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THE NOTCH RECEPTOR PROTEIN FAMILY AND ITS SIGNALLING PATHWAYS IN DIFFERENT MOLECULAR MECHANISMS OF NORMAL OR PATHOLOGICAL FUNCTIONING

FAMILIA DE RECEPTORI PROTEICI NOTCH ȘI CĂILE SALE DE SEMNALIZARE ÎN DIFERITE MECANISME MOLECULARE ALE FUNCȚIONĂRII NORMALE SAU PATOLOGICE

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ABSTRACT | REZUMAT

The paper reviews literature data considering the importance of the Notch receptor protein family and its signalling pathways in different molecular mechanisms of normal or pathological functioning. Notch proteins are single-pass transmembrane receptors encoded by four related genes in mammals, with a highly evolutionarily conserved structure from flies to mammalian species. They are involved in signalling pathways that were assumed with an important role in regulating various cellular processes of proliferation and differentiation, affecting reproductive, digestive, nervous, muscular, and hematopoietic systems. For example, the Notch pathway intervenes in the reproductive process, in terms of affecting the reproductive cycle, the survival rate of the offspring, the steroidogenesis process, or the mammary gland development. Others of its contribution are known in the process of placentation and normal intrauterine development, in Leydig cells differentiation in males, but also in the development of various types of cancer.

Keywords: receptors, ligands, interactions, domains, cellular growth, differentiation, neoplastic transformation

Această lucrare revizuiește datele din literatura de specialitate în acord cu importanța familiei de receptori proteici Notch și căile sale de semnalizare în diferite mecanisme moleculare ale funcționării normale și patologice. Proteinele Notch sunt receptori transmembranari de singură trecere, codificați de patru gene înrudite la mamifere, cu o structură bine conservată evolutiv, de la insecte la specii de mamifere. Aceste proteine sunt implicate în căi de semnalizare care au fost asumate cu un rol important in regularizarea diferitelor procese de proliferare și diferențiere, influențând sistemele reproductiv, digestiv, nervos, muscular și hematopoietic. De exemplu, calea Notch de semnalizare intervine în procesul reproducerii, în condițiile influențării ciclului reproductiv, rata de supraviețuire a descendenței, procesul de steroidogeneză sau cel de dezvoltare a glandei mamare. Alte contribuții ale sale sunt cunoscute în procesul de placentare și de dezvoltare normală intrauterină, în diferențierea celulelor Leydig la mascul, dar și în dezvoltarea diferitelor tipuri de cancer.

Cuvinte cheie: receptori, liganzi, interacțiuni, domenii, creștere celulară, diferențiere, transformare neoplazică

Notch proteins are single-pass transmembrane receptors with a molecular weight of ~300,000, encoded by four related genes in mammals, namely *Notch1/TAN1, Notch2, Notch3, Notch4/Int-3* (5,20). They act as an evolutionarily conserved pathway from flies to mammals, a signalling mechanism that is essential for cell fate regulation and lineage specification during embryonic and postembryonic development (1, 3, 8, 20, 22). An 86.04-98.75% *Notch2* amino acid sequence homology between bovine and other species is an argument for high conservatism of the gene structure, at least for this receptor protein (14).

An updated overview of various scientific reports revealed the *Notch* pathway and its role in the normal or pathological functioning of various organs and systems. There are studies that describe the contribution of the *Notch* pathway in the normal development of the mammary gland and the process of tumorigenesis at this level, its contribution in the process of placentation and normal intrauterine development, in Leydig cells differentiation in males, but also the involvement of different receptors, target genes, or ligands in certain types of blood, kidney or salivary gland cancers. This review presents the molecular functioning of the *Notch* pathway and its involvement in the physiology and pathology of different organs and systems.

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MATERIAL AND METHODS

The molecular mechanisms of Notch pathway signalling and its engagement opportunities in different mechanisms of normal or pathological functioning are

mainly debated in this review. To accomplish this aim of the work, 28 scientific papers selected by the criteria of scientific relevance for the subject matter were revised.

RESULTS AND DISCUSSIONS

1. Molecular insights in the Notch pathway

The Notch pathway is initiated by receptor-ligand interactions between neighbouring cells, in which the four receptors, Notch1-4, are bonded by five ligands located on adjacent cells, Jagged1 (Jag1), Jagged2 (Jag2), Delta-like1 (Dll1), Delta-like3 (Dll3), and Delta -like4 (Dll4) (1,4,19,21,22). The Notch proteins contain an extracellular domain and another one, of transmembrane / intracellular / cytoplasmic type. The extracellular domain comprises ~1,750 amino acids, with 36 tandem repeats of a sequence resembling the epidermal growth factor and three repeats of a motif designed as lin-12 (cysteine-rich) included. As regards the second domain, it contains ~750 amino acids without any apparent enzymatic activity but includes six tandem copies of an ankyrin-like repeat (CDC10/ ANK), a region rich in glutamine (OPA), and a region rich in glutamate, serine, and threonine (PEST). The first two of these have a supposed function of mediation of protein-protein interactions, while the third one may target proteins for degradation (5). Seven CDC10 / Ankyrin repeats were cited by (10) in the intracellular domain content of Notch receptors. The extracellular domain is non-covalently bound to the transmembrane/intracellular/cytoplasmic domain; the extracellular domain is involved in the ligand binding process (3). In an essential cell-to-cell contact mechanism, the Notch signalling is activated through two successive sequential proteolytic cleavages by tumour necrosis factor-alfa-converting enzyme (ADAM family – A Disintegrin And Metalloprotease) and gamma (γ) secretase/presenilin complex (1,4,19,21,22). As a result of these two proteolytic cleavages of the Notch receptor protein, a functionally active form of *Notch* is released, known as the Notch intracellular domain (NICD). In a subsequent step, the released NICD into the cytoplasm translocates to the nucleus to bind a DNA-binding protein that normally acts as a transcriptional repressor, namely CSL, also known as CBF1 (Centromere-binding protein 1) or RBP-Jκ (Recombinant Signal Binding Protein 1 for Jκ) in humans, and Su(H) – Suppressor of Hairless in Drosophila (1, 4, 10, 14, 18, 19, 21, 23, 27). This is a Notch-dependent transcriptional activation of targeted genes (7), including here members of the Hairy/Enhancer of Split (HES), the Hairy/Enhancer of Split related to YRPW motif (HEY) families, the Notch-regulated ankyrin repeat-containing protein (Nrarp) (4, 20-22), p21CI P1/WAF1, cyclin D (1,26), Akt, c-myc, COX-2 (CycloOXygenase-2), mTOR (mammalian target of rapamycin) (3). As an example, the HEY genes (*Hey1, Hey2, HeyL*), a new class of Notch signal transducers, were demonstrated as a group of basic helix-loop-helix transcription factors that possess RBP-Jκ binding sites to respond to the Notch signalling (17). On the other side, a protein that is encoded by a mutant bovine herpesvirus 1 containing stop codons at the amino-terminus of the open reading frame was shown to inhibit the Notch-mediated transcription and signalling (16).

2. Notch signalling pathway engagement opportunities

2.1. The Notch pathway, the development of the mammary gland, and its involvement in the process of lactation

The mechanism of Notch signalling and its involvement in both physiological and pathological pathways is a very complex one, based on the cell-specific type location and interactions, ligands, and receptors activities (20). *Notch* genes were reported to play a critical role in mammary gland growth and development, but also in its tumorigenesis (25). Considering the mouse gland as a model for mammary epithelial growth, morphogenesis, differentiation, and neoplastic transformation, several aspects of Notch signalling were debated by (25) in this regard: increased levels of *Notch 1-3* mRNAs in the mammary gland were found in the organ developed from five weeks of age through the early pregnancy, while in the organ of late pregnant mice, the levels of *Notch1-3* mRNAs were reported as decreased. The percentage of *Notch1* and *Notch3* expressing cells was quantified as the lowest during early pregnancy and lactation, the reduced number of cells expressing *Notch1* and *Notch3* in the mammary glands of pregnant and lactating mice being assumed as important for normal lobular and alveolar development and lactation (25). *Notch1-4* was detected in the parenchyma and stroma of the developing mammary gland of cows, with a predominant cytoplasmic subcellular location of *Notch1-3*, and a mostly nuclear one, for the *Notch4* receptor. The involvement of *Notch* receptors, their target genes, and ligands in the development of mammary gland was determined, with increased *Notch1* and *Notch2* active domains during pubertal stages, *HEY1* and *HEY2* mRNAs expression levels during prepuberty, and also of *Jagged1* and *Delta 1* mRNAs levels. By contrast, a decrease of *Notch3* and *Notch4* active domains was recorded during pubertal stages and a no variation in *HES1* mRNA levels (3).

2.2. Abnormal Notch regulation and the process of mammary tumorigenesis.

The presence of Notch receptors in normal mammary glands was identified at the level of its stroma

and epithelia while an abnormal Notch regulation was increasingly associated with cell transformation and tumorigenesis in the mammary gland (19,20). Activation of Notch signalling (including *Notch1, Notch3*, or *Notch4* but not *Notch2*) in mouse mammary epithelial cells were included as revised as causative factors for blocking the mammary gland normal development and mammary tumorigenesis. An intriguing model of *Notch2* expression and a decrease in breast tumour aggressiveness was also cited, as well as the Notch activation as a suppressor of cell transformation or tumorigenesis (6,20). Relation between obesity and an increased rate of various cancers was observed and a Notch signalling induced by leptin was presumably associated with higher incidence and aggressiveness, and poor prognosis of breast cancer in obese patients (2). In 20-30% of human breast carcinomas, the gene of epidermal growth factor receptor-2 (*HER2*) was cited as overexpressed. Based on its demonstrated involvement in two vital pathways for breast cancer (the Notch and the WNT – Wingless-type mouse mammary Tumour virus integration site), the NRARP (Notch-regulated ankyrin repeat protein) was checked and found as commonly overexpress in the rat's mammary carcinomas (6,12). An increase in the levels of *Notch1, Notch4,* and *Jagged1* expression was cited in human breast cancer tissue, where a *Jagged1* and *Notch1* high expression were correlated to poor patient survival (19, 20). The inhibition of p53 functions, such as those of cell growth arrest inducing, apoptosis, or senescence, is also a mechanism of the Notch-inducing oncogenesis. Specifically, *Notch1* is considered a tumour suppressor gene that is directly regulated by p53 (1,9).

2.3. Others involvements of Notch pathway in female and male reproductive systems

Various studies up to now showed the incursion of the Notch pathway also in other functions and activities of both female and male reproductive systems. Its involvement in normal placentation was described in humans, as being a process conditioned by low oxygen tension or hypoxia conditions in the uterine environment; for this condition, low oxygen levels of 1 to 3% were cited (22) as a promotion factor for trophoblast differentiation, migration, and invasion. Both pathological Notch signalling and inappropriate changes in oxygen tension were discussed as causative factors to the human placental insufficiency associated with preeclampsia (22). The *Notch2* signalling pathway also was reported to play a role in human decidualization, a process of transformation of the human uterine mucosa into the endometrium of pregnancy, ensuring thus the success of the implantation and embryonic development (21). Notch signalling was also found to promote endometrial regeneration and repair (28) and to play an important role in mammalian ovarian development. *Notch2* normally regulates the formation of primitive follicles in mice, its inhibition is associated with a reduction in cell proliferation and an increase in mouse follicular granulosa cells apoptosis (15). Although the bovine *Notch2* gene shared the lowest homology with rodents and the highest with buffaloes, its important role in regulating the development of bovine follicles luteinized granulosa cells was previously described (14). On the potential of oestrogens to modulate the Notch pathway, a signalling effect involved in the control of vasculogenesis and neo-angiogenesis was cited downstream of vascular endothelial growth factor A (VEGF-A) (4). Notch signalling is required during fetal life to maintain a balance between differentiated Leydig cells and their progenitors. However, the *Notch1* overexpression in the male gonads was reported as sufficient to block Leydig cell differentiation (8).

2.4. The involvement of the Notch pathway is not limited only to the level of the reproductive system

Notch is a general signalling pathway for stem cells of several tissues, for example, the central nervous and the hematopoietic systems, muscle cells, and of the intestinal mucosa, etc. (3, 13). The hypoxia was also reported as a promotor of the Notch signalling proteins expression, with implications in intervertebral disc cell proliferation (11). The inclusion of Notch signalling in both developments of melanocytes during embryogenesis and maintenance of melanocyte stem cells in adulthood was also described, with a gradual hair greying phenotype after birth when a reduced Notch signalling was initiated at the embryonic stage (13). The *Notch4* overexpression was cited in various renal cells in human immunodeficiency virus (HIV)-associated nephropathy (HIVAN) and also in rodent models for this pathology (23). An important role of *Notch2* and *Notch4* was described in salivary adenoid cystic carcinoma metastasis (9,24). However, the involvement of the Notch family in oncogenesis is a cell-type/organ-specific process. For example, a *Notch1* abnormal signalling is known as causative in T-cell lymphoblastic leukaemia, certain lymphomas, breast and kidney carcinomas (1).

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