

NUMB is a break of WNT - Notch signaling cycle

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Abstract. Notch, FGF and WNT signaling pathways cross-talk during embryogenesis, tissue regeneration and carcinogenesis. Notch-ligand binding to Notch receptors leads to the cleavage of Notch receptors and the following nuclear translocation of Notch intracellular domain (NICD) to induce transcriptional activation of Notch target genes. Notch signaling inhibitors, NUMB and NUMB-like (NUMBL), are docking proteins with PTB domain. We searched for the TCF/LEF-binding site within the promoter region of *NUMB* and *NUMBL* genes. Because two TCF/LEF-binding sites were identified within human *NUMB* promoter based on bioinformatics and human intelligence (Humint), comparative integromics analyses on *NUMB* orthologs were further performed. Chimpanzee *NUMB* gene, consisting of 13 exons, was identified within NW_115880.1 genome sequence. XM_510045.1 was not the correct coding sequence for chimpanzee NUMB. Chimpanzee *NUMB* gene was found to encode a 651-amino-acid protein showing 99.5, 93.9 and 82.6% total-amino-acid identity with human NUMB, mouse Numb and chicken numb, respectively. Human *NUMB* mRNA was expressed in placenta, ES cells, neural tissues, trachea, testis, uterus, thymus, coronary artery as well as in a variety of tumors, such as cervical cancer, tongue tumor, brain tumor, colorectal and breast cancer. Although distal TCF/LEF-binding site within human *NUMB* promoter was conserved only among primate *NUMB* orthologs, proximal TCF/LEF-binding site was conserved among primate and rodent *NUMB* orthologs. NUMB, JAG1, FGF18, FGF20 and SPRY4 are potent targets of the canonical WNT signaling pathway in progenitor cells. NUMB inhibits Notch signaling in progenitor cells to induce differentiation, while JAG1 activates Notch signaling in stem cells to maintain self-renewal potential. Because Notch signaling inhibitor NUMB was identified as the safe apparatus for the WNT - Notch signaling cycle,

epigenetic silencing, deletion and loss-of-function mutation of *NUMB* gene could lead to carcinogenesis through the dysregulation of the WNT - Notch signaling cycle.

Introduction

Notch signaling pathway is implicated in the maintenance of self-renewal potential in stem cells, binary cell-fate determination in progenitor cells, and induction of terminal differentiation in proliferating cells (1-7). Notch-ligand binding to Notch receptors leads to the cleavage of Notch receptors by metalloprotease and γ -secretase to induce nuclear translocation of Notch intracellular domain (NICD). Nuclear complex, consisting of CSL (RBPSUH), NICD, Mastermind (MAML), p300 and histone acetyltransferase (HAT), then induces transcriptional activation of Notch target genes, such as *HES1*, *HES5*, *HES7*, *HEY1*, *HEY2* and *HEYL*. HES/HEY family members are bHLH-type transcriptional repressors for tissue-specific transcription factors. Therefore, Notch signaling activation in stem cells leads to the maintenance of self-renewal potential.

Notch ligand JAG1 as well as FGF20 and FGF18 are evolutionarily conserved targets of the canonical WNT signaling pathway (7-9). *Notch4*, *Wnt1*, *Wnt3*, *Wnt10b*, *Fgf3*, *Fgf4* and *Fgf8* are up-regulated during mouse mammary carcinogenesis due to MMTV integration (10,11). Notch, FGF and WNT signaling pathways cross-talk during embryogenesis, tissue regeneration and carcinogenesis (1-31).

NUMB and NUMB-like (NUMBL), consisting of phosphotyrosine-binding (PTB) domain and SH3-binding proline-rich region, are docking proteins functioning as Notch signaling inhibitors (32,33). Here, we searched for the TCF/LEF-binding site within *NUMB* and *NUMBL* promoters. Because two TCF/LEF-binding sites were identified within human *NUMB* promoter, comparative integromics analyses on *NUMB* orthologs were further performed.

Materials and methods

WNT target gene screening. Genome sequences corresponding to human *NUMB* and *NUMBL* genes were searched for with BLAST programs (<http://www.ncbi.nlm.nih.gov>) as described previously (34-36). TCF/LEF-binding sites within the 5'-flanking promoter region of above genes were then searched for based on bioinformatics and human intelligence as described previously (37-39).

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Genetyx program revealed that nucleotide position 321-2276 was the coding region of chimpanzee NUMB complete CDS (Fig. 1B). Chimpanzee *NUMB* gene was found to encode a 651-amino-acid protein.

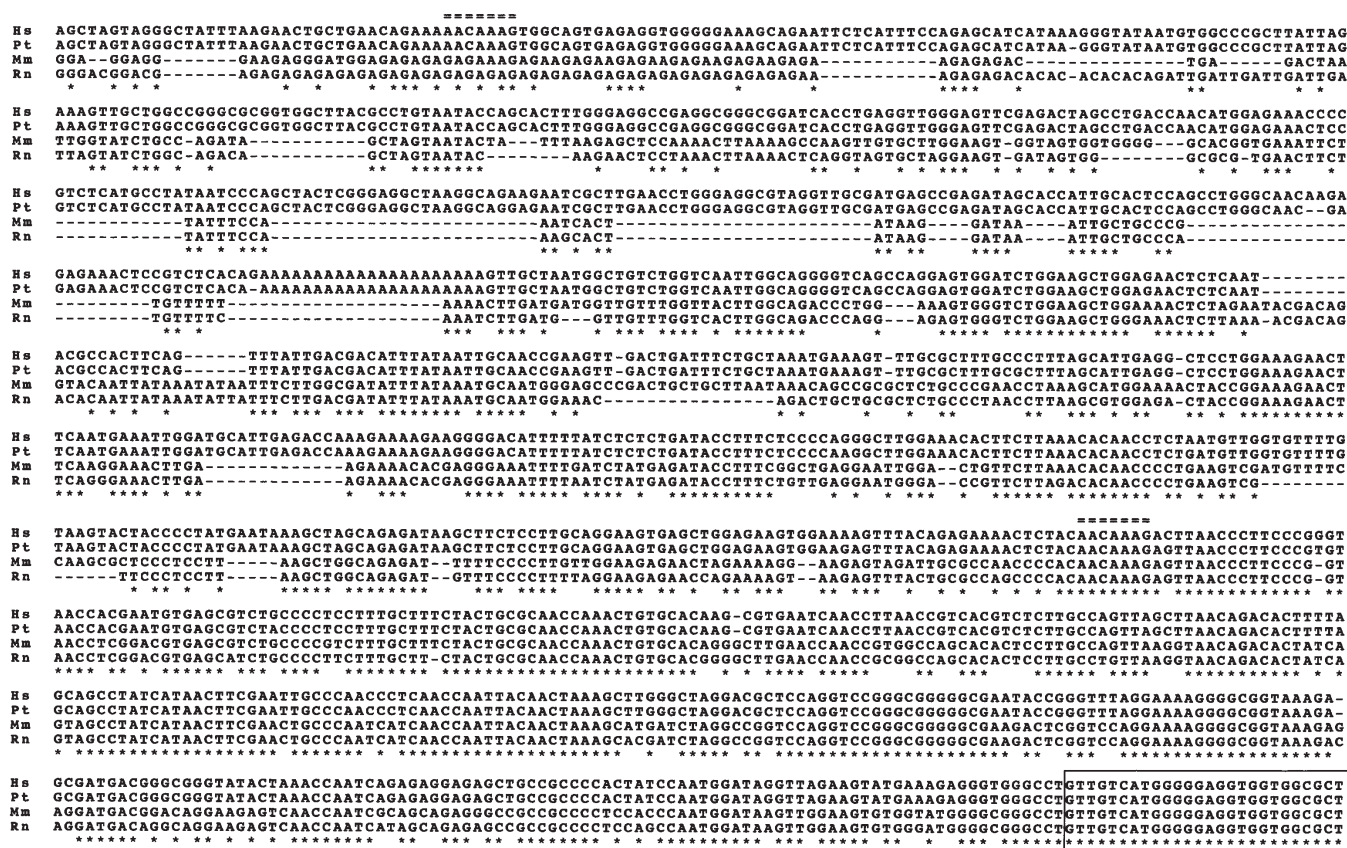


Figure 2. Alignment of mammalian *NUMB* promoters. Hs, human; Pt, chimpanzee; Mm, mouse; Rn, rat. Region corresponding to exon 1 of human *NUMB* gene is boxed. Two TCF/LEF-binding sites within human *NUMB* promoter are shown by double over-lines. Distal TCF/LEF-binding site within human *NUMB* promoter is conserved only among primate *NUMB* orthologs, while proximal TCF/LEF-binding site is conserved among primate and rodent *NUMB* orthologs.

Comparative integromics analysis on *NUMB* and *NUMBL* homologs. Intra-species comparative genomics revealed that *LTBP4-NUMBL-ADCK4* locus at human chromosome 19q13.2 and *NUMB-LTBP2-ADCK1* locus at human chromosome 14q24 were paralogous regions with recombinations within the human genome.

Phylogenetic analysis revealed that zebrafish *numb* was significantly divergent from mammalian *NUMB* and *NUMBL* orthologs (Fig. 1C).

Expression of human *NUMB* mRNA. *In silico* expression analysis revealed that human *NUMB* mRNA was expressed in placenta, ES cells, neural tissues, trachea, testis, uterus, thymus, coronary artery as well as in a variety of tumors, such as cervical cancer, tongue tumor, brain tumor, colorectal cancer and breast cancer.

Comparative genomics analyses on *NUMB* promoters. Human *NUMB* promoter and chimpanzee *NUMB* promoter were located within AC005280.3 and NW_115880.1 genome sequences, respectively, as mentioned above. BLAST programs using mouse *Numb* cDNA (BC033459.1) and rat *Numb* RefSeq (NM_133287.1) revealed that mouse *Numb* promoter and rat *Numb* promoter were located within AC133183.3 and NW_047762.2 genome sequences, respectively. GC content of human, chimpanzee, mouse and rat *NUMB* promoters were 51.3, 51.1, 53.2 and 52.7%, respectively.

Double TCF/LEF-binding sites within human *NUMB* promoter were located about 1100 and 350 bp upstream of the transcription start site (Fig. 2). Proximal TCF/LEF-binding site was conserved among primate and rodent *NUMB* orthologs, while distal TCF/LEF-binding site was conserved only among primate *NUMB* orthologs (Fig. 2).

Discussion

TCF/LEF-binding site within the promoter region of *NUMB* and *NUMBL* genes were searched for in this study. Because two TCF/LEF-binding sites were identified within human *NUMB* promoter based on bioinformatics and human intelligence, comparative integromics analyses on *NUMB* orthologs were further performed.

Chimpanzee *NUMB* gene, consisting of 13 exons, was identified within NW_115880.1 genome sequence (Fig. 1). XM_510045.1 was not the correct coding sequence for chimpanzee *NUMB*. Chimpanzee *NUMB* gene was found to encode a 651-amino-acid protein showing 99.5, 93.9 and 82.6% total-amino-acid identity with human *NUMB*, mouse *Numb* and chicken *numb*, respectively.

The human *NUMB*, chimpanzee *NUMB*, mouse *Numb* and rat *Numb* promoters were located within AC005280.3, NW_115880.1, AC133183.3 and NW_047762.2 genome sequences, respectively. Double TCF/LEF-binding sites were located about 1100 and 350 bp up-stream of the transcription

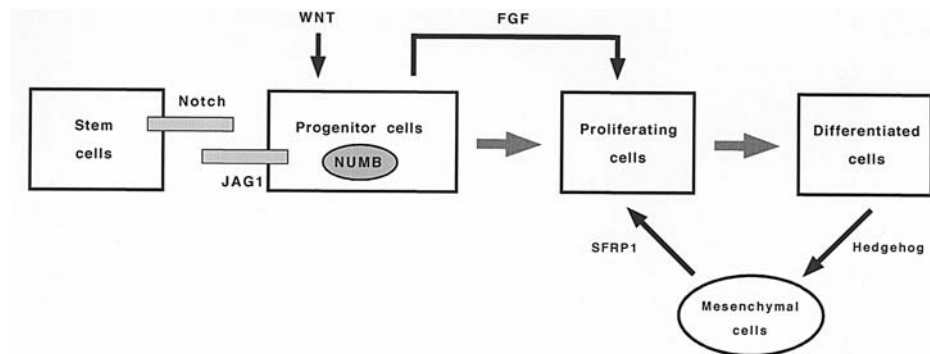


Figure 3. WNT and Notch signaling networks for the homeostasis of stem and progenitor cells. Canonical WNT activates the β -catenin - TCF signaling cascade to induce NUMB and JAG1 expression in progenitor cells. NUMB inhibits Notch signaling in progenitor cells to induce differentiation, while JAG1 activates Notch signaling in stem cells to maintain self-renewal potential.

start site of human *NUMB* gene. Although distal TCF/LEF-binding site was conserved only among primates, proximal TCF/LEF-binding site was conserved among primates and rodents (Fig. 2). NUMB and JAG1 are potent targets of the canonical WNT signaling pathway in progenitor cells. NUMB inhibits Notch signaling in progenitor cells to induce differentiation, while JAG1 activates Notch signaling in stem cells to maintain self-renewal potential (Fig. 3). Notch signaling inhibitor NUMB was identified as the safe apparatus for the WNT - Notch signaling cycle.

In silico expression analyses in this study revealed that human *NUMB* mRNA was expressed in placenta, ES cells, neural tissues, trachea, testis, uterus, thymus, coronary artery as well as a variety of tumors, such as cervical cancer, tongue tumor, brain tumor, colorectal cancer and breast cancer. NUMB-mediated Notch signaling inhibition is lost in a large proportion of human breast cancer due to ubiquitination-dependent NUMB degradation, which results in Notch signaling activation and induction of carcinogenesis (46,47). *NUMB* is a tumor suppressor gene maintaining homeostasis of stem and progenitor cells through the inhibition of Notch signaling.

Epigenetic changes of cancer-associated genes occur during chronic persistent inflammation, and then genetic alterations of cancer-associated genes occur during multi-stage carcinogenesis (48-51). Because epigenetic change and genetic alteration of *NUMB* gene could lead to carcinogenesis through the dysregulation of the WNT - Notch signaling cycle, epigenetic silencing, deletion and loss-of-function mutation of *NUMB* gene in a variety of human tumors should be investigated in the future.

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