN-12 Notch signaling during *Caenorhabditis elegans* vulval development, PLoS Biol. 6 (2008) e196.
[51] R. Kopan, M.X. Ilagan, The canonical Notch signaling pathway: unfolding the activation mechanism, Cell 137 (2009) 216–233.
[52] R.D. Kortschak, R. Tamme, M. Lardelli, Evolutionary analysis of vertebrate Notch genes, Dev. Genes Evol. 211 (2001) 350–354.
[53] L.T. Krebs, Y. Xue, C.R. Norton, J.R. Shutter, M. Maguire, J.P. Sundberg, D. Gallahan, V. Closson, J. Kitajewski, R. Callahan, G.H. Smith, K.L. Stark, T. Gridley, Notch signaling is essential for vascular morphogenesis in mice, Genes Dev. 14 (2000) 1343–1352.
[54] J. Laborda, The role of the epidermal growth factor-like protein dlk in cell differentiation, Histol. Histopathol. 15 (2000) 119–129.
[55] J.B. Larsen, C.H. Jensen, H.D. Schroder, B. Teisner, P. Bjerre, C. Hagen, Fetal antigen 1 and growth hormone in pituitary somatotroph cells, Lancet 347 (1996) 191.
[56] K. Lee, J.A. Villena, Y.S. Moon, K.H. Kim, S. Lee, C. Kang, H.S. Sul, Inhibition of adipogenesis and development of glucose intolerance by soluble preadipocyte factor-1 (Pref-1), J. Clin. Invest. 111 (2003) 453–461.
[57] J. Lewis, Notch signalling and the control of cell fate choices in vertebrates, Semin. Cell Dev. Biol. 9 (1998) 583–589.
[58] V. Limpt van, A. Chan, H. Caron, P.V. Sluis, K. Boon, M.C. Hermus, R. Versteeg, SAGE analysis of neuroblastoma reveals a high expression of the human homologue of the Drosophila Delta gene, Med. Pediatr. Oncol. 35 (2000) 554–558.
[59] V. Limpt van, A.J. Chan, P.G. Van Sluis, H.N. Caron, C.J. Van Noesel, R. Versteeg, High delta-like 1 expression in a subset of neuroblastoma cell lines corresponds to a differentiated chromaffin cell type, Int. J. Cancer 105 (2003) 61–69.
[60] J. Litten, T. Chen, R. Schultz, J. Schiffman, K. Hermann, G. Tomlinson, D. Rakheja, Activated NOTCH2 is overexpressed in hepatoblastomas: an immunohistochemical study, Pediatr. Dev. Pathol. (2011).
[61] K.M. Loomes, D.B. Taichman, C.L. Glover, P.T. Williams, J.E. Markowitz, D.A. Piccoli, H.S. Baldwin, R.J. Oakey, Characterization of Notch receptor expression in the developing mammalian heart and liver, Am. J. Med. Genet. 112 (2002) 181–189.
[62] D. Lopez-Terrada, Integrating the diagnosis of childhood malignancies, Adv. Exp. Med. Biol. 587 (2006) 121–137.
[63] D. Lopez-Terrada, P.H. Gunaratne, A.M. Adesina, J. Pulliam, D.M. Hoang, Y. Nguyen, T.A. Mistretta, J. Margolin, M.J. Finegold, Histologic subtypes of hepatoblastoma are characterized by differential canonical Wnt and Notch pathway activation in DLK+ precursors, Hum. Pathol. 40 (2009) 783–794.
[64] J.H. Luo, B. Ren, S. Keryanov, G.C. Tseng, U.N. Rao, S.P. Monga, S. Strom, A.J. Demetris, M. Nalesnik, Y.P. Yu, S. Ranganathan, G.K. Michalopoulos, Transcriptomic and genomic analysis of human hepatocellular carcinomas and hepatoblastomas, Hepatology 44 (2006) 1012–1024.
[65] J.R. Mann, N. Kasthuri, F. Raafat, J.R. Pincott, S.E. Parkes, K.R. Muir, L.C. Ingram, A.H. Cameron, Malignant hepatic tumours in children: incidence, clinical features and aetiology, Paediatr. Perinat. Epidemiol. 4 (1990) 276–289.
[66] B. McCright, X. Gao, L. Shen, J. Lozier, Y. Lan, M. Maguire, D. Herzlinger, G. Weinmaster, R. Jiang, T. Gridley, Defects in development of the kidney, heart and eye vasculature in mice homozygous for a hypomorphic Notch2 mutation, Development 128 (2001) 491–502.
[67] B. McCright, J. Lozier, T. Gridley, A mouse model of Alagille syndrome: Notch2 as a genetic modifier of Jag1 haploinsufficiency, Development 129 (2002) 1075–1082.
[68] R. McDaniell, D.M. Warthen, P.A. Sanchez-Lara, A. Pai, I.D. Krantz, D.A. Piccoli, N.B. Spinner, NOTCH2 mutations cause Alagille syndrome, a heterogeneous disorder of the notch signaling pathway, Am. J. Hum. Genet. 79 (2006) 169–173.
[69] B. Mei, L. Zhao, L. Chen, H.S. Sul, Only the large soluble form of preadipocyte factor-1 (Pref-1), but not the small soluble and membrane forms, inhibits adipocyte differentiation: role of alternative splicing, Biochem. J. 364 (2002) 137–144.
[70] Y. Miyaoka, M. Tanaka, T. Imamura, S. Takada, A. Miyajima, A novel regulatory mechanism for Fgf18 signaling involving cysteine-rich FGF receptor (Cfr) and delta-like protein (Dlk), Development 137 (2010) 159–167.

[71] Y.S. Moon, C.M. Smas, K. Lee, J.A. Villena, K.H. Kim, E.J. Yun, H.S. Sul, Mice lacking paternally expressed Pref-1/Dlk1 display growth retardation and accelerated adiposity, Mol. Cell. Biol. 22 (2002) 5585–5592.
[72] M. Nicolas, A. Wolfer, K. Raj, J.A. Kummer, P. Mill, N.M. van, C.C. Hui, H. Clevers, G.P. Dotto, F. Radtke, Notch1 functions as a tumor suppressor in mouse skin, Nat. Genet. 33 (2003) 416–421.
[73] M.L. Nueda, V. Baladron, J.J. Garcia-Ramirez, B. Sanchez-Solana, M.D. Ruvira, S. Rivero, M.A. Ballesteros, E.M. Monsalve, M.J. Diaz-Guerra, M.J. Ruiz-Hidalgo, J. Laborda, The novel gene EGFL9/Dlk2, highly homologous to Dlk1, functions as a modulator of adipogenesis, J. Mol. Biol. 367 (2007) 1270–1280.
[74] M.L. Nueda, V. Baladron, B. Sanchez-Solana, M.A. Ballesteros, J. Laborda, The EGF-like protein dlk1 inhibits notch signaling and potentiates adipogenesis of mesenchymal cells, J. Mol. Biol. 367 (2007) 1281–1293.
[75] R.A. Puertas-Avendano, M.J. Gonzalez-Gomez, M.D. Ruvira, M.J. Ruiz-Hidalgo, N. Morales-Delgado, J. Laborda, C. Diaz, A.R. Bello, Role of the non-canonical notch ligand delta-like protein 1 in hormone-producing cells of the adult male mouse pituitary, J. Neuroendocrinol. 23 (2011) 849–859.
[76] X. Qi, Z. Chen, D. Liu, J. Cen, M. Gu, Expression of Dlk1 gene in myelodysplastic syndrome determined by microarray, and its effects on leukemia cells, Int. J. Mol. Med. 22 (2008) 61–68.
[77] F. Radtke, A. Wilson, G. Stark, M. Bauer, M.J. van, H.R. MacDonald, M. Aguet, Deficient T cell fate specification in mice with an induced inactivation of Notch1, Immunity 10 (1999) 547–558.
[78] R. Raghunandan, M. Ruiz-Hidalgo, Y. Jia, R. Ettinger, E. Rudikoff, P. Riggins, R. Farnsworth, A. Tesfaye, J. Laborda, S.R. Bauer, Dlk1 influences differentiation and function of B lymphocytes, Stem Cells Dev. 17 (2008) 495–507.
[79] I. Rebay, R.J. Fleming, R.G. Fehon, L. Cherbas, P. Cherbas, S. Artavanis-Tsakonas, Specific EGF repeats of Notch mediate interactions with Delta and Serrate: implications for Notch as a multifunctional receptor, Cell 67 (1991) 687–699.
[80] W. Reik, M. Constancia, W. Dean, K. Davies, L. Bowden, A. Murrell, R. Feil, J. Walter, G. Kelsey, Igf2 imprinting in development and disease, Int. J. Dev. Biol. 44 (2000) 145–150.
[81] J.C. Rockett, P. Patrizio, J.E. Schmid, N.B. Hecht, D.J. Dix, Gene expression patterns associated with infertility in humans and rodent models, Mutat. Res. 549 (2004) 225–240.
[82] D.A. Ross, S. Hannenhalli, J.W. Tobias, N. Cooch, R. Shiekhattar, T. Kadesch, Functional analysis of Hes-1 in preadipocytes, Mol. Endocrinol. 20 (2006) 698–705.
[83] D.A. Ross, P.K. Rao, T. Kadesch, Dual roles for the Notch target gene Hes-1 in the differentiation of 3T3-L1 preadipocytes, Mol. Cell. Biol. 24 (2004) 3505–3513.
[84] J.M. Rowland, Hepatoblastoma: assessment of criteria for histologic classification, Med. Pediatr. Oncol. 39 (2002) 478–483.
[85] P. Ruck, J.C. Xiao, Stem-like cells in hepatoblastoma, Med. Pediatr. Oncol. 39 (2002) 504–507.
[86] P. Ruck, J.C. Xiao, T. Pietsch, S.D. Von, E. Kaiserling, Hepatic stem-like cells in hepatoblastoma: expression of cytokeratin 7, albumin and oval cell associated antigens detected by OV-1 and OV-6, Histopathology 31 (1997) 324–329.
[87] M.J. Ruiz-Hidalgo, E. Gubina, L. Tull, V. Baladron, J. Laborda, dlk modulates mitogen-activated protein kinase signaling to allow or prevent differentiation, Exp. Cell Res. 274 (2002) 178–188.
[88] S. Sakajiri, J. O'kelly, D. Yin, C.W. Miller, W.K. Hofmann, K. Oshimi, L.Y. Shih, K.H. Kim, H.S. Sul, C.H. Jensen, B. Teisner, N. Kawamata, H.P. Koeffler, Dlk1 in normal and abnormal hematopoiesis, Leukemia 19 (2005) 1404–1410.
[89] B. Sanchez-Solana, M.L. Nueda, M.D. Ruvira, M.J. Ruiz-Hidalgo, E.M. Monsalve, S. Rivero, J.J. Garcia-Ramirez, M.J. Diaz-Guerra, V. Baladron, J. Laborda, The EGF-like proteins DLK1 and DLK2 function as inhibitory non-canonical ligands of NOTCH1 receptor that modulate each other's activities, Biochim. Biophys. Acta 1813 (2011) 1153–1164.
[90] K. Schuster-Gossler, R. Cordes, A. Gossler, Premature myogenic differentiation and depletion of progenitor cells cause severe muscle hypotrophy in Delta1 mutants, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 537–542.
[91] H.Z. Sheng, H. Westphal, Early steps in pituitary organogenesis, Trends Genet. 15 (1999) 236–240.
[92] K. Shimizu, S. Chiba, K. Kumano, N. Hosoya, T. Takahashi, Y. Kanda, Y. Hamada, Y. Yazaki, H. Hirai, Mouse jagged1 physically interacts with notch2 and other notch receptors. Assessment by quantitative methods, J. Biol. Chem. 274 (1999) 32961–32969.
[93] K. Shimizu, S. Chiba, T. Saito, K. Kumano, H. Hirai, Physical interaction of Delta1, Jagged1, and Jagged2 with Notch1 and Notch3 receptors, Biochem. Biophys. Res. Commun. 276 (2000) 385–389.
[94] C.M. Smas, L. Chen, H.S. Sul, Cleavage of membrane-associated pref-1 generates a soluble inhibitor of adipocyte differentiation, Mol. Cell. Biol. 17 (1997) 977–988.
[95] C.M. Smas, H.S. Sul, Pref-1, a protein containing EGF-like repeats, inhibits adipocyte differentiation, Cell 73 (1993) 725–734.
[96] C.M. Smas, H.S. Sul, Molecular mechanisms of adipocyte differentiation and inhibitory action of pref-1, Crit. Rev. Eukaryot. Gene Expr. 7 (1997) 281–298.
[97] D.L. Suskind, K.F. Murray, Increasing the mutation rate for Jagged1 mutations in patients with Alagille syndrome, Hepatology 46 (2007) 598–599.
[98] P.J. Swiatek, C.E. Lindsell, F.F. del Amo, G. Weinmaster, T. Gridley, Notch1 is essential for postimplantation development in mice, Genes Dev. 8 (1994) 707–719.
[99] N. Tanimizu, A. Miyajima, Notch signaling controls hepatoblast differentiation by altering the expression of liver-enriched transcription factors, J. Cell Sci. 117 (2004) 3165–3174.
[100] N. Tanimizu, M. Nishikawa, H. Saito, T. Tsujimura, A. Miyajima, Isolation of hepatoblasts based on the expression of Dlk/Pref-1, J. Cell Sci. 116 (2003) 1775–1786.
[101] A. Theodosiou, S. Arhondakis, M. Baumann, S. Kossida, Evolutionary scenarios of Notch proteins, Mol. Biol. Evol. 26 (2009) 1631–1640.
[102] D. Tornehave, P. Jansen, B. Teisner, H.B. Rasmussen, J. Chemnitz, G. Moscoso, Fetal antigen 1 (FA1) in the human pancreas: cell type expression, topological and quantitative variations during development, Anat. Embryol. (Berl) 187 (1993) 335–341.
[103] P.N. Tsao, F. Chen, K.I. Izvolsky, J. Walker, M.A. Kukuruzinska, J. Lu, W.V. Cardoso, Gamma-secretase activation of notch signaling regulates the balance of proximal and distal fates in progenitor cells of the developing lung, J. Biol. Chem. 283 (2008) 29532–29544.
[104] E. Vasyutina, D.C. Lenhard, C. Birchmeier, Notch function in myogenesis, Cell Cycle 6 (2007) 1451–1454.
[105] E. Vasyutina, D.C. Lenhard, H. Wende, B. Erdmann, J.A. Epstein, C. Birchmeier, RBP-J (Rbpsuh) is essential to maintain muscle progenitor cells and to generate satellite cells, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 4443–4448.
[106] C.J. Vessey, P.M. de la Hall, Hepatic stem cells: a review, Pathology 33 (2001) 130–141.
[107] J.W. Voss, M.G. Rosenfeld, Anterior pituitary development: short tales from dwarf mice, Cell 70 (1992) 527–530.
[108] J.N. Waddell, P. Zhang, Y. Wen, S.K. Gupta, A. Yevtodiyenko, J.V. Schmidt, C.A. Bidwell, A. Kumar, S. Kuang, Dlk1 is necessary for proper skeletal muscle development and regeneration, PLoS One 5 (2010) e15055.
[109] Y. Wang, H.S. Sul, Ectodomain shedding of preadipocyte factor 1 (Pref-1) by tumor necrosis factor alpha converting enzyme (TACE) and inhibition of adipocyte differentiation, Mol. Cell. Biol. 26 (2006) 5421–5435.
[110] Y. Wang, L. Zhao, C. Smas, H.S. Sul, Pref-1 interacts with fibronectin to inhibit adipocyte differentiation, Mol. Cell. Biol. 30 (2010) 3480–3492.
[111] Y. Xue, X. Gao, C.E. Lindsell, C.R. Norton, B. Chang, C. Hicks, M. Gendron-Maguire, E.B. Rand, G. Weinmaster, T. Gridley, Embryonic lethality and vascular defects in mice lacking the Notch ligand Jagged1, Hum. Mol. Genet. 8 (1999) 723–730.
[112] H. Yanai, K. Nakamura, S. Hijioka, A. Kamei, T. Ikari, Y. Ishikawa, E. Shinozaki, N. Mizunuma, K. Hatake, A. Miyajima, Dlk-1, a cell surface antigen on foetal hepatic stem/progenitor cells, is expressed in hepatocellular, colon, pancreas and breast carcinomas at a high frequency, J. Biochem. 148 (2010) 85–92.
[113] A. Yevtodiyenko, J.V. Schmidt, Dlk1 expression marks developing endothelium and sites of branching morphogenesis in the mouse embryo and placenta, Dev. Dyn. 235 (2006) 1115–1123.
[114] H. Zhang, J. Noohr, C.H. Jensen, R.K. Petersen, E. Bachmann, B. Teisner, L.K. Larsen, S. Mandrup, K. Kristiansen, Insulin-like growth factor-1/insulin bypasses Pref-1/FA1-mediated inhibition of adipocyte differentiation, J. Biol. Chem. 278 (2003) 20906–20914.
[115] X. Zhu, J. Zhang, J. Tollkuhn, R. Ohsawa, E.H. Bresnick, F. Guillemot, R. Kageyama, M.G. Rosenfeld, Sustained Notch signaling in progenitors is required for sequential emergence of distinct cell lineages during organogenesis, Genes Dev. 20 (2006) 2739–2753.
[116] Y. Zong, A. Panikkar, J. Xu, A. Antoniou, P. Raynaud, F. Lemaigre, B.Z. Stanger, Notch signaling controls liver development by regulating biliary differentiation, Development 136 (2009) 1727–1739.
[117] P.A. Zweidler-McKay, Notch signaling in pediatric malignancies, Curr. Oncol. Rep. 10 (2008) 459–468.