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Review Article

Role of RBPJκ in Notch Dependent Signalling in Early Embryonic Development of Mice

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Abstract: Notch signalling is an intercellular signalling mechanism that belongs to a family of four highly conserved transmembrane proteins. Its core transcriptional regulator RBPJκ serves as a molecular centre for interactions with corepressor or coactivators, which inhibit or promote transcription, respectively. It is the main protein that control the expression of the target proteins involved in cell fate decisions by mediating signalling from Notch. Notch signalling pathway is an evolutionarily conserved signalling system that has been proven to be crucial for cell fate specification and embryonic development of all metazoans. Therefore, mutations or complete loss-of-function of Notch, RBPJκ, and associated genes may effects on embryonic development or sometimes also results in embryonic death. In the present study, we reviewed the previous literatures for the better understanding of the RBPJκ dependant notch signalling role in the embryonic development of the mice.

Keywords: RBPJκ, Notch signalling, endometrium, embryonic development

Introduction

The endometrium is a dynamic tissue that goes through continuous process of proliferation, differentiation, shedding, renewal and repair, regulated by ovarian hormones- estrogen and progesterone (Cousins *et al.,* 2021). During the follicular phase of the estrous cycle, the endometrial tissue proliferate and prepare itself to receive the ovulated oocyte in its dictyate state. During luteal phase exerts its effects on estrogen primed endometrial tissue induce altered morphological and biochemical milieu. Fertilization of the oocyte and formation of the zygote triggers the transformation of endometrial maternal tissue which is the *sine qua non* for attachment, growth and development of the embryo. The whole event requires an orchestrated support of gonadal steroid estrogen and progesterone as well as the growth factors in the fetal-maternal tissues (Saikia *et al*., 2017). The embryo implantation in the endometrial tissue is a "hallmark" event in the process of reproduction. During this, the embryo establish contact with the endometrium encompassing a complicated series of signalling activities that are critical for successful pregnancy establishment (Weberling and Zernicka-Goetz, 2021). Under the impact of ovarian hormones, a vast number of known molecular mediators such as growth factors, adhesion molecules, cytokines, lipids, and other compounds have been reported to be involved in the early fetal- maternal interactions (Achache and Revel, 2006). These molecular factors from blastocyst and endometrium mediates their effect through endocrine, autocrine, paracrine and juxtacrine signalling systems are essential in correct embryo implantation and progress in pregnancy (Soni *et al*., 2021).

The Notch signalling system is a juxtacrine cellular signalling found in numerous organs throughout the body including the reproductive tract. Notch signalling is hormone-regulated and mediates essential reproductive events in the female reproductive tract, such as ovarian and uterine function. It is also important for the development and function of both the male and female reproductive systems (Moldovan *et al.*, 2021). This pathway mediates its signalling by its transmembrane receptor protein which is known to be involved in both lateral and inductive signalling between local cells across a wide range of tissues and organisms (Artavanis-Tsakonas *et al.*, 1995).

The Notch signalling

Notch signaling pathway is evolutionary conserved from insects to mammals. This is an intercellular signaling mechanism that takes major part in cell survival, communication between neighbouring cells. It is also crucial in cell differentiation proliferation, and apoptotic programs that influences the process of organ formation and morphogenesis. It controls both the intrinsic and extrinsic developmental signals crucial for embryonic development in mammals (Artavanis-Tsakonas 1999). This pathway plays key role in tissue homeostasis and stem cell maintenance in adults as well (D'Souza *et al.*, 2010). It also regulates many developmental processes like embryonic hematopoiesis, neurogenesis, vasculogenesis, somitogenesis, miogenesis (Robert-Moreno, 2007), development of cardio-vascular, endocrine, central nervous system, epithelial-mesenchymal transition, cellular adhesion and migration (Bolos *et al.*, 2007). It maintains bone homeostasis (Tu *et al.*, 2012), angiogenesis (Fujikura *et al.*, 2006) and the development of the epithelial tissues in ovary, uterine endometrium, breast and cervix (Orzechowska *et al.*, 2020).

Notch pathway belongs to a family of highly conserved transmembrane receptor protein (Notch), Delta–Serrate–Lag (DSL) family ligands (Delta and Serrate/Jagged in *Drosophila* and vertebrates, Lag-2 in *Caenorhabditis elegans*), and DNA-binding nuclear protein CSL (CBF1/RBPJk in vertebrates, Su(H) in *Drosophila*, Lag-1 in *C. elegans*) (Raya *et al.*, 2003). In mammals, there are four heterodimeric transmembrane Notch receptors (Notch 1-4), five ligands (Jagged 1, 2, Delta-like 1, 3 and 4) and the nuclear transcription factor CSL (CBF1/RBPJκ) (Orzechowska *et al.*, 2020; Robert-Moreno, 2007). The Notch receptor contains an extracellular domain with multiple EGF-like repeats, a trans-membrane domain, and an intracellular domain (Saini and Sarin, 2019). This single-pass transmembrane Notch receptor, its ligands (DSL family) and nuclear transcription factor RBPJκ are important in uterine receptivity, implantation as well as embryonic-uterine interactions and remodelling of the decidua (Zhang *et al.*, 2014; Robinson and Fisher, 2014). The role of the Notch pathway in development to fine-tune morphogenetic events has been and continues to be extensively supported by research. Notch is pleiotropic not only in terms of the wide range of tissues it impacts during ontogeny, but also in terms of the fundamental developmental processes. Notch activity have a significant impact on differentiation, proliferation, and apoptotic cellular processes depending on the developmental setting. Notch activation in one cell, however, can have an indirect effect on distant cell populations. In many different species, Notch activation in one tissue drives cellular proliferation while it triggers apoptosis in another. Modulating Notch activity will cause cell fate modifications, at least in cells that are not terminally differentiated. The type of the subsequent cell fates is impossible to predict in advance because the fates affected will rely on both developmental environment (spatial and temporal) and the intensity of Notch signalling (Artavanis-Tsakonas and Muskavitch, 2010).

It is therefore, known that Notch controls both the intrinsic and extrinsic developmental signals which are necessary to understand and reveal specific developmental programs. The Notch activity influences the differentiation, proliferation, and also apoptotic programs, thus providing a general developmental tool to influence the process of organ formation and morphogenesis and is therefore crucial for embryonic development in mammals. Mutations in the Notch signaling pathway lead to disruption in cell fate specification and embryonic development of insects to mammals (Xue *et al.*, 1999, Artavanis-Tsakonas 1999).

RBPJκ- Notch signalling in embryogenesis

RBPJê is a sequence specific DNA binding factor that recognizes (C/T)GTGGGAA consensus sequence. The structure of RBPJκ is highly conserved throughout evolution. RBPJκ is involved in determination of cell fate in many cell lineages. It is the main protein that controls the expression of the target proteins involved in cell fate decisions by mediating signalling from Notch, a neurogenic transmembrane-type protein, to the nucleus in a unique manner. The Notch signalling is known to take part at every stage of embryogenesis in addition to oogenesis, neurogenesis, somatogenesis, and hematopoiesis (Tani *et al*., 1999). It impacts both the intrinsic and extrinsic developmental signals crucial for embryo as well as post-natal tissue homeostasis and stress response maintenance in adult (Artavanis-Tsakonas 1999; Malashicheva *et al*., 2020). It also regulates cellular activities such as cellular communication, differentiation, proliferation, apoptosis and stem cell maintenance. These processes are controlled via cell to cell communication that influences the process of organ formation and morphogenesis (Dutta *et al*., 2021). Therefore, mutations or complete loss-of-function of *Notch*, *RBPJκ* and associated genes results in embryonic death (Tani et al., 1999). Various earlier literatures suggested that the RBPJκ dependant notch signalling pathway plays a crucial role in the embryonic development of the mice.

Structure of RBPJκ

The RBPJκ protein has a highly conserved core region of around 420 amino acids surrounded by additional N- and C-terminal extensions of different lengths (Kovall and Blacklow, 2010). It has structural resemblances with the Rel transcription factor family. RBPJκ structure is composed of three domains namely N-terminal domain (NTD) similar to Rel homology region-N (RHR-N) domain, a central beta-trefoil domain (BTD) and C-terminal domain (CTD) resembling to an IPT/TIG domain (RHR-C) (Kovall and Hendrickson, 2004). NICD and the co-repressors interact with RBPJκ along the central region BTD. The NTD and BTD interact by binding to the major groove and the minor groove of the cognate DNA respectively whereas the CTD does not interact with the DNA at all. This side chain interaction of the NTD and BTD with the DNA contributes to structural justification of the DNA site specificity for RBPJκ transcription factor (Kovall, 2007). The NTD and CTD are also crucial for its association with the Notch's ANK (Ankyrin) repeats. The central domain of RBPJκ is critical for its binding with DNA and Notch's RAM domain. This interaction causes a significant conformational change in RBPJκ's N-terminal domain which increases the accessibility of Notch's ANK repeats to the CTD of RBPJκ. The consequent binding of the Notch ANK repeats to the CTD of RBPJκ establishes a full docking site for Mastermind, the co-activator nuclear protein. All of these interactions are required for RBPJκ -mediated Notch intracellular domain transactivation (Tanigaki and Honjo, 2010).

Different Roles of RBPJκ

RBPJκ- as the nuclear transducer

RBPJ was discovered thirty years ago and was named *RBPJκ* (Giaimo et al., 2020). The gene *RBPJκ* (*CBF1* or *KBF2*) is a member of the CSL protein family. CSL represents for CBF1 (human)/ RBPJκ (mice), Suppressor of Hairless (*Drosophila*), Lag-1 (*C. elegans*). RBPJκ is a 60 kDa nuclear protein first isolated from B-cells (Borggrefe and Oswald, 2009). This gene is evolutionarily conserved with 84% sequence identity between *Drosophila melanogaster* and humans (Kovall and Hendrickson, 2004). This potent DNA binding protein RBPJκ, has direct interaction with Notch/LIN-12/GLP-1 but doesn't consist of the known DNA-binding protein motifs like zinc finger, helix turn helix, helix loop helix and leucine zipper (Oka *et al*., 1995). As far as is known, only the CSL [CBF, RBPJ/Su(H)/Lag1] proteins operate as transcription factors to regulate the Notch signalling. The CSL proteins statically occupy regulatory regions of the gene regardless of the Notch signalling status, and upon activation of the pathway, NICD and other (co)activators are deployed to replace resident repressors (Castel *et al*., 2013*).*

RBPJκ is recognized for its role as a co-factor in Notch signalling where it converts extracellular signals into

changes in gene expression (Delacher *et al.*, 2019). It identifies the promoter-specific DNA sequence of the Notch target genes. It is a downstream signal molecule of the Notch signalling pathway. It binds to the Notch intracellular domain (NICD) complex in the nucleus regulating the expression of the target genes which are related to cell cycle or apoptosis (Cyclin D1, Cyclin-dependent kinase 2, p21, and Bcl-2) (Xiao *et al.*, 2019). The activated NICD has a domain named RAM (RBPJκ Associated Module) by which the NICD gets associated with RBPJκ. This RAM domain plays a role in target gene promoter activation in reporter gene assays. RBPJκ plays a key role in the regulation of the Notch target genes as mutations of RBPJκ binding sites in the promoters of HERP1, HERP2, and HES1 genes stop their activation (Iso *et al.*, 2003).

RBP-J acts as molecular switch in Notch pathway

In canonical Notch pathway, the transmembrane Notch receptor of a cell connects with one DSL (Delta/Serrate/LAG-2) ligand on a nearby cell via its extracellular domain. This triggers a signalling cascade of two proteolytic cleavages. The first cleavage is catalysed by tumour necrosis factor α-converting enzyme (TACE) namely disintegrin-metalloproteinase of ADAM family (Adam 10, Adam 17) which occurs in the extracellular domain and the second cleavage of the truncated receptor by intracellular α-secretase complex (comprising Psen 1, Psen 2, Pen 2, Aph 1 and nicastrin). These two cleavages results in the release of the NICD (Orzechowska *et al.*, 2020). NICD then translocates to the nucleus and interacts with downstream effector DNA-binding protein RBPJκ (CSL), through RAM domain and acts as a transcriptional activator. The RBPJκ gets ubiquitously expressed and associates with all the four types of Notch receptors. The co-repressors from RBPJκ gets replaced after the association of the NICD with RBPJκ and recruits a nuclear protein Mastermind and other cofactors. NICD- RBPJκ together with MAML 1 (mastermind-like 1) forms a trimeric co-activator complex upregulating the expression of various target genes viz. HES/HEY family (Hes 1, Hes 5, Hey 1, Hey 2 and HeyL) that play a critical role in regulation of cellular differentiation (Souilhol *et al.*, 2006; Tanigaki *et al.*, 2002). RBPJκ recruits a corepressor complex in the absence of Notch with distinct components at various Notch target genes. The corepressor complex is displaced in the presence of NICD and a coactivator complex containing Notch and Mastermind is recruited. The binding of RBPJκ to NICD is critical for transition from repressed to an active state (Borggrefe and Oswald, 2009). RBPJ therefore acts as a molecular switch, reversing the transcriptional activation or repression of Notch target genes (Kohyama *et al.*, 2005) (Fig. 1).

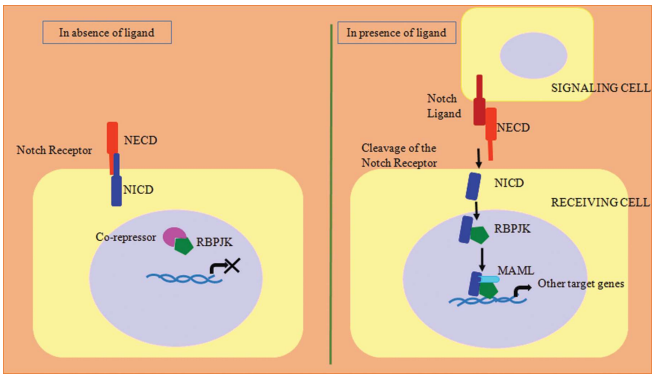

Fig. 1. Notch signaling pathway. In absence of Notch ligand the corepressor molecule stays bound to RBPJK. In the presence of ligand, the NECD binds with the ligand. NICD moves to the nucleus and binds to the RBPJK along with MAML and activates the target genes.

The Notch transcriptional activation complex (NTC) is formed when NICD, RBPJκ, and other co-activators are activated, allowing target genes to be expressed. In the absence of NICD, RBPJκ interacts with a number of transcriptional co-repressors, such as KYOT2 or MINT, to prevent Notch target genes from being expressed. As a result, RBPJκ's function is diverse and context-dependent. Loss of RBPJκ inhibits Notch target genes and limits the regulation of Notch-driven physiological states in some circumstances, such as marginal zone B cell development or muscle progenitor cell maintenance. Loss of RBPJκ leads to the "de-repression" of Notch target genes and the encouragement of biological processes that are otherwise inhibited in the absence of Notch signalling in other circumstances, such as the maintenance of adult neural stem cell populations or breast cancer. Identifying RBPJ's molecular partners will aid in a better understanding

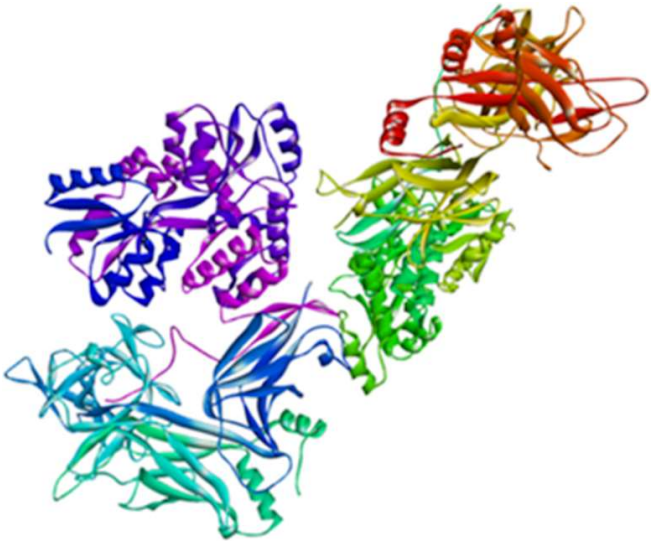

Fig. 2. Crystal structure of RBPJ-SHARP-DNA Repressor complex (PDB ID: 6DKS) (Source: RCSB PDB).

of the complicated and context-dependent role of RBPJ in Notch signalling control in both normal and disease states (Xu *et al.*, 2017). It is important to know the function of RBPJ in the presence and absence of Notch as it affects regulation of chromatin and thus target specificity (Giaimo *et al.*, 2020).

Crucial role of RBPJ- Notch signalling in early embryonic development in mice

RBPJκ and the other Notch pathway components are necessary for embryonic development. The functions of RBPJκ in early embryogenesis in mice were studied through replacement mutations. Those studies revealed that mutations impair the RBPJκ protein's DNA-binding capabilities. The alteration of the RBPJκ gene function was introduced in embryonic stem cells by homologous recombination. Homozygous RBPJκ-/- mutant mice had intense developmental delay during early day 8.5 post-coitum (pc) embryonic development compared to heterozygous mice litters. Abnormal development of the placenta takes place at day 8.5 pc as the allantois of homozygous RBPJκ-/- embryo is unsuccessful to merge with the chorion. The neural as well as somatic development of the homozygous RBPJκ-/- embryos have specific defects leading to embryo lethality before day 10.5 pc. About 10-25% of the inter-crossed *RBPJκ*+/- mice embryos get resorbed at day 8.5 pc. In some cases empty yolk sacs were observed at day 9.5 which indicates the embryos were already resorbed. Consequently embryos with the mutant RBPJκ (RBPJ*k*-/-) allele were dead or in the process of resorption before reaching 10.5 days of pregnancy. At day 12.5 homozygous mutant embryos were no more found. The lack of functional RBP-Jk expression causes embryonic mortality to begin at around day 8.5. Although homozygous mutant embryos demonstrate significant post-implantation development, they do so at a significantly slower rate than wild-type embryos. The absence of functional *RBPJk* shows retarded embryo development and reduced litter size. These findings showed that a functioning *RBPJk* gene is required for post-implantation development normally in mice (Oka *et al*, 1995).

Another study also confirms the possible role of RBPJκ in early development in mice based on the expression of several essential factors of the Notch pathway including RBPJκ in the oocytes and/or preimplantation embryos (fertilized egg to the implanting blastocyst stage), during which first cell fate specifications of the embryo take place. Zygotes that lack both maternal and zygotic expression of RBPJκ gives rise to normal blastocysts. These blastocysts have the ability to get implanted and develop until gastrulation stage but dies at around day 10-day 11 pc exhibiting developmental anomalies. These embryos shows similar phenotypic abnormalities as the embryos with homozygous null mutation of RBPJκ (Souilhol *et al.*, 2006).

The RBPJκ knock-out models in mice revealed the loss in expression of RBPJκ showed a lethal phenotype during the embryonic development at day 10.5 of pregnancy. In the hematopoietic system, conditional deletion results in a block in the development of T-cell and ectopic B-cell development in the thymus. This RBPJκ conditional deletion phenotype is alike to Notch1 conditional knock-out mice which points out that "canonical" Notch signalling (signalling through RBPJκ) is the principal pathway in the development of the lymphoid system (Borggrefe and Oswald, 2009).

RBPJκ regulates the determination of cell fate in various lineages as it is a key mediator in Notch signalling.

Notch plays an important role in the maintenance and proliferation of the hematopoietic stem cells and also regulates the development of the myeloid progenitor cell (Tanigaki *et al*, 2002). When the *RBPJκ* gene is disrupted in mice, the embryonic development changes in a similar way as Notch 1 gene knockout mice, with disorganized and delayed somitogenesis and embryonic death around day 10 of pregnancy. Induced deletion of Notch 1 and gene encoding RBPJκ in adult mice bone marrow cells results in impairment of early development of T cells and ectopic differentiation of B cells inhibiting its development (Han *et al*, 2002). The members of the Notch family controls the differentiation of the hematopoietic cells in many different lineages. The Notch is involved in the start of embryonic hematopoiesis and thus confirms that hematopoietic cells development from the hemogenic endothelium is a Notch1-regulated event and it is damaged in Notch1-deficient embryos. RBPJκ mutant embryos also show a lack of intra-embryonic hematopoiesis. Different Notch signals operate in the embryonic hematopoietic system as distinct expression patterns of several members of the Notch family can be observed in presumptive E9.5 and 10.5 hemogenic endothelium. Notch1/RBPJκ regulates and activates expression/transcription of Gata2 which is required for the determination of embryonic hematopoiesis i.e. generation and proliferation of HSCs. Therefore, for the generation of intra-embryonic hematopoiesis, Notch1 is dependent on the transcription factor RBPJκ and several Notch family members (Robert-Moreno *et al*., 2005).

Cell culture experiments of the embryonic retina of chicks, the embryonic *Xenopus* CNS, and mammalian cells revealed that the Notch signalling pathway is also involved in neurogenesis of vertebrates. Although phenotypes of mouse mutant in *Notch1* or its downstream effector *RBPJκ* does not exhibit the role of Notch signalling pathway in neurogenesis as mutations in both the genes causes death of the embryo at around day 9 during its development, just at the beginning of neuronal differentiation. The RBP-Jκ protein is localized in the nucleus throughout embryogenesis of the wild-type and *Notch1* mutant embryos. In mouse, the Notch signalling activation negatively regulates the neuron formation in the neural tube as more cells express early differentiation of neuronal markers in both *RBPJκ* and *Notch1* mutants. The *RBPJκ* mutants show a more severe neurogenic phenotype than the *Notch1* mutants indicating that there may be functional redundancy of the different Notch proteins of the mouse (Pompa *et al*, 1997).

The RBPJκ dependent Notch signalling pathway is also involved in organ development from the germ lines in invertebrates. In the vertebrates, the Notch receptors, ligands, and other components also get expressed in different organs from the germ lines. Deformity in different tissues plus central nervous system, heart, vessels, thymus, kidney, craniofacial region, rib, somite, limb, and hematopoietic cells can occur due to mutations in both Notch receptor and ligand. In metazoa, the Notch signalling controls cell fate by local cell-cell interactions, determines cellular proliferation, differentiation, and apoptosis. Binding of the ligand leads to proteolytic cleavage of the Notch receptors releasing the NICD (Notch intracellular domain). The signal transducing NICD migrates into the nucleus and connects with the nuclear proteins of the RBPJκ family (CSL or CBF1/Su(H)/Lag-1). The NICD-RBPJκ complex now acts as a transcriptional activator by activating primary target genes of Notch signalling such as HES and enhancer of split [E(spl)] families (Iso *et al,* 2003).

RBPJκ on the event and mechanism of endometrial receptivity

The role of *RBPJκ* on the event and mechanism of endometrial receptivity, simultaneously the decidual remodelling has been studied in vivo in mice model system. Following implantation of the mice embryo to the maternal receptive uterus, the uterine stromal cells surrounding the embryo undergo proliferation and differentiation into polyploid decidual cells, the phenomenon known as decidualization (Namiki *et al*., 2018). The decidualization regulates the microenvironment of the uterus and promotes the successful implantation of the blastocyst indicating its importance during early pregnancy. Complex signalling processes involving transcription factors,

cell-cycle genes, cytokines, growth factors, lipid mediators and other regulatory molecules regulate uterine decidualization in implantation (Gao *et al*., 2015). *RBPJκ* plays a major role in uterine endometrial receptivity and embryo implantation. *This* gene is involved in uterine transformation during the peri-implantation period in the mouse uterus. Embryo implantation can occur in the absence of *RBPJκ*, but at the time of implantation the embryonic orientation gets disrupted. Uterine-specific deletion of the nuclear transducer of Notch signalling, *RBPJκ*, via progesterone receptor (PR)-Cre recombination results in abnormal embryonic-uterine orientation and decidual patterning at post-implantation stages, which leads to substantial embryo loss, reduces the rate of pregnancy and size of the litters (Robinson and Fisher, 2014). Uterine RBPJκ is also important for normal female fertility as the pregnancy rate and litter size were observed to be markedly lower in the *RBPJκ d/d* females compared with the RBPJκ-loxp mice, *RBPJκ f/f* females mated with wild-type (WT) males. Major defects in pregnancy which leads to embryonic mortality may have begun at around the post-implantation to mid-gestation time as very clear defects were observed on pregnancy day 12 showing that a substantial number of implantation sites were completely absorbed while the pregnant uterus on days 5 and 8 were normal in the *RBPJκ d/d* mice. From day 6 to day 8, the *RBPJκ* mutant mice uterine tissues retained severely deflected uterine embryonic axis, abnormal uterine boundary and decidual shape. These decidual pattern changes are well evidenced in the pattern of expression in various decidual marker genes like *IL-11 Ra*, *Wnt 4* and *Bmp 2*. The defects in embryonic orientation and decidual patterning causes abnormal development of the embryo which can be characterized by significantly reduced length and area of the embryo, indicating restrained growth of the embryo and subsequent lethality. The uterine lumen had abnormal crypts rather than the normal vertical slit along a mesometrial-antimesometrial (M-AM) axis orientation, and the attached embryos were disoriented in detailed morphological analyses. Abnormal expression of E-cadherin in association with the uterine epithelia that lined these crypts observed at a molecular level. Spatiotemporal expression of *RBPJκ* in the peri-implantation mouse uterus by *in situ* hybridization analysis showed weak expression in the luminal epithelial cells on Day 1 of pregnancy. On day 4, *RBPJκ* gets expressed in the subepithelial stroma region and it expands to the entire stromal bed on the onset of day 5. On day 6, the *RBPJκ* was expressed in the decidual cells on the anti-mesometrial half of the uterus, and on day 8, the expression shifts to secondary decidual zone and mesometrium. In peri-implantation uterus, the expression of the Notch ligands and receptors overlaps with that of *RBPJκ* in a spatiotemporal manner suggesting its role in uterine functions during both pre- and post-implantation. *RBPJκ* physically interacts with uterine estrogen receptor (ERα) in a Notch pathway-independent manner before attachment with the embryo and grants on-time uterine lumen shape transformation which is crucial for the initial formation of embryo orientation in proper alignment with the uterine axis. Also, *RBPJκ* directly controls the uterine matrix metalloproteinase expression in a Notch pathway-dependent manner during post-implantation stages which are essential for normal remodelling of the decidua (Zhang *et al*, 2014).

During implantation in mice, loss of RBPJκ in the uterine tissues results in subfertility due to abnormal uterine–embryonic axis and failure in the process of decidualization. Induction of decidualization *in vivo* in RBPJκ c-KO mice was weakened due to the down regulation of decidual markers and also decrease in the signalling of progesterone receptor (Pgr). RBPJκ knockdown during *in vitro* decidualization of Human uterine fibroblast (HuF) cell results in the decrease in decidual marker genes expression along with PGR. During decidualization Notch signalling through RBPJκ controls both the expression of ovarian steroid receptor PGR and glucose transporter SLC2A1, and dysregulation imparts to failure in embryo implantation. Uterine RBPJκ is therefore indispensable for normal embryonic development by directing initial embryonic-uterine orientation and normal patterning of decidua in a stage-specific manner (Strug *et al*, 2018).

RBPJκ plays very essential roles in various aspects of implantation such as normal embryonic development,

decidual and vascular remodeling, uterine patterning, luminal closure, and vascular development via Notch-dependent and -independent signalling pathways providing understanding about its regulation during implantation RBPJκ is also important in the maintenance of stem cells, cell fate decisions, cellular proliferation and differentiation, embryonic patterning such as the establishment of dorsal-ventral axis and determination of left-right asymmetry. In mice, systemic deletion of RBPJκ results in the lethality of the embryo before gestational day 10.5 due to multiple abnormalities, like retardation of growth, defective somitogenesis, and abnormal development of the placenta (Zhang *et al*, 2014).

RBPJκ dependent Notch signalling is also involved in developmental and homeostatic functions, including organ self-renewal, development of the immune system through the conversion of RBP-Jk from a basal transcriptional repressor to an activator of downstream target genes and also plays role in decidualization in mice as well as in women. In mice, the endometrial Notch 1 gets induced during the implantation whereas, in primates, it gets induced by embryonic signal chorionic gonadotropin. In both the species, loss of Notch 1 results in inhibition of decidualization and increases apoptosis due to altered downstream signalling of the steroid hormone receptor. Reduced expression of RBPJκ in women with unexplained recurrent pregnancy loss links with increased IFN- " levels indicating that RBPJκ also plays role in regulating inflammation during endometrial repair that is important for conceiving in the future, whereas its dysregulation in women may assist as an anonymous contributor to unexplained recurrent pregnancy loss (Strug *et al*, 2018).

Conclusion

Embryonic development occurs in the maternal uterus in mammals. The endometrium undergoes stromal-decidual remodelling in response to blastocyst implantation in order to provide an adequate environment for the developing conceptus (Bao *et al*., 2021). The cycle-dependent physiological changes in the uterus necessitate the precise interaction of various hormones, cytokines, and signalling pathways to ensure successful embryo attachment and implantation (Zafir *et al*., 2021). The transcription factor RBPJκ is an integral part of the Notch signalling cascade. When Notch signalling is active, RBPJκ works as a coactivator, but when a Notch stimulus is absent, it represses the Notch signalling (Pan *et al*., 2021). RBPJκ is a transcription factor that is an essential component in Notch signalling and it is required in early embryogenesis. This transcription factor is known for its role in uterine decidualization to support the pregnancy and initial orientation of the embryo and uterine axis. The role of RBPJκ in early embryonic development during early gestation may help in understanding its importance in early gestation. It would be highly desirable to have novel compounds that specifically disrupt the RBPJ-corepressor function in certain disease settings like acute myeloid leukemia (AML). Similarly, compounds able to disrupt the activation function of RBPJ would be very helpful to avoid the serious off-target effects observed with γ -secretase inhibitors. In line with that, a recent study characterized a new RBPJ inhibitor that prevents both its repressive and activating function. Chromatin regulation is to be expected at the center of RBPJ-mediated repressive mechanisms (Giaimo *et al*., 2020).

Year	Events	Reference
1913	Discovery of Notch gene in *Drosophila melanogatser* by Thomas Hunt Morgan and his colleagues	Yamamoto *et al*., 2014
1914	First report on Notch defect in *Drosophila* by John S. Dexter	Dexter, 1914; Yamamoto *et al*., 2014
1917	The first Notch allele was discovered by Thomas Hunt Morgan	Yamamoto *et al*., 2014
1936	Donald F. Poulson's pioneering work on hemizygous Notch mutant embryos established the first relationship between Notch and development.	

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