

MINI-REVIEW

Notch Inhibitor: a Promising Carcinoma Radiosensitizer

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Abstract

Radiotherapy is an important part of modern cancer management for many malignancies, and enhancing the radiosensitivity of tumor cells is critical for effective cancer therapies. The Notch signaling pathway plays a key role in regulation of numerous fundamental cellular processes. Further, there is accumulating evidence that dysregulated Notch activity is involved in the genesis of many human cancers. As such, Notch inhibitors are attractive therapeutic agents, although as for other anticancer agents, they exhibit significant and potential side effects. Thus, Notch inhibitors may be best used in combination with other agents or therapy. Herein, we describe evidence supporting the use of Notch inhibitors as novel and potent radiosensitizers in cancer therapy.

Keywords: Notch inhibitor - radiotherapy - radiosensitizer

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Introduction

Surgery, radiation and chemotherapy are the major therapeutic options for treatment of human malignancies. In particular, more than 50% of newly diagnosed cancer patients worldwide receive radiotherapy (alone or in combination with chemotherapy or surgery) at some point during their treatment (Nygren et al., 2001). However, the curative potential of radiotherapy is often limited by intrinsic radioresistance of cancer cells and normal tissue toxicity (Bischoff et al., 2009). Therefore, there is increasing interest in enhancing the radiosensitivity of cancer cells for development of effective therapies. The Notch signaling pathway is an evolutionary conserved signaling pathway, which is also frequently activated in several human malignancies. In this review, we discuss the use of Notch inhibitors as radiosensitizers in cancer treatment.

Molecular Biology of Tumor Radioresistance

Radiotherapy has been the mainstay for treatment of human malignancies for the last century. However, resistance to radiation therapy remains a serious obstacle to effective cancer therapy. Intrinsic tumor radiosensitivity is regulated by the balance between DNA damage and DNA repair following irradiation. Exposure to ionizing radiation induces the formation of DNA double-strand breaks (DSBs), resulting in activation of complex damage recognition, repair and response machinery. Although the exact mechanisms of radioresistance remain unclear, radiation can trigger a series of cellular signaling and molecular changes in cancer cells. For example, in the

nucleus, cell cycle progression is halted and elicits a DNA damage response that may allow repair of damaged DNA. In the cytoplasm, inactivation of phosphatases by reactive oxygen species (ROS) can alter signaling at the receptor level, resulting in ligand-independent activation of receptor tyrosine kinases (RTKs) (Szumiel, 2008; Deorukhkar et al., 2010). Factors that can alter these processes including Chk2 (Gogineni et al., 2011), P53 (Canman et al., 1998; Squatrito et al., 2010) and survivin (Reichert et al., 2011) will thus affect radiosensitivity. Further, as ROS is a mediator of ionizing radiation-induced cellular damage, both superoxide dismutase (SOD2) (Hosoki et al., 2012) and hypoxia can alter radioresistance (Kato et al., 2011).

Carcinoma Stem Cells and Radioresistance

Solid tumors are histologically heterogeneous and include tumor cells, stroma, inflammatory infiltrates and vascular structures. These different cells exhibit different patterns of radiosensitivity. Importantly, there is increasing evidence that a small subpopulation of cancer stem cells (CSCs) in tumor cells contribute to radioresistance (Bao et al., 2006; Phillips et al., 2006; Woodward et al., 2007). 'Cancer stem cell' theory implies that CSCs are responsible for tumor initiation, progression, metastasis and resistance therapy. This theory assumes that CSCs have the following characteristics: (1) self-renewal, (2) heterogeneity (i.e., potential for multidirectional differentiation) and (3) resistance to apoptosis (Gil et al., 2008). The existence of CSCs and their ability to self-renew, differentiate into multiple lineages and proliferate extensively make them particularly insidious. To date, these cells have been distinguished from the bulk-tumor population by the

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expression pattern of cell surface proteins (e.g., CD24, CD44 and CD133) and cellular activities including the efflux of Hoechst dye (Keysar et al., 2010).

Resistance to radiotherapy has been observed in the CSCs of many solid tumors (Bao et al., 2006; Phillips et al., 2006; Woodward et al., 2007). The relative radioresistance of CSCs has been attributed to different intrinsic and extrinsic factors including quiescence, activated radiation response mechanisms (e.g., enhanced DNA repair (Mihatsch et al., 2011), upregulated cell cycle control mechanisms (Piao et al., 2012) and increased free-radical scavengers (Diehn et al., 2009)) and a surrounding microenvironment that enhances cell survival mechanisms (e.g., hypoxia (Yeung et al., 2011) and interaction with stromal elements (Tsuyada et al., 2012)). CSCs are slow growing and require signaling pathways for their proliferation and self-renewal. Although the exact molecular mechanisms that control these processes remain unclear, three major signaling pathways (Notch, Hedgehog and Wnt) have been identified, of which the Notch signaling pathway is considered most important.

Use of Radiosensitizers

The combination of radiotherapy with a radiosensitizer is a well-established experimental and clinical strategy to reduce radioresistance (Heidelberger et al., 1958). Radiosensitizers include traditional chemotherapeutic agents, which are widely used clinically and are considered to improve the local-regional effects of radiotherapy (Cooper et al., 2005), as well as newer molecular targeting agents (Kvols, 2005; Spalding et al., 2006). Typical chemotherapeutic agents include 5-g-fluorouracil, analogs of platinum, gemcitabine and DNA topoisomerase I-targeting drugs, which are targeted towards DNA. A number of novel molecular radiosensitizer agents that do not target DNA have also been found, including epidermal growth factor receptor blockade (Czito et al., 2006), farnesyltransferase inhibitors (Cohen-Jonathan et al., 1999) and COX-2 inhibitors (Pyo et al., 2001), as well as various naturally occurring compounds such as sulforaphane (Kotowski et al., 2011), tetrandrine (Yu et al., 2011) and honokiol (He et al., 2011).

Although the exact mechanisms of action remain unclear, the majority of radiosensitizers can induce radiosensitization via the following pathways: (1) increased primary radiation damage (e.g., BrdU) (Dextraze et al., 2009), (2) inhibition of DNA damage repair (e.g., HSP90 inhibitors NVP-AUY922) (Zaidi et al., 2012), (3) alteration of the cell cycle (e.g., tetrandrine; enhances radiosensitivity by relief of radiation-induced G2/M arrest (Yu et al., 2011)) and (4) enhanced apoptosis (e.g., Paclitaxel) (Zhang et al., 2007). Further, as CSCs contribute to radioresistance, targeting CSCs is another approach to decrease radioresistance. For example, cucurbitacin I can reduce CSCs and enhance tumor cell radiosensitivity (Chen et al., 2010). It is important to note that some radiosensitizers may have multiple mechanisms and targets.

Notch Signaling Pathway and Carcinoma

Notch signaling plays a pivotal role in the regulation of many fundamental cellular processes, including proliferation, stem cell maintenance, differentiation during embryonic and adult development and homeostasis of adult self-renewing organs (Artavanis-Tsakonas et al., 1999; Lino et al., 2010). Dysfunction of Notch signaling results in a tremendous variety of developmental defects and adult pathologies (Lai, 2004). The Notch receptor is a transmembrane protein expressed on the cell surface as a heterodimer of an EGF-like repeat-rich extracellular domain and an intracellular domain with a single pass transmembrane domain (Artavanis-Tsakonas et al., 1999). There are four distinct isoforms of Notch receptor (Notch1-4) in mammals, whereas five different ligands (Dll-1, Dll-3, Dll-4, Jagged-1 and Jagged-2) have been identified (Suwanjune et al., 2008). Signaling is initiated by ligand-receptor interaction, thereby inducing a series of cleavages termed S2, S3 and S4. Ligand-dependent proteolysis at the S2 site removes the bulk of the extracellular domain of Notch by the metalloprotease tumor necrosis factor α -converting enzyme (TACE) (Brou et al., 2000; Lee et al., 2011). The S3/4 cleavage is an intramembranous cleavage mediated by the presenilin-dependent γ -secretase, resulting in the translocation of the Notch intracellular domain (NICD) into the nucleus. The nuclear NICD then interacts with a transcriptional factor CSL (C protein binding factor 1/Suppressor of Hairless/Lag-1, CBF1/RBPJ κ in mammals) to activate downstream target genes such as Hes-1 (Figure 1) (Nam et al., 2002; Tien et al., 2009).

Accumulating data indicate that abnormal activation of the Notch gene is also involved in the genesis of many human cancers. Human Notch was first identified in T-cell acute lymphoblastic leukemia (T-ALL), in

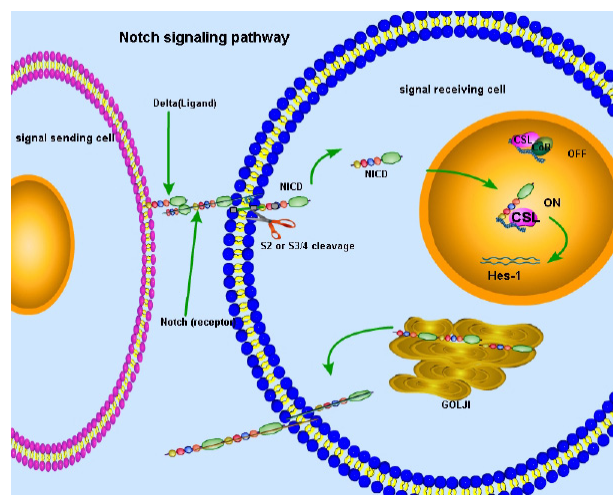


Figure 1. Illustration of the Notch Signaling Pathway.

Notch receptors are synthesized as single precursor proteins, and are then modified in the Golgi apparatus and endoplasmic reticulum, and targeted to the cell surface. Notch signaling is initiated through ligand binding on the EGF-like repeats, which induce a series of cleavage at the site S2 and S3/S4. The cleavage at S3/S4 releases NICD, which translocates to the nucleus to activate CSL. The CSL co-repressor (CoR) complex is displaced by a co-activator complex containing NICD, which mediates activation of genes targeted by Notch

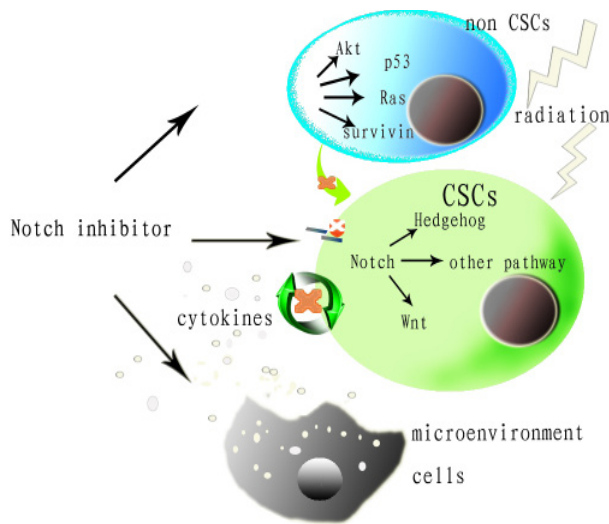


Figure 2. Potential Mechanism of Notch Inhibitor-induced Radiosensitivity. Notch inhibitors inhibit Notch and other pathways that crosstalk with Notch (such as Wnt and Hedgehog), resulting in a reduction in CSCs. Notch inhibitors also can inhibit other key oncogenic pathways and factors in all cancer cells. Further, Notch inhibitors affect the microenvironment of cancer cells, resulting in retardation of functional angiogenesis and synthesis of some inflammatory cytokines

which abnormal Notch activation results in halting of the differentiation process and accumulation of immature cells vulnerable to additional oncogenic mutations (Ellisen et al., 1991). Dysregulated Notch signaling with upregulated expression of Notch receptors and their ligands is also frequently observed in human malignancies in glioma (Jiang et al., 2011), breast cancer (Guo et al., 2011), colon cancer (Zhang et al., 2010), cervical cancer (Maliekal et al., 2008), nasopharyngeal carcinoma (Wang et al., 2005; Zhang et al., 2010), head and neck cancer (Lin et al., 2010), acute myeloid (Xu et al., 2011) and lymphomas and pancreatic cancer (Wang et al., 2008, 2009). Notch signaling pathway is widely considered to play an important factor in tumor angiogenesis, which drive to the hyperplasia in the malignancies (Dufraigne et al., 2008). It should be noted, however, that Notch can also act as a tumor suppressor in a few malignancies including some hepatocellular carcinoma (Qi et al., 2003) and small cell lung cancer (Sriuranpong et al., 2001), which may be associated with cell type- and tissue-specific cross-talk of Notch signaling with other pathways or regulatory molecules (Dotto, 2008).

Use of Notch Inhibitors in Cancer Therapy

Due to the strong evidence of a role for Notch signaling in the pathogenesis of many cancers, this pathway forms an attractive target for the development of novel therapeutics. Several key steps in the activation of this pathway may be pharmacologically targeted, and a number of agents including γ -secretase inhibitors (GSIs), α -secretase inhibitors (ASIs), Notch antisense, anti-Notch monoclonal antibodies and RNA interference have been suggested (Purow, 2009). GSIs are the only form of Notch inhibitors used in clinical trials, with several GSIs including RO4929097 (Strosberg et al., 2012), MK-

0752 (Whitehead et al., 2012) and PF03084014 (Wei et al., 2010) undergoing Phase I or Phase II trials. By contrast, although the other types of Notch inhibitor are theoretically feasible, they have not been used clinically (Morrison et al., 2011).

Notch inhibitors have been reported to exhibit good inhibitory effects on many solid and hematopoietic tumors, including T-ALL (Jehn et al., 1999), breast (Debeb et al., 2012), endometrial (Mori et al., 2012), head and neck squamous (Liu et al., 2011), pancreatic (Cook et al., 2012), nasopharyngeal (Yu et al., 2011), gastric (Nickoloff et al., 2003) and lung carcinomas (Li et al., 2011), leukemia and hepatoma (Suwanjonee et al., 2008), gliomas (Krop et al., 2012), lymphomas (Hajdu et al., 2010), and multiple myeloma (Chen et al., 2011). Nevertheless, as for other anticancer agents, Notch inhibitors are associated with significant and potential side effects, including gastrointestinal toxicity and diarrhea (Searfoss et al., 2003; Garber, 2007), and hepatotoxicity and nephrotoxicity (Searfoss et al., 2003; Wu et al., 2010). The development of clinically approved highly specialized Notch antibodies or RNA interference may be able to reduce these side effects, although other potential side effects of blocking Notch signaling should be considered, including reduction in the normal self-renewal of stem cells in bone marrow and brain, induction of hematopoietic collapse, and subtle cognitive decline (Purow, 2012). As such, Notch inhibitors may be best used in combination with other agents or therapy, including radiotherapy.

Notch Inhibitors Act as Radiosensitizers

There is increasing evidence that Notch inhibitors may provide radiosensitization. For example, we previously reported that a Notch inhibitor could enhance the radiosensitivity of NPC cells (Yu et al., 2011), while other studies have confirmed a radiosensitivity effect in glioblastoma, breast cancer, colorectal carcinoma and glioma (Hirose et al., 2010; Hovinga et al., 2010; Lin et al., 2010; Wang et al., 2010; Liu et al., 2011). The mechanisms by which Notch inhibitors induce radiosensitivity are likely to include the following mechanisms (Figure 2).

(1) Targeting of CSCs

The theory of the 'cancer stem cell' indicates that radiation sensitizers that target CSCs can provide a strong radiosensitization effect. Further, although there are only a few radiosensitizers that target CSCs, there is evidence that such compounds can overcome radioresistance (Teimourian et al., 2006; Zhang et al., 2008; Kurrey et al., 2009). Irradiation has been shown to activate the Notch signaling pathway, which may result in increased numbers of CSCs and increased radioresistance (Phillips et al., 2006). Notch is a critical pathway in CSC self-renewal and survival, while its inhibition was reported to deplete CSCs and inhibit tumor growth (Zhang et al., 2008; Fan et al., 2010; Harrison et al., 2010; Hovinga et al., 2010; Lin et al., 2010; Sikandar et al., 2010; Zhen et al., 2010). While CSCs can promote radioresistance (Bao et al., 2006; Phillips et al., 2006; Woodward et al., 2007), a decreasing percentage of CSCs causes tumor cells to be more susceptible to killing by ordinary methods such as

radiotherapy. Radiation also can reprogram differentiated cancer cells into CSCs, which can be partially prevented by Notch inhibition (Lagadec et al., 2012). Notch, Hedgehog and Wnt signaling are key factors in regulation of self-renew and survival of CSCs, and a crosstalk between these pathways has been suggested in normal stem cells as well as CSCs. Notch signaling can also crosstalk with other important signal pathway and cell metabolism in the CSCs, including STAT3 and Akt (Wang et al., 2010; Lin et al., 2011; Dai et al., 2011). These factors may contribute to the reduced CSCs and enhanced the radiosensitivity of the tumor following Notch inhibition.

2) Inhibition of other key cancer pathways and oncogene factors

Notch inhibitors can also inhibit other key cancer pathways, proteins and factors in all cancer cells. Numerous studies have shown that Notch inhibitors can induce apoptosis by inhibiting PI3K/Akt (Ramakrishnan et al., 2012), p53 (Lin et al., 2011), Ras (Ordentlich et al., 1998) and MEK signaling (Chen et al., 2011). Further, Notch signaling can regulate expression of myc oncogene (Allen et al., 2011), survivin (Chen et al., 2011) and Bcl-2 and Bax (Rasul et al., 2009). These signaling and oncogene factors play an important role in oncogenesis and growth of cancers. In some instances, other oncogene signaling pathways, such as hypoxia-inducible factor 1 (HIF-1), can also activate Notch signaling (Bedogni et al., 2008). In fact, the majority of the best-known oncogenic pathways have been shown to crosstalk with the Notch signaling pathway at some level (Purow, 2012).

3) Alteration of the cancer cell microenvironment

The tumor stroma, or microenvironment, is comprised of a variety of mesenchymal cell types and extracellular matrix components (McAllister et al., 2010). The microenvironment plays a critical role in tumor growth, metastasis and the tumor response to treatment modalities. Notch inhibitors can alter this microenvironment to regulate the tumor response to radiotherapy. For example, ionizing radiation can disrupt tumor vasculature, and Notch signaling pathway inhibition can interfere with functional angiogenesis (Liu et al., 2011). Notch signaling can regulate endothelial sprouting, and its inhibition results in disordered and unproductive endothelial growth (Noguera-Troise et al., 2006; Hellstrom et al., 2007), while Notch signaling can also regulate aspects of vascular development such as arterial versus venous fate (Purow, 2012). Further, Notch signaling pathways can crosstalk with vascular endothelial growth factor (VEGF), a key receptor for vascular formation, to promote tumor angiogenesis (Noguera-Troise et al., 2006; Kuhnert et al., 2001). Finally, Notch inhibitors can inhibit stromal and cancer cell synthesis of some inflammatory cytokines including TNF, IL-6 and IL-8 in the microenvironment (Debeb et al., 2012). These cytokines are associated with radioresistance, metastasis, proliferation and the maintenance of CSCs (Efimova et al., 2009; Iliopoulos et al., 2011; Sethi et al., 2011).

Conclusion and Perspectives

The Notch signaling pathway is one of the most important pathways in the regulation of many fundamental cellular processes. Further, dysregulation of Notch signaling has been observed in many types of diseases, and contributes to the survival and tumorigenesis of numerous cancer types. Notch inhibitors are attractive therapeutic agents, and there is strong evidence that Notch inhibitors have tremendous potential as a novel radiosensitizer agents in cancer therapy. Nevertheless, the differing roles of the various Notch receptors in different cancers needs to be elucidated, as despite an oncogenic role in most cancers, Notch acts as a tumor suppressor in some malignancies. Different Notch isoforms are also expressed in different cancers, and it remains unclear how to use specific Notch inhibitors to target these isoforms in specific cancers. GSIs are the most common Notch inhibitors used in pre-clinical and clinical studies, as all Notch receptors require γ -secretase for processing and signaling (Suwanjonee et al., 2008). However, this non-specificity is also a potential drawback of GSIs. The toxicity of GSIs may be avoided by blockade of individual Notch receptors and ligands with specific antibodies (Liu et al., 2011), although it remains unclear how to select specific antibodies to specific cancers. The optimal clinical regime for use of Notch inhibitors as radiosensitizers is also unknown, and may depend upon the context. As Notch inhibitors can targets CSCs, we suggest the use of Notch inhibitors at a low concentration for a few days to reduce CSCs, followed by radiotherapy. Finally, further in vivo pre-clinical and clinical studies are required using Notch inhibitors as radiosensitizers.

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