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Targeting Notch signaling pathway to overcome drug-resistance for cancer therapy

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Abstract

Chemotherapy is an important therapeutic strategy for cancer treatment and remains the mainstay for the management of human malignancies; however, chemotherapy fails to eliminate all tumor cells because of intrinsic or acquired drug- resistance, which is the most common cause of tumor recurrence. Recently, emerging evidences suggest that Notch signaling pathway is one of the most important signaling pathways in drug-resistant tumor cells. Moreover, down-regulation of Notch pathway could induce drug sensitivity, leading to increased inhibition of cancer cell growth, invasion, and metastasis. This article will provide a brief overview of the published evidences in support of the roles of Notch in drug-resistance, and will further summarize how targeting Notch by “natural agents” could become a novel and safer approach for the improvement of tumor treatment by overcoming drug-resistance.

Keywords

Notch; drug resistance; cancer; EMT

1. Introduction

The Notch signaling pathway is a conserved ligand–receptor signaling pathway that plays critical mechanistic roles in cell proliferation, survival, apoptosis, and differentiation which affects the development and function of many organs [1]. Notch genes encode single-pass transmembrane proteins which can be activated by interacting with a family of its ligands. To date, four Notch receptors have been identified in mammals, including human, such as Notch-1-4. The mammalian canonical ligands are designated as either Delta-like (Delta-like 1, Delta-like 3, and Delta-like 4) or Serrate-like ligands, known as Jagged-1 and Jagged-2 [2]. All four Notch receptors are very similar except subtle differences in their extracellular and cytoplasmic domains. The extracellular domains of Notch contain many repeated copies of an epidermal growth factor (EGF)-like motif, which are involved in ligand interaction. Both Notch-1 and Notch-2 proteins have 36 arranged repeats of EGF-like domain, whereas Notch-3 and Notch-4 contain 34 and 29 EGF-like repeats, respectively [3]. The amino-

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terminal EGF-like repeats are followed by cysteine-rich Notch Lin12 repeats (N/Lin12) that modulate interactions between the extracellular and the membrane-tethered intracellular domains. The cytoplasmic region of Notch contains a Recombination Signal-Binding Protein 1 for J-kappa (RBP-J)-association molecule (RAM) domain, ankyrin (ANK) repeats, nuclear localization signals (NLS), a trans-activation domain (TAD) and a region rich in proline, glutamine, serine and threonine residues (PEST) sequence. It is well known that ANK repeats are necessary and sufficient for Notch activity. PEST sequence is involved in Notch protein turnover [4] and the cytoplasmic region of conveys the signal to the nucleus. Notch ligands have multiple EGF-like repeats in their extracellular domain and a cysteine-rich region (CR) in Serrate which are absent in Delta. Jagged-1 and Jagged-2 have almost two-fold numbers of EGF-like repeats compared to Delta [4] (Figure-1A).

Notch signaling is activated after ligand binding to an adjacent Notch receptor between two neighboring cells. Upon activation, Notch receptors undergo a series of proteolytic cleavages by the metalloprotease, tumor necrosis factor-α-converting enzyme (TACE) and γ-secretase complex (comprised of presenilin-1/2, nicastrin, Pen-2, and Aph-1). The first cleavage is mediated by TACE, which leads to cleave the receptor in the extracellular domain. The released extracellular domain is then trans-endocytosed by the ligand-expressing cell. The second cleavage caused by the γ-secretase complex releases the Notch intracellular domain (NICD) into the cytoplasm, which can subsequently translocate into the nucleus because of the presence of nuclear localization signals located within it [5]. Therefore, inhibiting γ-secretase function would prevent the cleavage of the Notch receptor, blocking Notch signal transduction, and thus γ-secretase inhibitor (GSI) could be useful for the treatment of human malignancies [6]. Consistent with this rationale, GSI are now undergoing clinical trials (see website: clinicaltrials.gov). In the absence of NICD, transcription of Notch target genes is inhibited by a repressor complex mediated by the CSL (C protein binding factor 1/Suppressor of Hairless/Lag-1). When NICD is in the nucleus, it forms an active transcriptional complex due to displacing the histone deacetylase-corepressor complex and recruiting the protein mastermind-like 1 (MAML1) and histone acetyltransferases to the CSL complex, leading to convert it from a transcriptional repressor into a transcription activator complex [2] (Figure-1B). A few Notch target genes have been identified, including Hes (Hairy enhance of split) family, Hey (Hairy/enhancer of spit related with YRPW motif), nuclear factor-kappa B (NF-κB), vascular growth factor receptor (VEGF), mammalian target of rapamycin (mTOR), cyclin D1, c-myc, p21, p27, Akt, etc, all of which have been well documented for their roles in tumor development and progression [7-10].

2. Notch in cancer development and progression

It has been well known that Notch signaling plays important roles in maintaining the balance involved in cell proliferation, survival, apoptosis, and differentiation which affects the development and function of many organs. Therefore, dysfunction of Notch prevents differentiation, ultimately guiding undifferentiated cells toward malignant transformation. Indeed, many observations suggest that alterations in Notch signaling are associated with many human cancers [11-17]. Moreover, Notch receptors and ligands have been found as prognostic markers in human cancers [18;19].

2.1. Notch functions as oncogene or tumor suppressor

Very interestingly, the function of Notch signaling in tumorigenesis could be either oncogenic or anti-proliferative, and the function could be context dependent. Notch signaling has been shown to be anti-proliferative in a limited number of tumor types, including skin cancer, human hepatocellular carcinoma, medullary thyroid, cervical cancer, and small cell lung cancer [20-24]. For example, Nicolas et al. used a tissue-specific

inducible gene-targeting approach to study the physiological role of the Notch-1 receptor in the mouse epidermis and the corneal epithelium of adult mice. They unexpectedly found that ablation of Notch-1 results in epidermal and corneal hyperplasia followed by the development of skin tumors and facilitated chemical-induced skin carcinogenesis through beta-catenin-mediated signaling [24]. Recently, studies have also demonstrated that Notch-1 loss in epidermal keratinocytes promotes tumorigenesis by impairing skin-barrier integrity and creating a wound-like microenvironment in the skin. Using mice with a chimeric pattern of Notch-1 deletion, the authors have found that Notch-1 was insufficient to suppress tumor-promoting effect, and the tumor-promoting effect of Notch-1 loss involves a crosstalk between barrier-defective epidermis and its stroma [25]. More recent findings obtained in melanoma and non-melanoma skin cancers show that Notch signaling has a dual action (either as an oncogene or as a tumor suppressor), depending on the tumor cell type and involving synchronous activation of other intracellular signaling mechanisms [26].

However, most of the studies have shown oncogenic function of Notch in many human carcinomas. Emerging evidence suggest that the Notch signaling network is frequently deregulated in human malignancies with up-regulated expression of Notch receptors and their ligands were found in breast, lung, colon, head and neck, renal carcinoma, acute myeloid, Hodgkin and large-cell lymphomas and pancreatic cancer [8;9;15;27-30]. Notch signaling pathway has also been found to cross-talk with multiple oncogenic signaling pathways, such as NF-κB, Akt, Sonic hedgehog (Shh), mTOR, Ras, Wnt, estrogen receptor (ER), androgen receptor (AR), epidermal growth factor receptor (EGFR) and platelet-derived growth factor (PDGF) [31-36]. Thus it is believed that the cross-talk between Notch and other signaling pathways may play critical roles in tumor aggressiveness. From the literature, Notch may act either as a tumor suppressor or tumor promoter depending on the cell type and tissue context, suggesting the complexity of Notch signaling pathways [26]. The functions of Notch signaling have recently been reviewed [7;10;16;23;27;37-40], and thus the readers who are interested in learning more details on the functions of Notch signaling pathway could also consult well-published review articles because the focus of the current article is restricted to overcoming drug-resistance.

2.2. Notch as diagnostic and prognostic markers in human cancers

Notch signaling pathway has been shown to play a role in cancer patient survival. Patients with tumors expressing high levels of Jagged-1 or Notch-1 had a significantly poorer overall survival compared with patients expressing low levels of these genes. Jagged-1 was also found to be highly expressed in metastatic prostate cancer compared to localized prostate cancer or benign prostatic tissues [41]. Furthermore, high Jagged-1 expression in a subset of clinically localized tumors was significantly associated with recurrence, suggesting that Jagged-1 may be a useful marker in distinguishing indolent *vs*. aggressive prostate carcinomas [41]. Recently, high level co-expression of Jagged-1 and Notch-1 has been observed in human breast cancer and the expression was found to be associated with poor overall survival. Moreover, Jagged-1 expression is associated with a basal phenotype and recurrence in lymph node-negative breast cancer [42-44]. Very recently, it was found that Notch-1 and Notch-4 receptors could serve as prognostic markers in breast cancer [18]. Shi et al. also found that the Notch family expression pattern in papillary bladder transitional cell carcinoma which was different from that in invasive bladder transitional cell carcinoma. Therefore, the expression of Notch-1 and Jagged-1 could potentially be a useful marker for survival of patients diagnosed with papillary bladder transitional cell carcinoma [45]. More recently, it was reported that patients with cervical carcinomas positive for nuclear Notch-3 expression had significantly shorter overall survival than their peers whose tumors did not express nuclear Notch-3, suggesting that Notch-3 could be a prognostic marker in cervical

carcinomas [46]. Further research toward exploration of the Notch signal as diagnostic and prognostic markers in human cancers requires in-depth investigations.

3. The role of Notch in drug-resistance

Recently, Notch pathway has been reported to be involved in drug-resistance. More importantly, the studies have demonstrated that Notch regulates the formation of cancer stem cells (CSCs) and contributes to the acquisition of the epithelial-mesenchymal transition (EMT) phenotype, which are critically associated with drug-resistance [40;47]. Experimental evidence also revealed that Notch was involved in anticancer drug-resistance, indicating that targeting Notch could be a novel therapeutic approach for the treatment of cancer by overcoming drug-resistance of cancer cells, which may lead to the elimination of CSCs or EMT type cells which are typically drug-resistant, and are believed to be the “root cause” of tumor recurrence. Therefore, in the following sections, we have attempted to summarize the state-of-our-knowledge on the functional role of Notch signaling pathway in drug-resistance, and approaches by which one could overcome drug-resistance for the successful treatment of most human malignancies.

3.1 Drug-resistance

Chemotherapy is an important therapeutic strategy for cancer treatment and remains the mainstay for the management of human malignancies; however, chemotherapy fails to eliminate all tumor cells because of intrinsic or acquired drug-resistance, which is the most common cause of tumor recurrence. Human cancers are generally initially responsive to standard chemotherapies; however; response is almost inevitably followed by the development of drug-resistant phenotype [48], which leads to tumor recurrence and metastasis. The mechanisms responsible for drug-resistance are complex and still poorly understood. It may be due to either the specific nature and genetic background of the cancer cell itself, or the genetic changes that follow toxic chemotherapy [49]. Drug-resistance to therapy is classified by two categories: intrinsic (*de novo*) and acquired. Intrinsic resistance would make the therapy ineffective because prior to receiving the therapy, the cancer cells have already resistant to anti-cancer drugs due to multiple mechanisms. Acquired resistance develops during the treatment, although the tumor cells were not initially resistant to anti-cancer drugs. The most common reasons for the acquisition of resistance to anti-cancer drugs are due to expression of one or more energy-dependent transporters that detect and eject anti-cancer drugs from cells, insensitivity to drug-induced apoptosis and the induction of drug-detoxification mechanisms [50]. For example, the ATP-binding cassette (ABC) drug transporters have been shown to protect tumor cells from chemotherapeutic agents. ABC transporters eject toxic drugs from cancer cells, leading to reducing the effect of drug’s ability to kill the cancer cells. There are three ABC protein members that have been identified, which are ABCB1 (PGP, P-glycoprotein), ABCG2 (BCRP, breast cancer resistant protein) and ABCC1 (MRP1, multidrug resistance associated protein) [49;50].

Studies over the past years have shown that a number of genes are involved in chemotherapy drug-resistance. These genes include: K-ras, TOP1 (topoisomerase 1), ERCC1 (excision repair cross complementation 1), LRP (lung resistance-related protein), COX-2, cyclin D1, Bcl-2, Survivin, etc [50-53]. Recently, many signaling pathways have been found to be involved in drug-resistance such as PTEN, Akt, mTOR, NF-κB, EGFR, FGFR (fibroblast growth factor receptor), Raf/MEK/ERK, MAPK (mitogen-activated protein kinase), IGF (insulin-like growth factor), and Notch signaling pathway [54-61]. The main roles of these pathways (except Notch signaling pathway) in drug-resistance have recently been reviewed [53-60]. Therefore, in this review article, we will focus our discussion on describing the role of Notch in drug-resistance and summarize approaches by which one could overcome drug-resistance.

3.2 Notch regulates EMT in drug-resistance

Recent studies have shown that EMT is associated with drug-resistance and cancer cell metastasis. It is now widely accepted that epithelial cells can acquire mesenchymal phenotype by a fundamental yet complex processes. The processes of EMT is a unique process by which epithelial cells undergo remarkable morphologic changes characterized by a transition from epithelial cobblestone phenotype to elongated fibroblastic phenotype (mesenchymal phenotype) leading to increased motility and invasion [62]. During the acquisition of EMT characteristics, cells lose epithelial cell-cell junction, actin cytoskeleton reorganization and the expression of proteins that promote cell-cell contact such as E-cadherin and γ-catenin, and gains in the expression of mesenchymal markers such as vimentin, fibronectin, α-smooth muscle actin (SMA), N-cadherin as well as increased activity of matrix metalloproteinases (MMPs) like MMP-2, MMP-3 and MMP-9, leading to an invasive phenotype [63]. Indeed, increasing evidence has shown the relationship between drug-resistance and the existence of EMT phenotype. Fox instance, epithelial but not mesenchymal gene signature has been associated with sensitivity to the EGFR inhibitor erlotinib mediated growth inhibition in lung cancer cells [64]. These results were confirmed in other types of tumors like head and neck squamous cell carcinoma and hepatocellular carcinoma as well as for the treatment of cancer with other EGFR inhibitors such as gefitinib and cetuximad [65;66]. The processes of EMT has also been shown to be important on conferring drug-resistance characteristics to cancer cells against conventional therapeutics including taxol, vincristine and oxaliplatin [67]. Consistent with these observations, recent studies has also shown the link between EMT and gemcitabine-resistant pancreatic cancer cells with increased invasive capacities, oxaliplatin-resistant colorectal cancer cells, lapatinib-resistant breast cancer, and paclitaxel-resistant ovarian carcinoma cells [68-71]. Therefore, the discovery of precise mechanisms that governs the acquisition of EMT phenotype in cancer cells would likely be useful for devising targeted therapeutic approaches in combination with conventional therapeutics for the treatment of human malignancies.

Notch signaling pathway has been reported to be involved with the acquisition of EMT in drug-resistant cancer cells. Our recently published data showed that pancreatic cancer cells that are gemcitabine-resistant (GR) have acquired EMT phenotype as evidenced by elongated fibroblastoid morphology, lower expression of epithelial marker E-cadherin, and higher expression of mesenchymal markers such as zinc-finger E-box binding homeobox 1 (ZEB1) and vimentin [70;72]. We also found that Notch-2 and Jagged-1 are highly up-regulated in GR cells. Moreover, down-regulation of Notch signaling by siRNA approach led to partial reversal of the EMT phenotype, resulting in the mesenchymal-to-epithelial transition (MET), which was associated with decreased expression of vimentin, ZEB1, Slug, Snail, and NF-κB [72]. These results provide molecular evidence indicating that the activation of Notch signaling is mechanistically linked with chemoresistance phenotype, which is consistent with the acquisition of EMT phenotype by pancreatic cancer cells, and further suggesting that the inactivation of Notch signaling by novel strategies could be a potential targeted therapeutic approach for overcoming chemoresistance toward the prevention of tumor progression and/or treatment of human cancer for which current conventional therapeutic strategies are highly disappointing.

3.3 Notch regulates cancer stem cell in drug-resistance

Current cancer therapeutic strategies based on tumor regression may target and kill differentiated tumor cells, which constitute the bulk of the tumor, while sparing the rare cancer stem cell population. Cancer stem cells (CSCs) constitute a small subset of cancer cells that are a reservoir of self-sustaining cells with the exclusive ability to self-renew capacity leading to the maintenance of the tumor mass. The CSCs have been identified and

isolated from tumors of the hematopoietic system, breast, lung, prostate, colon, brain, head and neck, and pancreas [73]. The CSCs are able to self-renew, differentiate, and regenerate to phenotypic cells of the original tumor when implanted into the severe combined immunodeficient mouse. Recently, CSCs have been believed to play critical roles in drug-resistance and cancer metastasis especially because CSCs express drug transporters and DNA repair systems, which allow CSCs to resist the killing effects of the drug. For instance, ABC drug transporters have been shown to protect CSCs from chemotherapeutic agents [74;75]. Another mechanism is that CSCs accumulate mutations over time as a consequence of a long-term exposure to drug, which confer drug-resistance phenotype acquired by the daughter cancer cells [76]. Thus, the molecular knowledge of drug-resistance and metastasis with respect to CSCs in human cancer is considered very important, and the gain of such knowledge is likely to be helpful not only in the discovery of newer drugs but also in the design of novel therapeutic strategies for the treatment of human cancer with better treatment outcome.

Emerging evidence are clearly showing that Notch signaling plays critical roles in both stem cells and progenitor cells, suggesting that abnormal Notch signaling may contribute to carcinogenesis by deregulating the self-renewal of normal stem cells. For example, Phillips *et al.* have reported that CSCs can be identified by phenotypic markers and their fate is controlled by the Notch pathway in breast cancer [77]. Recombinant human erythropoietin receptor increased the numbers of stem cells and self-renewing capacity in a Notch-dependent fashion by induction of Jagged-1. Inhibitors of the Notch pathway blocked this effect, suggesting the mechanistic role of Notch signaling in the maintenance of cancer stem-like cell phenotype [77]. Farnie *et al.* also provided evidence for breast cancer stem cells, and their studies have consistently shown that stem-like cells and breast cancer initiating populations can be enriched using cell surface markers CD44+/CD24− and, as such, these cells showed up-regulated genes including Notch that are known to contribute to cancer stem-like cells characteristics [78]. It has also been reported that glioma stem cells have elevated chemo-resistance because of the high expression levels of drug-transporter proteins such as ABCG2. Furthermore, ABCG2 expression is also associated with proliferation, and the ABCG2 positive cells preferentially express several “stemness” genes such as Notch-1 [79]. Therefore, eradication of CSCs is an important goal for curing cancer, and thus the Notch pathway is considered an attractive target for treatment of cancer because targeting Notch will not only kill differentiated cancer cells but could also kill CSCs by overcoming drug-resistance.

3.4 Notch cross-talks with miRNAs in drug-resistance

Recently evidences suggest that microRNAs (miRNAs) play important roles in the regulation of drug-resistance [80]. It is well known that the miRNAs elicit their regulatory effects in post-transcriptional regulation of genes by binding to the 3′ untranslated region (3′UTR) of target messenger RNA (mRNA). Either perfect or near perfect complimentary base pairing results in the degradation of the mRNA, while partial base pairing leads to translational inhibition to functional proteins [81]. Very interestingly, some miRNAs are thought to have oncogenic activity while others have tumor suppressor activity. Oncogenic miRNAs are up-regulated in cancer and contribute to its pathology through various mechanisms such as targeting tumor suppressor genes. In contrast to the oncogenic miRNAs, other miRNAs are considered to have tumor suppressor activity and are down-regulated in cancer [82]. Recent studies have suggested altered expression of specific miRNAs in drug-resistant tumor cells. For example, the expression of three miRNAs (miR-192, miR-424 and miR-98) was significantly up-regulated while the expression of three other miRNAs (miR-194, miR-200b and miR-212) was down-regulated in docetaxel-resistant NSCLC cells [83]. Recently, Song et al. found that the expression of miR-140 was

associated with chemo-sensitivity to 5-fluorouracil (5-FU) and methotrexate in osteosarcoma. Specifically, blocking endogenous miR-140 sensitized resistant cancer cells to 5-FU treatment, whereas overexpression of miR-140 made tumor cells more resistant to 5-FU, suggesting that miR-140 could be a novel target to develop therapeutic strategy to overcome drug-resistance [84]. Increasing evidence clearly implicating the role of miRNAs in drug-resistance, and in a recent review article, we have summarized the implication of miRNAs in drug-resistance for designing novel cancer therapy [80]. Here, we will discuss further how miRNAs could crosstalk with Notch pathway leading to drug-resistance and how and what novel agents could be useful to overcome such a drug-resistance phenotype of cancer cells.

One miRNA, namely miR-34, has been found to participate in Notch pathways regulation, and has been reported to be involved drug-resistance [85]. The miR-34 family is composed of three processed miRNAs: miR-34a is encoded by its own transcript, whereas miR-34b and miR-34c share a common primary transcript [86]. The expression of miR-34a has been found to be lower or undetectable in pancreatic cancer, osteosarcoma, breast cancer and non-small cell lung cancer, suggesting that miR-34a could function as a tumor suppressor gene [86]. Recently, Li et al. reported that transfection of miR-34a to glioma cells down-regulated the protein expression of Notch-1, Notch-2, and CDK6 [87]. More recently, Ji et al reported that human gastric cancer cells with miR-34 restoration reduced the expression of target gene Notch [88]. In parallel, the same group reported that Notch-1 and Notch-2 are downstream genes of miR-34 in pancreatic cancer cells because restoration of miR-34 expression in the pancreatic cancer cells down-regulated the expression of Notch-1 and Notch-2. Moreover, they reported that pancreatic cancer stem cells are enriched with tumor-initiating cells or CSCs with high levels of Notch-1/2 and loss of miR-34 [89], suggesting that miR-34 may be involved in pancreatic cancer stem cell self-renewal mediated by Notch signaling. More recently, Fujita et al. demonstrated that miR-34a is down-regulated in drug-resistant prostate cancer cells, and ectopic over-expression of miR-34a resulted in growth inhibition and attenuated chemoresistance to the anti-cancer drug camptothecin [85]. Very recently, another study determined that miR-34a was down-regulated in doxorubicin and verapamil resistance MCF-7 breast cancer cells [90]. Collectively, these reports clearly suggest the role of miR-34 in drug-resistance, which is in part mediated through the regulation of Notch signaling; however, further in-depth research is needed in order to fully understanding how miR-34 regulates the Notch pathway in drug-resistant cells and finding novel avenues by which one could up-regulate miR-34 would be highly innovative for designing novel treatment strategies for eliminating tumor cells that are the root cause of tumor recurrence and metastasis.

Another miRNA, miR-1, was markedly reduced in primary human hepatocellular carcinoma, prostate cancer, head and neck, and lung cancer [91;92]. Recently, miR-1 was also found to alter sensitivity of cancer cells to therapeutic agents. Nasser et al. reported that ectopic miR-1 expression sensitize lung cancer cells to anti-cancer drug doxorubicin, suggesting that up-regulation of miR-1 has potential as a target for therapy against lung cancers [92]. It has been reported that Notch ligand Dll-1 protein levels are negatively regulated by miR-1 [93]. In parallel, miR-1 directly targets the Notch ligand delta for repression [94], suggesting that miR-1 may regulate drug-resistance in part via regulating the Notch signaling pathway. Recently, the alteration of miR-200 family was also found in drug-resistant cells. The miR-200 family has five members: miR-200a, miR-200b, miR-200c, miR-141 and miR-429. The expression of miR-200b was significantly down-regulated in docetaxel-resistant NSCLC cells [83]. Recently, many studies have shown that the miR-200 family regulates EMT which is associated with drug-resistance. One study discovered that miR-200 expression regulates EMT in bladder cancer cells and reverses resistance to EGFR therapy [95]. Another recent study reported that miR-200c restored

microtubule-binding chemotherapeutic agents in breast and ovarian cancer cells [96]. We also found that miR-200a, miR-200b, and miR-200c were down-regulated in gemcitabine-resistant pancreatic cancer cells, which show the acquisition of EMT phenotype. Furthermore, we have shown that re-expression of miR-200 family resulted in the down-regulation of ZEB1, slug, E-cadherin, and vimentin, and increased cell sensitivity to gemcitabine [97]. In addition, we found that Notch-1 could be one of miR-200b targets because over-expression of miR-200 family significantly inhibited Notch-1 expression in gemcitabine-resistant pancreatic cancer cells and prostate cancer cells (unpublished data), suggesting that re-expression of miR-200 could increase drug-sensitivity, which indeed could be mediated through the regulation of Notch signaling pathway. Thus, it is our belief that more and more miRNAs will be discovered, whose re-expression will make drug-resistant cells drug-sensitive, and such strategy could be useful in eliminating cancer cells with propensity of recurrence and metastasis.

3.5. Notch pathway in specific chemoresistance

Chemotherapy is critically important for cancer therapy; however, chemotherapy fails to eliminate all tumor cells due to chemoresistance either the *de novo* or acquired chemo-resistance. Currently, chemo-resistance is still the most common cause of tumor progression. Many cellular pathways have been found to be involved in drug-resistance. Recent studies have demonstrated that Notch pathway plays a critical role in anti-cancer drug-resistance as documented in the previous paragraphs. Here, we will further discuss the roles of Notch pathway in chemoresistance and a comprehensive list of Notch pathway that is involved in chemoresistance is presented in Table 1.

3.5.1 The role of Notch in anti-cisplatin resistance—Cisplatin is the most important chemotherapeutic agent for the treatment of human carcinoma including lung, ovarian, bladder and testicular cancers. However, acquired resistance to cisplatin therapy is still a critical problem in the clinical management of cancer patients. Recent studies have shown that Notch may play a role in the mechanisms of cisplatin resistance. One such study by Zhang et al. demonstrated that the positive rate of Notch-1 was significantly higher in head and neck squamous cell carcinoma (HNSCC) than in normal squamous epithelium, and it was negatively correlated with cisplatin-sensitivity [98]. Moreover, Notch-1 was highly expressed in cisplatin resistance HNSCC patients [99]. Further, cisplatin resistance of HNSCC was decreased after inhibition of Notch signaling [99]. In addition, combination of GSI and cisplatin elicits a striking induction of colorectal cancer cell death [100]. Human ovarian cancer-initiating cells enhanced chemoresistance to cisplatin and up-regulation of Notch-1 compared with parental tumor cells [101]. These results support the notion that inactivation of Notch pathway could be a novel strategy for patients who is likely respond to such chemotherapy.

3.5.2 The role of Notch in anti-gemcitabine resistance—Gemcitabine monotherapy (2′,2′-difluorodeoxycytidine), a deoxycytidine analogue, or its combination with other agents has become standard chemotherapy for the treatment of advanced human cancers. However, the effect of gemcitabine on survival has been disappointing, which could be due to many factors including intrinsic drug-resistance or acquired drug-resistance. For example, gemcitabine showed only about 5% partial response rate and imparts a progression-free survival interval ranging from 0.9 to 4.2 months in pancreatic cancer. This disappointing outcome strongly suggests that a better understanding of the mechanism by which chemo-resistance arises is likely to lead to novel therapeutic strategies for the successful treatment of cancer patients. Recently, Notch signaling pathway was found to play a critical role in gemcitabine-resistant cancer cells. Yao et al. demonstrated that Notch-3 siRNA suppressed Notch-3 expression, and increased gemcitabine-induced, caspase-mediated apoptosis in

pancreatic cancer. Moreover, inhibition of Notch-3 enhances sensitivity to gemcitabine in pancreatic cancer through an inactivation of PI3K/Akt-dependent pathway [102]. We also found that Notch-2 and Jagged-1 are highly up-regulated in gemcitabine-resistant pancreatic cancer cells. Moreover, down-regulation of Notch signaling by siRNA approach led to partial reversal of the EMT phenotype, which was associated with increased gemcitabine sensitivity [72].

3.5.3 The role of Notch in anti-taxotere resistance—Taxotere (Docetaxel), a member of the taxane family, has shown high efficacy in the treatment of a wide spectrum of solid tumors including prostate, breast, and gastric cancer [103]. It has been found that taxotere inhibits cell growth and induces apoptosis with down-regulation of some genes for cell proliferation, transcription factors, and oncogenesis, and up-regulation of some genes related to the induction of apoptosis and cell cycle arrest in tumor cells, suggesting pleiotropic effects of taxotere on tumor cells. Clinical trials have shown that the combination chemotherapy using taxotere with other agents improves survival in cancer patients [103]. However, the effect of taxoetere is also disappointed due to drug-resistant. Recently, we found that taxotere-resistant DU145 prostate cells have high expression of Notch-1 (unpublished data), suggesting that Notch pathway is involved in taxotere-resistance. Another group also reported that down-regulation of Notch-1 signaling increased chemosensitivity to taxotere and doxorubicin in breast cancer [104], indicating that Notch signaling may be a promising target for overcoming taxotere-resistant in breast cancer and other cancers.

3.5.4 The role of Notch in anti-taxol resistance—Taxol (Paclitaxel) is another anti-cancer chemotherapy drug. It is used for the treatment of breast, ovarian, lung, bladder, prostate, melanoma, esophageal, as well as other types of solid tumors. It has been reported that taxol could enhance the expression of Notch downstream gene CBF1 in erythroleukemia K562 cells and cervical carcinoma HeLa cells [105]. Recently, it was found that GSIs could enhance taxol-induced mitotic arrest and apoptosis of colon cancer cells both *in vitro* and *in vivo*, suggesting that GSIs could be useful for the treatment of taxol-resistant colorectal cancers [106]. More recently, Mine et al. reported that targeting Notch-1 was significant for novel treatments to eliminate taxol-resistant ovarian cancer stem cells [107]. These limited emerging evidences suggests that overcoming taxol-resistance could be achieved by inactivation of Notch signaling which would become a rational approach for the treatment of most human malignancies.

3.5.5 The role of Notch in anti-tamoxifen resistance—Tamoxifen is a well known anti-cancer drug for the treatment of breast cancer. Certain types of breast cancer require estrogen to grow and tamoxifen blocks the actions of estrogen. Tamoxifen works by blocking the effect of estrogen, resulting in inhibiting gene transcription and tumor growth that are activated by estrogen. Resistance to tamoxifen is often seen in tumor cells that become estrogen independent, thus tamoxifen can not inhibit tumor growth. In a recent study, Rizzo et al found that down-regulation of Notch-1 by siRNA or GSI potentiated the effects of tamoxifen in breast cancer cells. Moreover, GSI in combination with tamoxifen caused regression of breast cancer cell growth in mice [27]. These data indicate that the combinations of tamoxifen and Notch inhibitors may be effective in ERα (+) breast cancer, and such a combination treatment could eliminate the emergence of Tamoxifen-resistance, which certainly would improve the treatment outcome of patients diagnosed with breast cancer.

3.5.6 The role of Notch in anti-oxaliplatin resistance—Oxaliplatin is a platinum-compound chemotherapy drug that acts as an alkylating agent. Oxaliplatin is used to treat

colorectal cancer, and it is often given in combination with other anticancer drugs (5-fluorouracil and leucovorin). It has been shown that Notch-1 is up-regulated in colon cancer. Further, oxaliplatin or 5-FU could induce NICD protein and activated Hes-1 though an increase in the activity and expression of gamma-secretase complex. Therefore, GSI could sensitize cells to chemotherapy, which has been demonstrated showing synergistic activity with oxaliplatin and 5-FU [108]. The authors have summarized that colon cancer cells with up-regulated expression of Notch-1 could function as a protective mechanism in response to chemotherapy [108], further suggesting that combining GSIs with chemotherapy may be a novel strategy for overcoming chemoresistance in colon cancer.

3.5.7 The role of Notch in anti-trastuzumab resistance—Trastuzumab is the humanized, monoclonal antibody that directed against ErbB-2. It has shown efficacy causing improved overall survival for breast cancer patients. However, resistance to trastuzumab remains a major concern, specifically in women with metastatic breast cancer. It has been found that Notch-1 could contribute to trastuzumab resistance in breast cancer [61]. Notch-1 signaling regulates ErbB-2 transcription in ErbB-2-overexpressing breast carcinoma tumor-initiating cells, therefore affecting their self-renewal properties [109]. Trastuzumab increased the Notch-1 activity and its target gene expression. The expression of Notch-1, Hey-1, and Hes-5 was highly expressed in trastuzumab-resistant BT474 compared to trastuzumab-sensitive BT474 [110]. Moreover, down-regulation of Notch-1 increased efficacy of trastuzumab in BT474 sensitive cells and restored sensitivity in resistant cells. Furthermore, the growth of both trastuzumab sensitive and resistant cells was completely inhibited by combining trastuzumab plus Notch-1 siRNA. The Notch-1 siRNA or a GSI re-sensitized trastuzumab-resistant BT474 cells to trastuzumab [110], suggesting that Notch-1 might play a novel role in resistance to trastuzumab, which could be prevented or reversed by inhibiting Notch-1.

3.5.8 The role of Notch in other chemoresistance drugs—Notch signaling pathway was also found in many other chemo-resistant cancer cells [111-113]. For example, Notch-3 was up-regulated and contributed to the anti-cancer drug doxorubicin resistance through regulating p53 expression and DNA damage in human hepatocellular carcinoma (HCC) cell lines, suggesting that Notch-3 silencing in combination with chemotherapeutics could conceivably provide a novel strategy for HCC treatment [114]. Another study determined that Notch-1 signaling was involved in bone marrow stroma-mediated *de novo* melphalan and mitoxantrone resistance of myeloma [115]. Moreover, GSI significantly improved the cytotoxicity of the chemotherapeutic drugs doxorubicin and melphalan in myeloma cells, demonstrating that inhibition of Notch signaling prevents bone marrow mediated drug-resistance and sensitizes to chemotherapy [116]. There are currently more and more studies being done to uncover the resistance mechanism by Notch signaling pathway.

4. Targeting Notch to increase drug-sensitivity

Notch signaling has been reported to be involved in drug-resistance as documented in the previous paragraphs. Therefore, targeting Notch pathway for cancer therapy is a novel strategy for optimizing treatment outcome of conventional chemotherapy. Strategies to regulate Notch expression in cancers could be at many different levels. It is possible to interfere with Notch-ligand interactions, receptor activation, mono-ubiquitination, NICD nuclear complex formation and inhibition of its translocation to the nuclear compartment (Figure 1B). Notch signaling is activated *via* the activity of γ-secretase which became a target in cancer therapy. Several forms of γ-secretase inhibitors (GSIs) have been tested for anti-tumor effects. The GSI inhibits cell growth and could induce apoptosis in many human cancer cells, such as hepatoma cells, breast cancer cells, pancreatic cancer cells, and myeloma cells [1; 7]. Recently, it was found that inhibition of Notch signaling with GSI

sensitized cells to chemotherapy and was synergistic with oxaliplatin and 5-FU, suggesting that combining GSI with chemotherapy may represent a novel approach for treating metastatic colon cancers as indicated above [108]. Recently, Gu et al reported that cisplatin resistance of HNSCC was decreased by inhibition of Notch signaling, suggesting that inactivation of Notch-1 could help HNSCC response to chemotherapy [99]. Very recently, Song et al. evaluated the effects of Notch-1 silencing on cisplatin induced cytotoxicity in CaSki cervival cancer cells. They found that Notch-1 knockdown by siRNA significantly potentiated cisplatin-induced cytotoxicity, lowering the IC50 value of cisplatin in CaSki cells by almost two orders of magnitude [28]. Collectively, all the published data suggest that targeting Notch pathway could increase drug-sensitivity in human cancers; however, one of the major challenges is to eliminate unwanted toxicity associated with the GSI, especially the cytotoxicity in the gastrointestinal tract. Shih *et al.* reported the possible mechanisms underlying the unwanted cytotoxicity of GSI [39]. Notch signaling pathway is known to widely participate in cellular physiology in normal tissues, therefore, it is plausible that inactivation of γ-secretase may lead to the dysfunction of vital organs. Moreover, GSI do not exclusively target the Notch signaling pathways because γ-secretase has many substrates in addition to Notch receptors, such as several Notch ligands, v-erb-a erythroblastic leukemia viral oncogene homolog 4 (ErbB4), CD44, etc. Further, GSI may target proteases other than γ-secretase. Therefore, GSI may have widespread adverse effects *in vivo* because proteases participate in a wide array of cellular functions [39].

In order to overcome such limitations, recent studies have shown that “natural agents”, which are typically non-toxic to humans, including isoflavone, resveratrol, curcumin, withaferin-A, and others could inhibit the Notch-1 expression. The studies from our laboratory have shown that genistein and curcumin down regulated the transcription and translation of Notch-1 and its downstream genes, Hes-1, cyclin D1, Bcl-X_L and NF-κB. Over-expression of Notch-1 by Notch-1 cDNA transfection abrogated genistein- and curcumin-induced apoptosis to a certain degree. Therefore, we strongly believe that down-regulation of Notch-1 by genistein and curcumin is mechanistically linked to cell proliferation and apoptotic processes [9;117-119]. In addition, studies from other laboratories have shown that resveratrol could induce apoptosis by inhibiting the Notch pathway mediated by p53 and PI3K/Akt in T-ALL [120]. Moreover, one Chinese herb antitumor B was also found to inhibit Notch expression in a mouse lung tumor model [121]. Recently, it was reported that withaferin-A could inhibit Notch-1 signaling and thereby down-regulates pro-survival pathways, such as Akt/NF-κB/Bcl-2, in colon cancer cells [122]. Furthermore, recent studies have shown that natural agents could alter the expression of specific miRNAs that could regulate Notch signaling pathway. We found that re-expression of miR-200 by pre-miR-200 transfection or treatment of GR pancreatic cancer cells with isoflavone resulted in the up-regulation of miR-200, leading to increased sensitivity of GR cells to gemcitabine. Isoflavone also induced the expression of let-7, which could be linked to the treatment effects [97]. Considering the relatively non-toxic nature of natural agents, targeting the Notch pathway by these natural agents combined with conventional chemotherapy could be a novel and safer approach for achieving better treatment outcome; however, further in-depth preclinical and clinical studies are warranted in order to appreciate the value of natural agents in overcoming drug-resistance to eliminate cancer cells that are the root cause of tumor recurrence and metastasis.

5. Conclusion and overall perspectives

In this review article we attempted to summarize the role of Notch pathway in drug-resistance; however we could not cite all the published studies, and thus we sincerely apologize to those whose work has not been cited here due to space limitations. In conclusion, recent studies demonstrate that Notch signaling pathway may play critical roles

in the regulation of anti-cancer drug-sensitivity and resistance. Since Notch signaling pathway has been found to be involved in EMT and CSCs and deregulated expression of miRNAs, suggesting that up-regulation and down-regulation of specific miRNA that are intimately associated with Notch signaling could become a novel approach for overcoming drug-resistance (Figure-2). As such, high expression of Notch pathway can reduce response to anti-cancer agents such as cispltin, doxorubicin, 5-fluorouracil, gemcitabine, tamoxifen, etc., and thus, down-regulation of Notch signaling by multiple approaches appears to be a novel strategy for increasing drug-sensitivity of cancer cells to conventional chemotherapeutics. To that end, natural agents such as genistein, curcumin, resveratrol, and others could be very useful for the inhibition of Notch signaling pathway, which could lead to the inhibition of cancer growth, induction of apoptosis, reversal of EMT phenotype, elimination of drug-resistant CSCs, and thereby increasing drug-sensitivity, which would be useful for treatment of cancer patients with better treatment outcome. In summary, our findings together with those reported in the literature are becoming an exciting area for further in-depth research toward targeted inactivation of Notch signaling proteins, especially by natural agents, as a novel therapeutic approach for increasing the drug-sensitivity, and thereby improving the treatment outcome of cancer patients, which is believed to be due to eliminating the cancer cells that are the root cause of tumor recurrence and metastasis.

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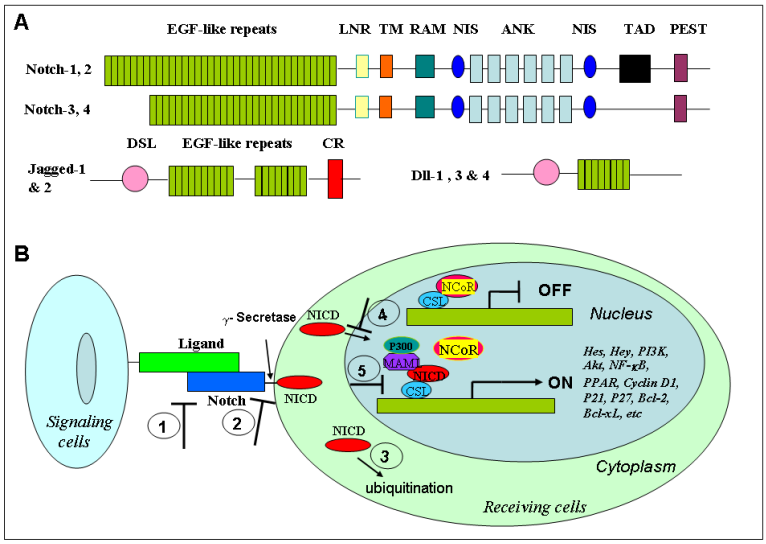

Figure-1. A, Structure of Notch receptors (1-4) and ligands (Jagged-1, 2, Dll-1, 3, 4)
Both receptors and ligands contain multiple conserved domains. Notch is a single-pass transmembrane receptor. The extracellular domain contains EGF-like repeats and a cysteine-rich region. The intracellular domain contains the RAM domain, NLS, ANK, TAD and PEST domain. Notch ligands have multiple EGF-like repeats in their extracellular domain and a CR in Jagged which are absent in Delta. **B, Schematic of Notch signaling.** Notch signaling is activated after ligand binding to an adjacent Notch receptor between two neighboring cells. Upon activation, Notch receptors undergo a series of proteolytic cleavages by the metalloprotease, TACE, and γ-secretase complex. The cleavage releases the NICD into the cytoplasm, which can subsequently translocate into the nucleus. In the absence of NICD, transcription of Notch target genes is inhibited by a repressor complex mediated by the CSL. When NICD is in the nucleus, it forms an active transcriptional complex due to displacing the histone deacetylase-corepressor complex and recruiting the protein MAML1 and histone acetyltransferases to the CSL complex, leading to convert it from a transcriptional repressor into a transcription activator complex, leading to activation of Notch target genes. Diagram of putative therapeutic target in the Notch pathway. Notch signaling could be inhibited theoretically at many different levels. It is possible to (1) interfere with Notch-ligand interactions, (2) inhibit receptor activation, (3) promote Notch ubiquitination and degradation, (4) inhibit its translocation to the nuclear compartment and (5) inhibit NICD nuclear complex formation.
ANK: Ankyrin repeat, CR: Cysteine rich region, DSL: Delta-serrate-lag2, EGF: Epidermal growth factor, LNR: Lin12/Notch repeats, NLS: Nuclear localization signals, PEST: Proline, glutamine, serine,threonine, RAM: RBP-J association molecule domain, TAD: Transcriptional activator domain, TM: Transmembrane domain. CSL: C protein binding factor 1/Suppressor of Hairless/Lag-1, NICD: Notch intracellular domain, TACE: tumor necrosis factor-α-converting enzyme, MAML1: mastermind-like 1.

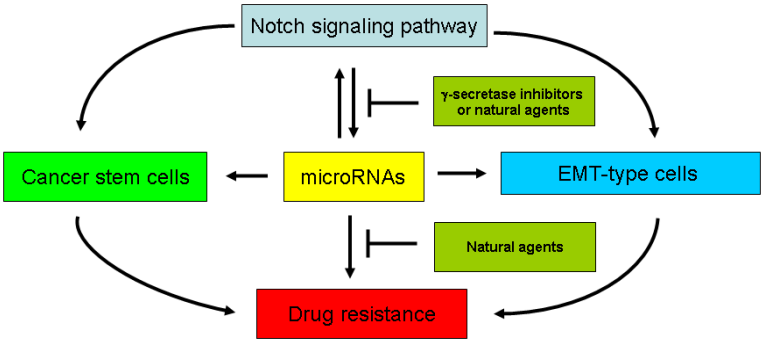

Figure-2.

The role of Notch signaling pathway in the progression of cancer, and during the acquisition of EMT phenotype, and the formation of cancer stem cells, leading to drug resistance. Natural agents and γ-secretase inhibitors could be useful for targeting Notch signaling pathway proteins, which could enhance the sensitivity of chemotherapeutic drugs.

Table 1
A comprehensive list of Notch pathway involved in chemoresistance

Drug	Targeted genes	Cell or tissue	Reference
Cisplatin	Notch-1 was highly expressed in cisplatin resistance cells	Head and neck squamous cell, colorectal and ovarian cancer cells	[98-101]
Doxorubicin	Notch-3 was up-regulated and contributed to the anti-cancer drug doxorubicin resistance through regulating p53 expression and DNA damage.	Hepatocellular carcinoma cells, myeloma cells	[114;116]
	GSI improved the cytotoxicity of the doxorubicin		
Erlotinib	GSI enhance the EGFR tyrosine kinase inhibitor erlotinib anti-tumor activity	Lung cancer	[112]
5-fluorouracil	5-FU induced NICD protein and activated Hes-1	Colon cancer cells	[108]
Gemcitabine	Inhibition of Notch-3 enhances sensitivity to gemcitabine.	Pancreatic cancer cells	[72;102]
	Notch-2 and Jagged-1 are highly up-regulated in gemcitabine-resistant.		
Gefitinib	Over-expression of Notch-1 contributes to the gefitinib resistance	Breast cancer cells	[111;113]
Melphalan	GSI improved the cytotoxicity of the melphalan	Myeloma cells	[116]
Mitoxantrone	Activation of Notch-1 resulted in the protection from mitoxantrone-induced apoptosis	Myeloma cells and malignant lymphoid cell lines	[115]
Oxaliplatin	oxaliplatin induced NICD protein and activated Hes-1	Colon cancer cells	[108]
	GSI sensitized cells to oxaliplatin		
Paclitaxel	Taxol enhanced the expression of Notch downstream gene CBF1.	Erythroleukemia, cervical, colorectal, ovarian cancer cell	[105-107]
	GSIs are useful for the chemotherapeutic treatment of taxol-resistant cancer cells		
Tamoxifen	Down-regulation of Notch-1 or GSI potentiated the effects of tamoxifen	Breast cancer cells	[27]
Taxol	GSIs enhance taxol-induced mitotic arrest and apoptosis of colon cancer cells	erythroleukemia cells, cervical , colon, ovarian cancer cells	[105-107]
Taxetere	Down-regulation of Notch-1 signaling increased chemosensitivity to taxotere	Breast and prostate cancer cells	[104]
Trastuzumab	Notch-1 signaling regulates ErbB-2 transcription.	Breast cancer cells	[61;109;110]
	Down-regulation of Notch-1 increased efficacy of trastuzumab and restored sensitivity in resistant cells		