The Impact of Notch Pathway on The Occurrence and Development of Cancer

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Abstract. The Notch pathway (NP) controls the growth and development of organisms through intercellular interactions, and is highly conserved. NP extensively affects human life activities. It has an involvement in a range of cellular processes, including the process of the differentiation and proliferation. For example, the normal development of neuronal cells, germ cells and sensory cells in living organisms depends on Notch-mediated inhibition of differentiation. Neurological growth, organ formation, immune system and tumorigenesis are also dependent on NP. Furthermore, available studies show that NP is critical not only in tumorigenesis and progression, but also in prognosis. Currently, the NP has become a hotspot for research in several fields. This article describes some of the roles played by the NP in the nervous system, organogenesis, and immune system. Some of the research advances in tumors related to NP are collated, and the research on the impact of interventions targeting Notch pathway on cancer treatment is summarized.

Keywords: Notch, Signaling Pathway, Cancer.

1. Introduction

Cancer is a disease result of the over proliferation of cells in the body due to the loss of normal regulation. About 4.064 million new cancer cases in China in 2016.

Different signaling pathways are implicated in the oncogenesis. Wnt/β-catenin pathway is involved in a range of cellular mechanisms, e.g., cell proliferation, differentiation, apoptosis, early embryonic development, and tissue regeneration, and is linked to cancer formation [1]. In a variety of cancers, the Hedgehog signaling pathway is expressed in varying degrees. For example, overexpression of the forkhead box protein M1, a Hedgehog signaling pathway downstream target gene, promotes colorectal carcinogenesis [2]. Varied types of tumors have different oncogenic roles for Notch pathway (NP). For example, aberrantly activated NP is implicated in most of the essential features of cancer, such as regulation of tumor angiogenesis, maintenance of tumor stem cell stemness, infiltration of immune cells, and therapeutic resistance [3]. In addition, the NP is vital in therapy and has a lot of potential as a tumor therapeutic target. The NP has emerged as among cancer therapy's most promising targets in recent years, and its aberrant expression in a variety of tumor treatments, such as chemotherapy, radiation, and targeted therapies, further demonstrates its prospective as a drug strategy for tumors.

A thorough understanding of the NP can provide us with more potential molecular targets to design effective targeted drugs. Therefore, this article starts from the basic concept of NP, introduces some functions of NP, and collates some oncology research advances related to NP.

2. Definition of NP and its common functions

2.1. Structure of the NP

The NP plays a role in cellular activity and influences many life activities. The underlying structure of the Notch signaling system is becoming clearer as research continues (Figure1).

Notch is a gene found in Drosophila, named because deletion of part of the gene's function can lead to defects caused by the edges of Drosophila wings. Notch is a single type I transmembrane protein of approximately 300 kDa. Three cysteine-rich LNR-12 repeats and 36 EGF repeats make up the extracellular portion of the Notch. The 11th-12th repeat of the EGF repeats is a critical region for

ligand binding, and the LNR promotes protein dimerization upon ligand binding. There are multiple functional structural domains in Notch's intracellular portion: one RAM region that binds to CSL transcription factors, seven CDC10 epidermal repeats, one nuclear localization sequence, and one C-terminal PEST sequence, in that order, from near the cell membrane. The degradation of the Notch protein is linked to the PEST sequence (Figure2).

In mammals, Notch receptors can be classified into four types (Notch1-4). The main differences between the four mammalian Notch isoforms are the length of the intracellular domain and the abundance of EGF repeats. The ligands of Notch receptor proteins can be divided into the Delta-1 and Jagged/Serrate families. Delta-like ligands do not have this region (Figure3). The ligands of Notch receptors are known to be Jagged1, Jegged2, Delta1, Delta3, Delta4 in mammals. Studies have shown that Delta1 and Jagged1 are located on the surface of hematopoietic cells, bone marrow mesenchymal cells, lymphocytes, and antigen-presenting cells and can induce differentiation of lymphocytes. Delta1 induces Delta1 induces thymocyte differentiation to T cells, while Jagged1 induces thymocyte differentiation to NK cells. Binding of the DSL structural domain of the ligand to the 11th-12th EGF repeat sequence of the receptor activates the NP. The complete Notch protein is synthesized in the endoplasmic reticulum, where the glycosyltransferase OFUT1 enables the binding of rockulose to EGF in the extracellular region of Notch, followed by the Golgi N-acetyl glucosyltransferase Fringe.

Further modification of EGP by Fringe, an N-acetyl glucosyltransferase in the Golgi apparatus, cleaves the Notch receptor into two fragments and thus has the ability to bind specifically to different ligands [5]. After cleavage of Notch precursor protein into heterodimers in the Golgi apparatus, the ligand and receptor are binding, resulting in a TACE metalloproteinase cleavage site for the receptor. After primary hydrolysis outside the cell by TACE, the ligand-expressing cells endocytosed the N-terminal segment, and the C-terminal cleavage fragment undergoes secondary hydrolysis by γ-secretase, released the Notch receptor's active form (NICD/ICN), and through endocytosis and membrane vesicle transport, the ICN fragment enter the nucleus through the nuclear pore. The ICN fragments produced include RAM and Ankyrin, in which RAM binds to CSL/CBF1/RBP-Jk and co-activator Co-A (MAML) to generate transcriptional activators that control the expression of target genes [6].

The CSL transcription factor sequence is located on the promoter of Notch target genes, and when ICN is not present, CSL is a transcriptional repressor, and the binding of both can induce the expression of the corresponding gene.

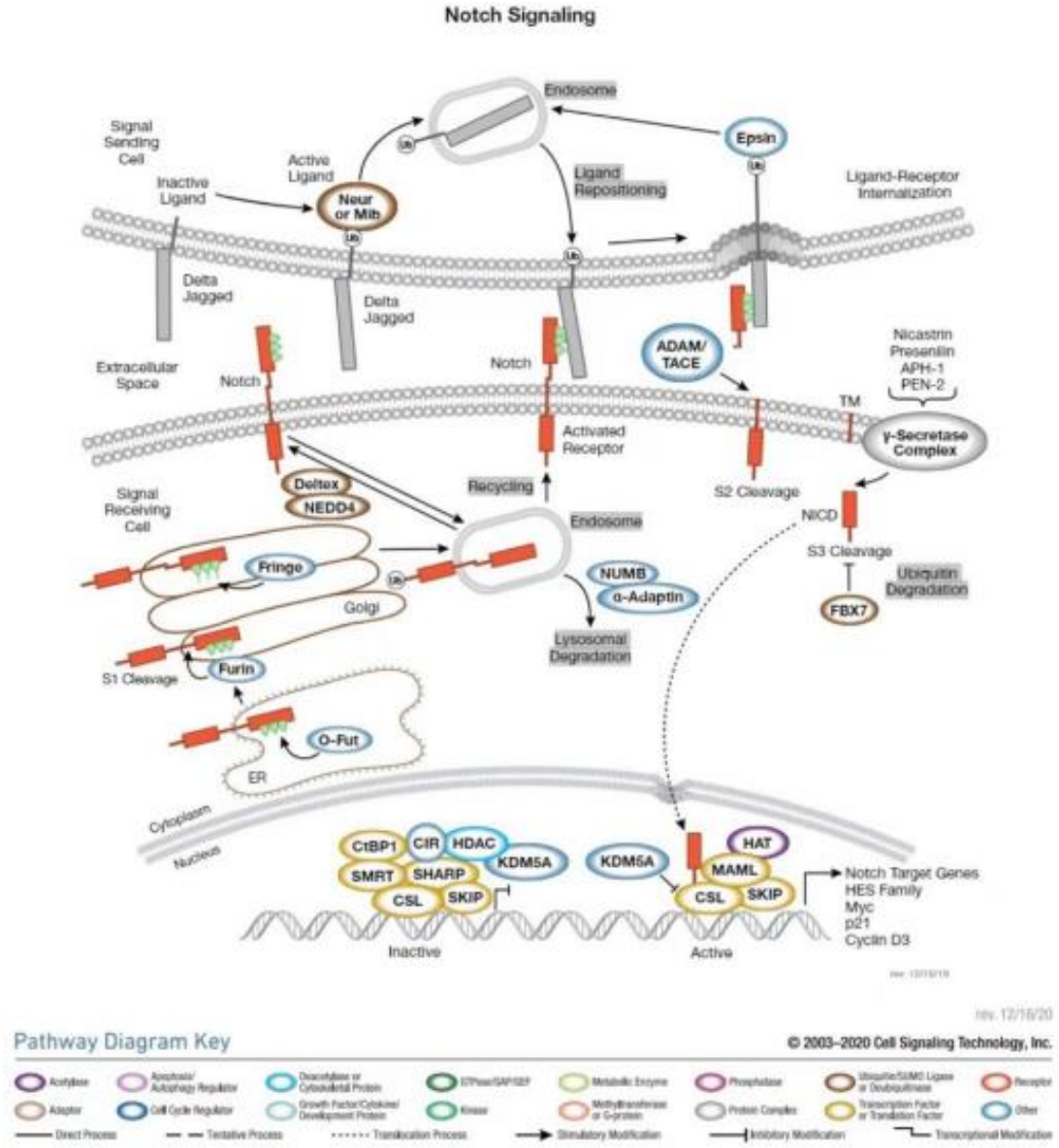


Figure 1. The Notch Pathway [4]

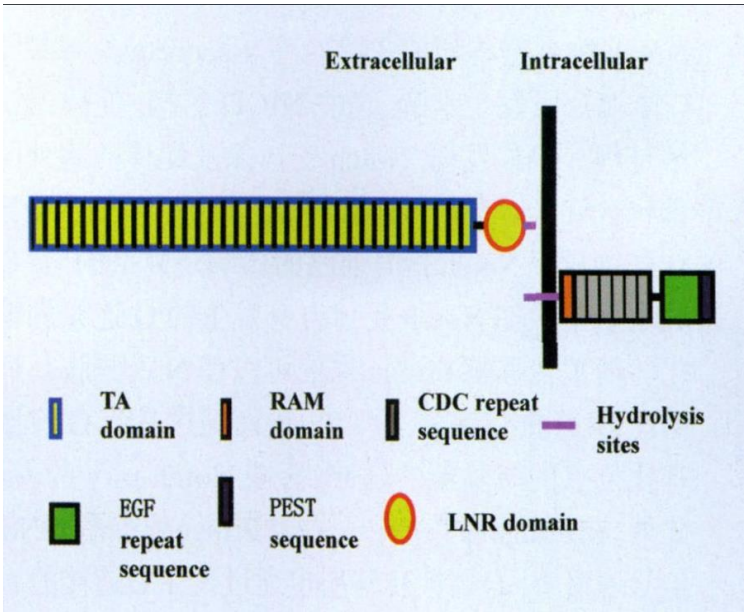


Figure 2. The structure of Notch [4]

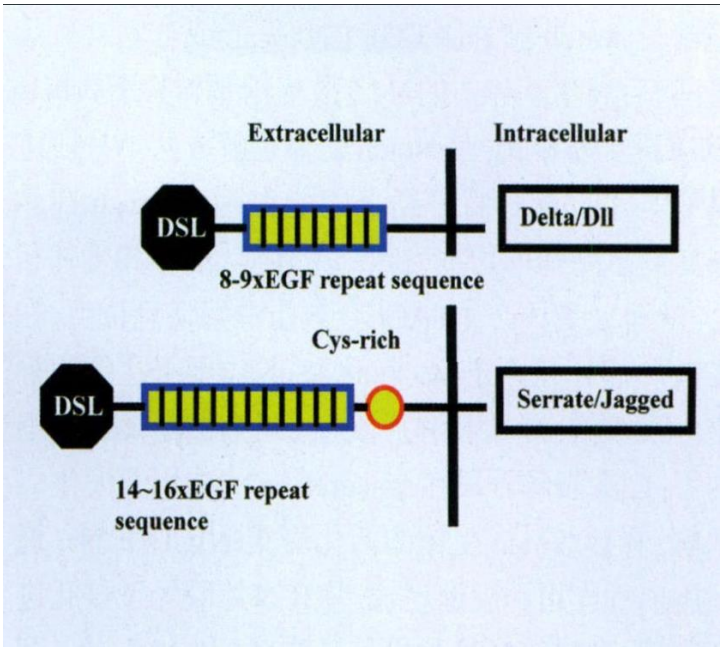


Figure 3. The ligand of Notch [4]

2.2. Partial role of NP in the nervous system

The NSP determines neuronal cell differentiation through paracrine inhibition. The normal development of neuronal cells, germ cells and sensory cells in organisms is dependent on Notch-mediated inhibition of differentiation. A study by Dias et al. found that overexpression of NP by heat shock was able to inhibit motor neuron regeneration and progenitor cell proliferation after zebrafish spinal cord injury, and inhibition of NP by DAPT was able to increase the number of motor neuron regeneration in the zebrafish model [7]. These results suggest that in zebrafish capable of neural regeneration, activation of NP can inhibit neuronal regeneration.

2.3. NP affects organ development

Not only does the NP affect nervous system development, but it also affects organ development. It was found that the expression of Notch signaling appears early in the development of mouse embryonic lungs and the expression level increases with gestational age [8]. NP is involved in preserving the undifferentiated condition of smooth muscle cells and controlling smooth muscle cell proliferation [9]. High Notch3 expression can lead to increased pulmonary blood pressure, which can be addressed with the γ-secretase inhibitor DAPT.

2.4. NP influences tumorigenesis

NP is involved in tumorigenesis. the Tumor microenvironment (TME) includes the peritumor vasculature, immune infiltrating cells, fibroblasts and extracellular matrix. the interaction between TME and tumor cells affects tumor progression, resistance to therapy, invasion and metastasis [10]. One of the important signaling pathways for tumor cell and TME interaction is the NP.

NP regulates tumors by also inducing tumor angiogenesis, which provides nutrition to tumor cells. NP regulates vascular outgrowth by regulating VEGFR2, affecting the differentiation selection of epithelial cells (ECs) toward tip cells versus stem cells [11]. Since Dll4 and Jag1 have opposite functions in controlling outgrowth angiogenesis, the balance of expression of Dll4 and Jag1 in ECs is critical for the formation of tumor vascular structures [12].

3. Study of the effect of interventions targeting the Notch pathway on cancer treatment

The NP is a highly evolutionarily conserved that is involved in various processes of cellular activity. As an important pathway of cellular activity, NP plays different oncogenic roles in different types of tumors, interfering with tumor cell differentiation, proliferation, apoptosis and self-renewal, and participating in tumor angiogenesis. According to existing research, the NP is important not just in tumor lesions but also in prognosis. It also holds promise as a new idea for curing tumors.

3.1. Breast cancer

Breast cancer (BC) is the most threatening tumor for women in recent times, accounting for 7-10% of all malignant tumors in the population. In the advancement of BC, the NP system plays a critical role [13]. The Notch4 locus, which was inserted by the MMT virus, was found in 1987, establishing a relationship between the Notch and BC for the first time [13]. In breast cancer tissues, Notch receptors and ligands also found to be overexpressed, Ras-transformed human cells require Notch1 to retain their tumor phenotype [13].

LncRNA is a NP downstream target, and by studying the relationship between LncRNA and Notch1 protein levels, Pei et al. identified LncRNA CAS5, which is most associated with Notch1 in breast cancer, and then by studying the 5-year survival analysis of 30 breast cancer patients, they discovered that elevated Notch1 in BC tended to predict a poorer prognosis. Notch1 also regulates the LncRNA CAS5, which increases breast cancer cell proliferation [14].

3.2. Lung cancer

Lung cancer and lung cancer treatment are both reliant on NP. Hassan et al. indicated that Notch1 overexpression in small cell lung cancer (SCLC) limits Epithelial-mesenchymal transitions [15]. In SCLC cells, the NP is also implicated in drug resistance, and it was found that knockdown of Notch1 in SBC-3 cell lines in vitro reduced resistance to high concentrations of doxorubicin and activated the apoptotic pathway in cancer lines. After knockdown of Notch1 in the H69AR cell line, expression of MRP-1 was increased, maintaining cellular chemoresistance [16]. In clinical practice, radiation therapy is now commonly employed in the treatment of lung cancer. Upregulation of the NP system and HIF-1a protein expression has been demonstrated to improve tumor cell susceptibility to radiation therapy, indicating that the Notch pathway might be a viable therapeutic target for radiation therapy [17].

Notch2 in lung cancer is also linked to the progression of the disease. Hu et al. used bioinformatics analysis to identify that Notch2 expression in lung cancer tissues differs from that in normal lung tissues. Notch2 expression was shown to be considerably lower in SCLC than in the control group, and significantly greater in NSCLC (including LUAD and LUSC) than in SCLC. Subgroup analysis showed that Notch2 expression was not contrasting in LUAD versus LUSC (Figure 4). These findings show that Notch2 might be employed as a possible new biomarker for forecasting NSCLC patient prognosis.

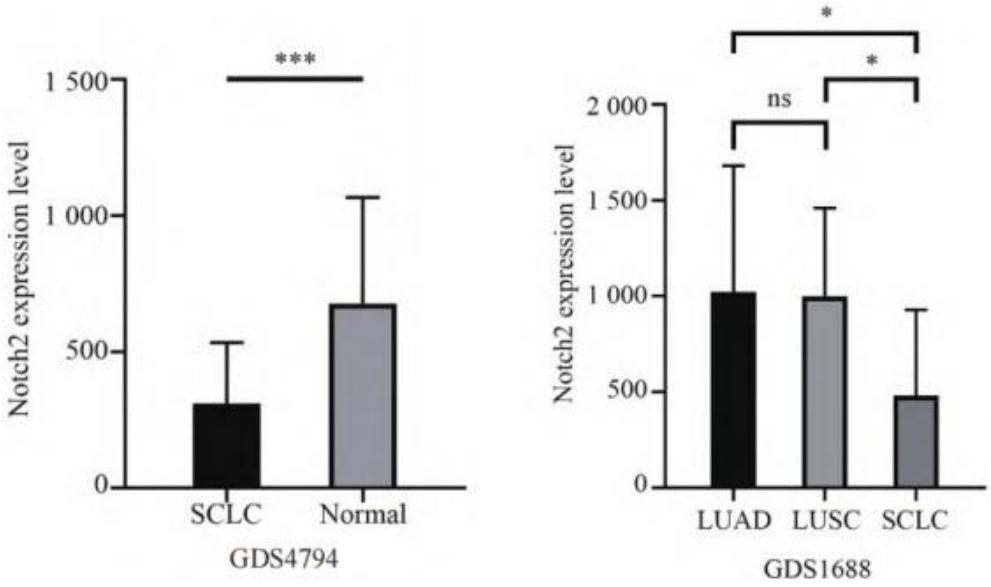


Figure 4. Notch2 expression in lung cancer tissues [18]

3.3. Colorectal cancer

With a growing incidence and mortality rate, colorectal cancer (CRC) is already the second leading cause of cancer-related deaths [19]. VEGF-A is a pro-angiogenic factor whose increased expression contributes to angiogenesis, and Increased level of pro-angiogenic factors is required for early CRC development. Endothelial cells express the Notch ligand DLL-4, which may contribute to the increased production of VEGF via HZF-1a under hypoxic conditions [19]. DLL-4 was discovered to be high-level expressed in CRC endothelial cells, and this expression was linked to VEGF and hypoxia, indicating that the NP of DLL-4 might be a viable therapeutic target for colon cancer [19].

Notch1 and Notch2 expression levels have been established to be an independent factor in determining the prognosis of colorectal cancer patients [20]. Notch2 was associated with higher overall survival, but Notch1 was the opposite. In CRC, people who showed Notch1-positive performance worse than to those with Notch1-negative expression (Figure 5A and B).

Furthermore, the findings confirmed that Notch1 and Notch2 expression were negatively correlated in CRC. Therefore, in defining the biological role of CRC, Notch1 has an opposite role to Notch2: Notch1 is a carcinogen and Notch2 is a carcinogen [20]. Therefore, combining Notch1 and Notch2 can predict prognosis more accurately and allow patients to receive more personalized treatment, thus avoiding over- and ineffective treatment.

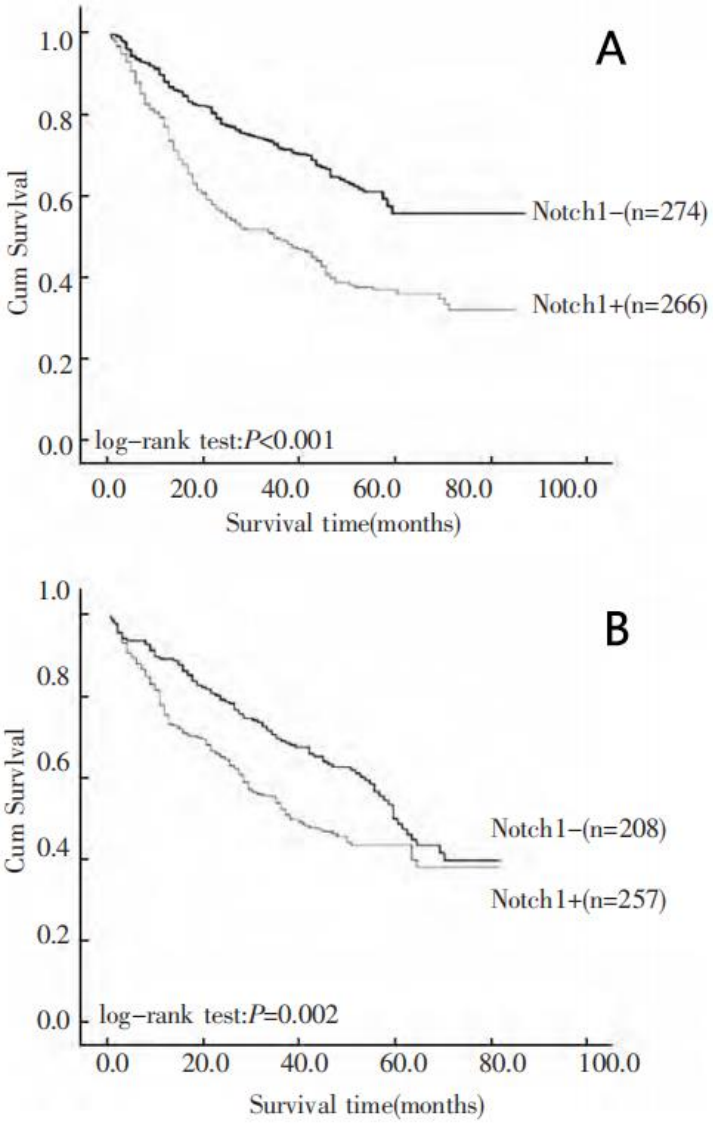


Figure 5. Correlation of Notch1 Expression with Survival [20]

3.4. Pancreatic cancer

One of the most dangerous tumors is pancreatic cancer (PC), and abnormal activation of the Notch pathway is widespread in this disease.

PanINs are precursor lesions of pancreatic cancer. Mazur et al. found that in pancreatic follicular cells, Notch1 was high-level expressed and Notch2 was highly expressed in pancreatic ductal cells during the progression of PanINs, indicating that the NP is important in the advancement of PanINs [21].

Notch ligands, including high-level of Jagged2 and DLL-4, can be detected in most pancreatic cancer cell lines. In the treatment of PC, anti-Notch2/3 was found to be efficacious as monotherapy or in combination with chemotherapeutic drugs in research using patient-derived xenograft tumor cells. The anti-tumor effect of anti-notch2/3 that combining with gemcitabine plus albumin-bound paclitaxel was better than that of the gemcitabine plus albumin-bound paclitaxel group alone. This suggests that the inclusion of considerations regarding Notch in the treatment ideas may be helpful in improving the efficacy compared to previous treatment regimens.

4. Conclusion

Although cancer is difficult to cure, the oncogenic role of the NP in cancer suggests that starting with the NP or combining it with other pathways may be an effective therapeutic option, and it may

well be an effective way to cure cancer. Therefore, it's critical to comprehend the structure of NP, its functions and regulatory mechanisms. However, some related issues cannot be ignored, such as whether Notch-targeted therapies are safe or not, and whether such treatments are effective, still need further research. Due to the complexity of Notch pathway interactions with other pathways, antibodies targeting Notch receptors alone may not be sufficient to inhibit tumor growth or metastasis. This requires further research in related studies. In addition, the adverse effects and toxicity of some drugs are also hindering factors that cannot be ignored in the treatment process. It is hoped that future research on Notch pathway will go further and open an effective pathway for cancer treatment.

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