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-gfluorouracil, 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 (Suwanjunee 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/RBPJκ 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

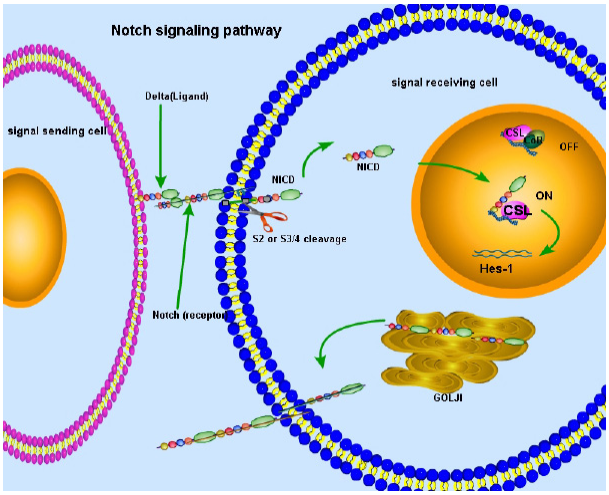

Figure 1. Illustration of the Notch Signaling Pathway. Notch receptors are synthesized as single precursor proteins, and are then modified in the Golgi apparatus and endoplasmic reticulum, and targeted to the cell surface. Notch signaling is initiated through ligand binding on the EGF-like repeats, which 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

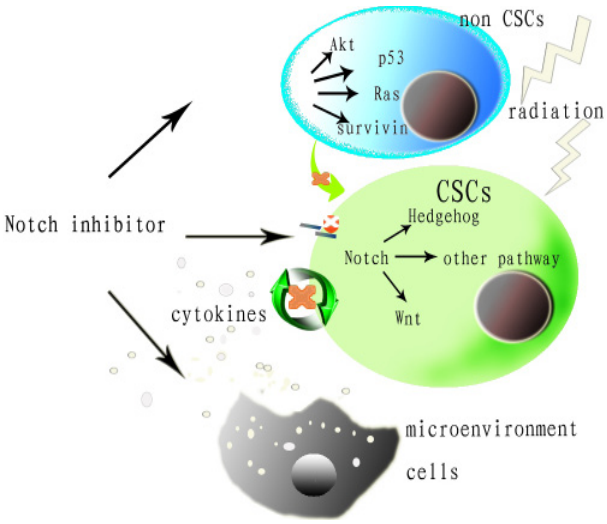

Figure 2. Potential Mechanism of Notch Inhibitor-induced Radiosensitivity. Notch inhibitors inhibit Notch and other pathways that crosstalk with Notch (such as Wnt and Hedgehog), resulting in a reduction in CSCs. Notch inhibitors also can inhibit other key oncogenic pathways and factors in all cancer 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 (Dufraine 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 (Suwanjunee 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 morrow 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 tumors 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 tumorogenesis 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 (Suwanjunee 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.

References

Allen TD, Rodriguez EM, Jones KD, Bishop JM (2011). Activated Notch1 induces lung adenomas in mice and cooperates with Myc in the generation of lung adenocarcinoma. *Cancer Res*. **71**, 6010-8.

Artavanis-Tsakonas S, Rand MD, Lake RJ (1999). Notch signaling: cell fate control and signal integration in development. *Science*, **284**, 770-6.

Bao S, Wu Q, McLendon RE, et al (2006). Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature*, **444**, 756-60.

Bedogni B, Warneke JA, Nickoloff BJ, Giaccia AJ, Powell MB (2008). Notch1 is an effector of Akt and hypoxia in melanoma development. *J Clin Invest*, **118**, 3660-70.

Bischoff P, Altmeyer A, Dumont F (2009). Radiosensitising agents for the radiotherapy of cancer: advances in traditional and hypoxia targeted radiosensitisers. *Expert Opin Ther Pat*, **19**, 643-62.

Brou C, Logeat F, Gupta N, et al (2000). A novel proteolytic cleavage involved in Notch signaling: the role of the disintegrin-metalloprotease TACE. *Mol Cell*, **5**, 207-16.

Canman CE, Lim DS, Cimprich KA, et al (1998). Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. *Science*, **281**, 1677-9.

Chen F, Pisklakova A, Li M, et al (2011). Gamma-secretase inhibitor enhances the cytotoxic effect of bortezomib in multiple myeloma. *Cell Oncol (Dordr)*, **34**, 545-51.

Chen SM, Liu JP, Zhou JX, et al (2011). Suppression of the notch signaling pathway by gamma-secretase inhibitor GSI inhibits human nasopharyngeal carcinoma cell proliferation. *Cancer Lett*, **306**, 76-84.

Chen Y, Li D, Liu H, et al (2011). Notch-1 signaling facilitates survivin expression in human non-small cell lung cancer cells. *Cancer Biol Ther*, **11**, 14-21.

Chen YW, Chen KH, Huang PI, et al (2010). Cucurbitacin I suppressed stem-like property and enhanced radiation-induced apoptosis in head and neck squamous carcinoma--derived CD44(+)ALDH1(+) cells. *Mol Cancer Ther*, **9**, 2879-92.

Cohen-Jonathan E, Toulas C, Ader I, et al (1999). The farnesyltransferase inhibitor FTI-277 suppresses the 24-kDa FGF2-induced radioresistance in HeLa cells expressing wild-type RAS. *Radiat Res*, **152**, 404-11.

Cook N, Frese KK, Bapiro TE, et al (2012). Gamma secretase inhibition promotes hypoxic necrosis in mouse pancreatic ductal adenocarcinoma. *J Exp Med*, **209**, 437-44.

Cooper JS, Ang KK (2005). Concomitant chemotherapy and radiation therapy certainly improves local control. *Int J Radiat Oncol Biol Phys*, **61**, 7-9.

Czito BG, Willett CG, Bendell JC, et al (2006). Increased toxicity with gefitinib, capecitabine, and radiation therapy in pancreatic and rectal cancer: phase I trial results. *J Clin Oncol*, **24**, 656-62.

Dai L, He J, Liu Y, et al (2011). Dose-dependent proteomic analysis of glioblastoma cancer stem cells upon treatment with gamma-secretase inhibitor. *Proteomics*, **11**, 4529-40.

Debeb BG, Cohen EN, Boley K, et al (2012). Pre-clinical studies of Notch signaling inhibitor RO4929097 in inflammatory breast cancer cells. *Breast Cancer Res Treat*, **134**, 495-510.

Deorukhkar A, Krishnan S (2010). Targeting inflammatory pathways for tumor radiosensitization. Biochem. *Pharmacol*, **80**, 1904-14.

Dextraze ME, Cecchini S, Bergeron F, et al (2009). Reaching for the other side: generating sequence-dependent interstrand cross-links with 5-bromodeoxyuridine and gamma-rays. *Biochemistry*, **48**, 2005-11.

Diehn M, Cho RW, Lobo NA, et al (2009). Association of reactive oxygen species levels and radioresistance in cancer stem cells. *Nature*, **458**, 780-3.

Dotto GP (2008). Notch tumor suppressor function. *Oncogene*, **27**, 5115-23.

Dufraine J, Funahashi Y, Kitajewski J (2008). Notch signaling regulates tumor angiogenesis by diverse mechanisms. *Oncogene*, **27**, 5132-37.

Efimova EV, Liang H, Pitroda SP, et al (2009). Radioresistance of Stat1 over-expressing tumour cells is associated with suppressed apoptotic response to cytotoxic agents and increased IL6-IL8 signalling. *Int J Radiat Biol*, **85**, 421-31.

Ellisen LW, Bird J, West DC, et al (1991). TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell*, **66**, 649-61.

Fan X, Khaki L, Zhu TS, et al (2010). NOTCH pathway blockade depletes CD133-positive glioblastoma cells and inhibits growth of tumor neurospheres and xenografts. *Stem Cells*, **28**, 5-16.

Garber K (2007). Notch emerges as new cancer drug target. J. Natl. *Cancer Inst*, **99**, 1284-85.

Gil J, Stembalska A, Pesz KA, Sasiadek MM. (2008). Cancer stem cells: the theory and perspectives in cancer therapy. *J Appl Genet*, **49**, 193-9.

Gogineni VR, Nalla AK, Gupta R, et al (2011). Chk2-mediated G2/M cell cycle arrest maintains radiation resistance in malignant meningioma cells. *Cancer Lett*, **313**, 64-75.

Guo S, Liu M, Gonzalez-Perez RR (2011). Role of Notch and its oncogenic signaling crosstalk in breast cancer. *Biochim Biophys Acta*, **1815**, 197-213.

Hajdu M, Kopper L, Sebestyen A (2010). Notch-regulation upon Dll4-stimulation of TGFb-induced apoptosis and gene expression in human B-cell non-Hodgkin lymphomas. *Scand J Immunol*, **71**, 29-37.

Harrison H, Farnie G, Howell SJ, et al (2010). Regulation of breast cancer stem cell activity by signaling through the Notch4 receptor. *Cancer Res*, **70**, 709-18.

He Z, Subramaniam D, Ramalingam S, et al (2011). Honokiol radiosensitizes colorectal cancer cells: enhanced activity in cells with mismatch repair defects. *Am J Physiol Gastrointest Liver Physiol*, **301**, G929-37.

Heidelberger C, Griesbach L, Cruz O, Schnitzer RJ, Grunberg E. (1958). Effects of 5-fluorouridine and 5-fluoro-2'-deoxyuridine on transplanted tumors. *Proc Soc Exp Biol Med*, **97**, 470-5.

Hellstrom M, Phng LK, Hofmann JJ, et al (2007). Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. *Nature*, **445**, 776-80.

Hirose H, Ishii H, Mimori K, et al (2010). Notch pathway as candidate therapeutic target in Her2/Neu/ErbB2 receptor-negative breast tumors. *Oncol Rep*, **23**, 35-43.

Hosoki A, Yonekura S, Zhao QL, et al (2012). Mitochondria-targeted superoxide dismutase (SOD2) regulates radiation resistance and radiation stress response in HeLa cells. *J Radiat Res*, **53**, 58-71.

Hovinga KE, Shimizu F, Wang R, et al (2010). Inhibition of notch signaling in glioblastoma targets cancer stem cells via an endothelial cell intermediate. *Stem Cells*, **28**, 1019-29.

Iliopoulos D, Hirsch HA, Wang G, Struhl K (2011). Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. *Proc Natl Acad Sci U S A*, **108**, 1397-402.

Jehn BM, Bielke W, Pear WS, Osborne BA (1999). Cutting edge: protective effects of notch-1 on TCR-induced apoptosis. *J Immunol*, **162**, 635-8.

Jiang L, Wu J, Chen Q, et al (2011). Notch1 expression is upregulated in glioma and is associated with tumor progression. *J Clin Neurosci*, **18**, 387-90.

Kato Y, Yashiro M, Fuyuhiro Y, et al (2011). Effects of acute and chronic hypoxia on the radiosensitivity of gastric and esophageal cancer cells. *Anticancer Res*, **31**, 3369-75.

Keysar SB, Jimeno A (2010). More than markers: biological significance of cancer stem cell-defining molecules. *Mol Cancer Ther*, **9**, 2450-57.

Kotowski U, Heiduschka G, Brunner M, et al (2011). Radiosensitization of head and neck cancer cells by the phytochemical agent sulforaphane. *Strahlenther Onkol*, **187**, 575-80.

Krop I, Demuth T, Guthrie T, et al (2012). Phase I Pharmacologic and Pharmacodynamic Study of the Gamma Secretase (Notch) Inhibitor MK-0752 in Adult Patients With Advanced Solid Tumors. *J Clin Oncol*, **30**, 2307-13 .

Kuhnert F, Kirshner JR, Thurston G (2011). Dll4-Notch signaling as a therapeutic target in tumor angiogenesis. *Vasc Cell*, **3**, 20.

Kurrey NK, Jalgaonkar SP, Joglekar AV, et al (2009). Snail and slug mediate radioresistance and chemoresistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells. *Stem Cells*, **27**, 2059-68.

Kvols LK (2005). Radiation sensitizers: a selective review of molecules targeting DNA and non-DNA targets. *J Nucl Med*, **46**, S187-90.

Lagadec C, Vlashi E, Della DL, Dekmezian C, Pajonk F (2012). Radiation-induced reprogramming of breast cancer cells.

Stem Cells, **30**, 833-44.
Lai EC (2004). Notch signaling: control of cell communication and cell fate. *Development,* **131**, 965-73.
Lee SF, Srinivasan B, Sephton CF, et al (2011). Gamma-secretase-regulated proteolysis of the Notch receptor by mitochondrial intermediate peptidase. *J Biol Chem,* **286**, 27447-53.
Li C, Zhang Y, Lu Y, et al (2011). Evidence of the cross talk between Wnt and Notch signaling pathways in non-small-cell lung cancer (NSCLC): Notch3-siRNA weakens the effect of LiCl on the cell cycle of NSCLC cell lines. *J Cancer Res Clin Oncol*, **137**, 771-8.
Lin H, Xiong W, Zhang X, et al (2011). Notch-1 activation-dependent p53 restoration contributes to resveratrol-induced apoptosis in glioblastoma cells. *Oncol Rep*, **26**, 925-30.
Lin J, Zhang XM, Yang JC, Ye YB, Luo SQ (2010). Association of high levels of Jagged-1 and Notch-1 expression with poor prognosis in head and neck cancer. *Arch Med Res,* **41**, 519-29.
Lin JT, Chen MK, Yeh KT, et al (2010). Association of high levels of Jagged-1 and Notch-1 expression with poor prognosis in head and neck cancer. *Ann Surg Oncol*, **17**, 2976-83.
Lin L, Fuchs J, Li C, et al (2011). STAT3 signaling pathway is necessary for cell survival and tumorsphere forming capacity in ALDH/CD133 stem cell-like human colon cancer cells. *Biochem Biophys Res Commun*, **416**, 246-51.
Lino MM, Merlo A, Boulay JL (2010). Notch signaling in glioblastoma: a developmental drug target. *BMC Med*, **8**, 72.
Liu SK, Bham SA, Fokas E, et al (2011). Delta-like ligand 4-notch blockade and tumor radiation response. *J Natl Cancer Inst*, **103**, 1778-98.
Maliekal TT, Bajaj J, Giri V, Subramanyam D, Krishna S (2008). The role of Notch signaling in human cervical cancer: implications for solid tumors. *Oncogene*, **27**, 5110-14.
McAllister SS, Weinberg RA (2010). Tumor-host interactions: a far-reaching relationship. *J Clin Oncol*, **28**, 4022-8.
Mihatsch J, Toulany M, Bareiss PM, et al (2011). Selection of radioresistant tumor cells and presence of ALDH1 activity in vitro. *Radiother Oncol*, **99**, 300-6.
Mori M, Miyamoto T, Yakushiji H, et al (2012). Effects of N-[N-(3, 5-difluorophenacetyl-L: -alanyl)]-S-phenylglycine t-butyl ester (DAPT) on cell proliferation and apoptosis in Ishikawa endometrial cancer cells. *Hum Cell*, **25**, 9-15.
Morrison R, Schleicher SM, Sun Y, et al (2011). Targeting the mechanisms of resistance to chemotherapy and radiotherapy with the cancer stem cell hypothesis. *J Oncol*, **2011**, 941876.
Nam Y, Aster JC, Blacklow SC (2002). Notch signaling as a therapeutic target. *Curr Opin Chem Biol*, **6**, 501-9.
Nickoloff BJ, Osborne BA, Miele L (2003). Notch signaling as a therapeutic target in cancer: a new approach to the development of cell fate modifying agents. *Oncogene*, **22**, 6598-608.
Noguera-Troise I, Daly C, Papadopoulos NJ, et al (2006). Blockade of Dll4 inhibits tumour growth by promoting non-productive angiogenesis. *Nature*, **444**, 1032-7.
Nygren P, Glimelius B (2001). The Swedish Council on Technology Assessment in Health Care (SBU) report on Cancer Chemotherapy--Project objectives, the working process, key definitions and general aspects on cancer trial methodology and interpretation. *Acta Oncol,* **40**, 155-65.
Ordentlich P, Lin A, Shen CP, et al (1998). Notch inhibition of E47 supports the existence of a novel signaling pathway. *Mol Cell Biol,* **18**, 2230-39.
Phillips TM, McBride WH, Pajonk F (2006). The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. *J Natl Cancer Inst*, **98**, 1777-85.
Piao LS, Hur W, Kim TK, et al (2012). CD133+ liver cancer stem cells modulate radioresistance in human hepatocellular carcinoma. *Cancer Lett*, **315**, 129-37.
Purow B (2009). Notch inhibitors as a new tool in the war on cancer: a pathway to watch, Curr Pharm Biotechnol. *Curr Pharm Biotechnol,* **10**, 154-60.
Purow B (2012). Notch inhibition as a promising new approach to cancer therapy. *Adv Exp Med Biol*, **727**, 305-19.
Pyo H, Choy H, Amorino GP, et al (2001). A selective cyclooxygenase-2 inhibitor, NS-398, enhances the effect of radiation in vitro and in vivo preferentially on the cells that express cyclooxygenase-2. *Clin Cancer Res*, **7**, 2998-3005.
Qi R, An H, Yu Y, et al (2003). Notch1 signaling inhibits growth of human hepatocellular carcinoma through induction of cell cycle arrest and apoptosis. *Cancer Res*, **63**, 8323-9.
Ramakrishnan V, Ansell S, Haug J, et al (2012). MRK003, a gamma-secretase inhibitor exhibits promising in vitro pre-clinical activity in multiple myeloma and non-Hodgkin's lymphoma. *Leukemia*, **26**, 340-8.
Rasul S, Balasubramanian R, Filipovic A, et al (2009). Inhibition of gamma-secretase induces G2/M arrest and triggers apoptosis in breast cancer cells. *Br J Cancer*, **100**, 1879-88.
Reichert S, Rodel C, Mirsch J, et al (2011). Survivin inhibition and DNA double-strand break repair: a molecular mechanism to overcome radioresistance in glioblastoma. *Radiother Oncol*, **101**, 51-8.
Searfoss GH, Jordan WH, Calligaro DO, et al (2003). Adipsin, a biomarker of gastrointestinal toxicity mediated by a functional gamma-secretase inhibitor. *J Biol Chem*, **278**, 46107-16.
Sethi N, Dai X, Winter CG, Kang Y (2011). Tumor-derived JAGGED1 promotes osteolytic bone metastasis of breast cancer by engaging notch signaling in bone cells. *Cancer Cell,* **19**, 192-205.
Sikandar SS, Pate KT, Anderson S, et al (2010). NOTCH signaling is required for formation and self-renewal of tumor-initiating cells and for repression of secretory cell differentiation in colon cancer. *Cancer Res*, **70**, 1469-78.
Spalding AC, Lawrence TS (2006). New and emerging radiosensitizers and radioprotectors. *Cancer Invest*, **24**, 444-56.
Squatrito M, Brennan CW, Helmy K, et al (2010). Loss of ATM/Chk2/p53 pathway components accelerates tumor development and contributes to radiation resistance in gliomas. *Cancer Cell,* **18**, 619-29.
Sriuranpong V, Borges MW, Ravi RK, et al (2001). Notch signaling induces cell cycle arrest in small cell lung cancer cells. *Cancer Res*, **61**, 3200-5.
Strosberg JR, Yeatman T, Weber J, et al (2012). A phase II study of RO4929097 in metastatic colorectal cancer. *Eur J Cancer,* **48**, 997-1003.
Suwanjunee S, Wongchana W, Palaga T (2008). Inhibition of gamma-secretase affects proliferation of leukemia and hepatoma cell lines through Notch signaling. *Anticancer Drugs,* **19**, 477-86.
Szumiel I (2008). Intrinsic radiation sensitivity: cellular signaling is the key. *Radiat Res*, **169**, 249-58.
Teimourian S, Jalal R, Sohrabpour M, Goliaei B (2006). Down-regulation of Hsp27 radiosensitizes human prostate cancer cells. *Int J Urol*, **13**, 1221-5.
Tien AC, Rajan A, Bellen HJ (2009). A Notch updated. *J Cell Biol*, **184**, 621-9.
Tsuyada A, Chow A, Wu J, et al (2012). CCL2 mediates crosstalk between cancer cells and stromal fibroblasts that regulates breast cancer stem cells. *Cancer Res*, **72**, 2768-79 .
Wang J, Wakeman TP, Lathia JD, et al (2010). Notch promotes radioresistance of glioma stem cells. *Stem Cells*, **28**, 17-28.
Wang M, Li JT, Zeng YX, Hou JH, Lin QQ. (2005). Expression

and Significance of Notch1, P21WAF1 and involucrin in nasopharyngeal carcinoma. *Ai Zheng*, **24**, 1230-4.
Wang Z, Li Y, Banerjee S, Sarkar FH (2008). Anticancer Res. Exploitation of the Notch signaling pathway as a novel target for cancer therapy. *Anticancer Res*, **28**, 3621-30.
Wang Z, Li Y, Banerjee S, Sarkar FH (2009). Emerging role of Notch in stem cells and cancer. *Cancer Lett,* **279**, 8-12.
Wei P, Walls M, Qiu M, et al (2010). Evaluation of selective gamma-secretase inhibitor PF-03084014 for its antitumor efficacy and gastrointestinal safety to guide optimal clinical trial design. *Mol Cancer Ther*, **9**, 1618-28.
Whitehead J, Thygesen H, Jaki T, et al (2012). A novel Phase I/IIa design for early phase oncology studies and its application in the evaluation of MK-0752 in pancreatic cancer. *Stat Med*, **31**, 1931-43 .
Woodward WA, Chen MS, Behbod F, et al (2007). WNT/beta-catenin mediates radiation resistance of mouse mammary progenitor cells. *Proc Natl Acad Sci U S A*, **104**, 618-23.
Wu Y, Cain-Hom C, Choy L, et al (2010). Therapeutic antibody targeting of individual Notch receptors. *Nature*, **464**, 1052-7.
Xu X, Zhao Y, Xu M, et al (2011). Activation of Notch signal pathway is associated with a poorer prognosis in acute myeloid leukemia. *Med Oncol*, **28**, S483-9.
Yeung TM, Gandhi SC, Bodmer WF (2011). Hypoxia and lineage specification of cell line-derived colorectal cancer stem cells. P*roc Natl Acad Sci U S A*, **108**, 4382-7.
Yu J, Liu F, Sun M, Sun Z, Sun S (2011). Enhancement of radiosensitivity and the potential mechanism on human esophageal carcinoma cells by tetrandrine. *Cancer Biother Radiopharm*, **26**, 437-42.
Yu S, Zhang R, Liu F, et al (2011). Down-regulation of Notch signaling by a gamma-secretase inhibitor enhances the radiosensitivity of nasopharyngeal carcinoma cells. *Oncol Rep*, **26**, 1323-8.
Zaidi S, McLaughlin M, Bhide SA, et al (2012). The HSP90 Inhibitor NVP-AUY922 Radiosensitizes by Abrogation of Homologous Recombination Resulting in Mitotic Entry with Unresolved DNA Damage. *PLoS One*, **7**, e35436.
Zhang AL, Russell PJ, Knittel T, Milross C (2007). Paclitaxel enhanced radiation sensitization for the suppression of human prostate cancer tumor growth via a p53 independent pathway. *Prostate*, **67**, 1630-40.
Zhang X, Komaki R, Wang L, Fang B, Chang JY (2008). Treatment of radioresistant stem-like esophageal cancer cells by an apoptotic gene-armed, telomerase-specific oncolytic adenovirus. *Clin Cancer Res*, **14**, 2813-23.
Zhang XP, Zheng G, Zou L, et al (2008). Notch activation promotes cell proliferation and the formation of neural stem cell-like colonies in human glioma cells. Mol. Cell. Biochem. 307, 101-8.
Zhang Y, Li B, Ji ZZ Zheng PS. (2010). Notch1 regulates the growth of human colon cancers. Cancer. 116, 5207-18.
Zhang Y, Peng J, Zhang H, et al. (2010). Notch1 signaling is activated in cells expressing embryonic stem cell proteins in human primary nasopharyngeal carcinoma. *J Otolaryngol Head Neck Surg*, **39**, 157-66.
Zhen Y, Zhao S, Li Q, Li Y, Kawamoto K (2010). Arsenic trioxide-mediated Notch pathway inhibition depletes the cancer stem-like cell population in gliomas. *Cancer Lett*, **292**, 64-72.