MINIREVIEW

Notch1 in Cancer Therapy: Possible Clinical Implications and Challenges

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ABSTRACT

The Notch family consists of four highly conserved transmembrane receptors. The release of the active intracellular domain requires the enzymatic activity of γ -secretase. Notch is involved in embryonic development and in many physiologic processes of normal cells, in which it regulates growth, apoptosis, and differentiation. Notch1, a member of the Notch family, is implicated in many types of cancer, including breast cancer (especially triple-negative breast cancer), leukemias, brain tumors, and many others. Notch1 is tightly connected to many signaling pathways that are therapeutically involved in tumorigenesis. Together, they impact apoptosis, proliferation, chemosensitivity, immune response, and the population of cancer stem cells. Notch1 inhibition can be achieved through various and diverse methods, the most common of which are the γ -secretase inhibitors, which produce a pan-Notch inhibition, or the use of Notch1 short interference RNA or Notch1 monoclonal antibodies, which produce a more specific

blockade. Downregulation of Notch1 can be used alone or in combination with chemotherapy, which can achieve a synergistic effect and a decrease in chemoresistance. Targeting Notch1 in cancers that harbor high expression levels of Notch1 offers an addition to therapeutic strategies recruited for managing cancer. Considering available evidence, Notch1 offers a legitimate target that might be incorporated in future strategies for combating cancer. In this review, the possible clinical applications of Notch1 inhibition and the obstacles that hinder its clinical application are discussed.

SIGNIFICANCE STATEMENT

Notch1 plays an important role in different types of cancer. Numerous approaches of Notch1 inhibition possess potential benefits in the management of various clinical aspects of cancer. The application of different Notch1 inhibition modalities faces many challenges.

Introduction

Notch proteins are transmembrane receptors that were first identified in *Drosophila melanogaster*. They exert an important role in the transfer of cell-to-cell signals and in the regulation of embryonic development. They determine the fate of cells by regulating their differentiation, apoptosis, and

proliferation. The Notch receptor, after post-translational modifications, consists of an extracellular part [with multiple tandem epidermal growth factor (EGF)-like repeats] that binds to ligands expressed on an adjacent cell, a transmembrane region, and an intracellular part that transports the signal (Bianchi et al., 2006). When the extracellular domain binds to any of the Delta/Serrate/ or LAG-2 ligands, it forms a complex that is internalized into the cytoplasm by endocytosis and mediates a metalloprotease cleavage of the extracellular domain. Notch intracellular domain (NICD) is released by

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ABBREVIATIONS: ADAM, a disintegrin and metalloproteinase; Bcl-2, B-cell lymphoma 2; CK2, casein kinase 2; CLL, chronic lymphocytic leukemia; CRC, colorectal cancer; DAPT, *N*-[*N*-(3,5-difluorophenyl)-L-alanyl]-S-phenylglycine t-butyl ester; Dll, delta-like; EGF, endothelial growth factor; EMT, epithelial-to-mesenchymal transition; Erb-B2, epidermal growth factor receptor B2; ERK, extracellular signal-regulated kinase; GBM, glioblastoma; GSI, γ -secretase inhibitor; Hes, hairy and enhancer of split; Hey, hairy/enhancer-of-split related with YRPW motif protein; JAG, jagged; KRAS, Kirsten rat sarcoma; lncRNA, long noncoding RNA; mAb, monoclonal antibody; MAPK, mitogen-activated protein kinase; MCF-7, Michigan cancer foundation-7; miRNA, microRNA; NF- κ B, nuclear factor- κ B; N1-ICD, Notch1 active intracellular domain protein; NICD, notch intracellular domain; NSCLC, non-small-cell lung cancer; PDX, patient-derived xenograft; PRKCI, protein kinase C iota; POFUT1, protein O-fucosyltransferase-1; PPI, protein-protein interaction; PTEN, phosphatase and tensin homolog; RAS, rat sarcoma; SERCA, sarco/endoplasmic reticulum calcium ATPase; shRNA, short hairpin RNA; siRNA, short interference RNA; T-ALL, T-cell acute lymphoblastic leukemia; TNBC, triple-negative breast cancer.

γ -secretase cleavage of the fragment that is bound to the membrane, which in turn is translocated to the nucleus and binds to the transcription complex that removes the repression of Notch targets, the Hes and Hey proteins (Fig. 1). Cis-inhibitory complexes that limit the Notch activity are formed when the Notch receptor and ligands that are expressed on the same cell surface interact (Chillakuri et al., 2012).

There are four Notch receptors and five transmembrane Notch ligands that belong to Delta/Delta-like (Dll1, 3, and 4) and Serrate/Jagged (Jagged1 and Jagged2) ligand families (Lindsell et al., 1996). Although the Notch receptors share ligands and structural similarities, they have different functions. In humans, Notch1 and 4 are crucial for T-cell development (Radtke et al., 1999; Hozumi et al., 2008). Notch3 mutations cause cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy syndrome (Joutel, 2011); Notch4, on the other hand, does not seem to be an important player, but it overlaps with Notch1 (Krebs et al., 2000).

The Notch pathway is an important key player in malignancies; abnormalities in Notch signaling are involved in many hallmarks of cancer (Aster et al., 2017). The role of Notch signaling pathway in cancer is context-dependent and varies from being oncogenic to tumor suppressive (Li et al., 2010).

Although most of the members of the Notch receptor family are generally involved in many cancers, targeting individual Notch receptors offers many advantages, including reduced toxicity and enhanced effects on specific receptors that are involved in certain types of cancer (Wu et al., 2010). Notch1 activating mutations were identified as an oncogene in many cancers, including triple-negative breast cancer (Stoeck et al., 2014) and T-cell acute lymphoblastic leukemia (T-ALL) (Pear

et al., 1996; Ferrando, 2009). Conversely, blockade of Notch1 in the skin of mice resulted in the formation of skin tumors, which may be mediated through an increase in β -catenin signaling (Nicolas et al., 2003). The disparities in the role of Notch signaling suggest an effect that is dependent on tissue type, development stage, and coinvolving signaling pathways.

Tumorigenesis

The role of Notch1 in the tumorigenesis of many types of cancer was explored extensively.

Prostate Cancer. The oncogenic effect of Notch1 was confirmed in prostate cancer when Notch1 expression was discovered in cultured prostate cancer cells that originated from primary tumors, lymph node metastasis, brain metastasis, and bone metastasis (Leong and Gao, 2008). EMT markers were investigated in metastatic prostate cancer, and data analysis obtained from primary and bone metastasis formalin-fixed paraffin-embedded samples showed that metastatic samples had a significantly higher frequency of Notch1-positive cells, and Notch-1 expression was also higher in bone metastatic prostate cancer tissue samples compared with those of the primary prostate cancer ($P = 0.057$) (Sethi et al., 2010).

The relationship between Notch1 and prostate cancer was further complicated by the finding that dysregulation of this signaling pathway also occurs in prostate cancer. Analysis of data from the Gene Logic data base revealed that the expression of Notch1 and the downstream target gene Hey1 was downregulated in prostate adenocarcinoma samples when compared with normal prostate samples in normal people or adjacent to prostate cancer cells. The expression of many genes was changed as a result of Notch1 ablation; these

Fig. 1. Notch signaling pathway. Figure was created using BioRender.com. ANK, ankyrin repeats; CO-A, coactivator; HD, heterodimerization domain; LNR, Lin-12 and Notch repeats; MAML, coactivator of the Mastermind-like family; NLS, nuclear localization signal; NNR, negative regulatory region; PEST, proline, glutamic acid, serine, and threonine domain that controls the ubiquitin-mediated degradation; RAM, RBP-j-associated molecule; RBPJ, DNA binding factor—recombination signal binding protein for immunoglobulin κ J region; TACE, tumor necrosis factor- α -converting enzyme (also called ADAM17); TAD, trans-activation domain.

were important for proliferation, cell cycle, DNA repair, DNA replication, cell growth, cancer, and many other functions (Wang et al., 2006). Additionally, Whelan et al. (2009) demonstrated that, in prostate cancer, diminished Notch1 signaling was correlated with adenocarcinoma through dysregulation of PTEN. Despite the previous conflicting data, which do not confirm whether Notch1 is a tumor suppressor or an oncogene in prostate cancer, O'Brien and Marignol (2017) suggested that Notch inhibition at the localized prostate stage could prevent progression of cancer.

T-Cell Acute Lymphoblastic Leukemia. The role of Notch1 in T-cell acute lymphoblastic leukemia is more well defined and was well established by Weng et al. (2004). In their analysis of human T-ALLs, more than 50% had activating mutations in the Notch1 gene. The study proposed Notch1 as a possible therapeutic target, especially for patients who are refractory. One of the Notch1 target genes was *c-myc*, where increased expression of intracellular Notch1 was associated with increased *c-myc* mRNA levels in primary mouse T-cell tumors. Additionally, inhibition of Notch1 [with γ -secretase inhibitor (GSI)] caused a reduction in *c-myc* mRNA levels and inhibited leukemic cell growth (Sharma et al., 2006). Another Notch1 target was mTOR which was deactivated by the γ -secretase inhibitor DAPT, a well known inhibitor of Notch. This effect was rescued by increased expression of the intracellular domain of Notch and expression of *c-myc*, which suggests that *myc* is an intermediate between Notch1 and mTOR (Chan et al., 2007). Cell cycle is also influenced by Notch1; it enhances the progression of T cells through the G1/S phase of the cell cycle by increasing the expression of cyclin D3 and CDK4. Notch-dependent human T-ALL cell lines treated with a γ -secretase inhibitor (LY-411575) were rescued from cell cycle arrest by increased expression of cyclin D3 (Joshi et al., 2009). Notch1 may also increase cell cycle progression by reducing the levels of protein p27 Kip1 through promotion of its degradation by E3 ubiquitin ligase SKP2 in T-ALL cells (Dohda et al., 2007).

Breast Cancer. In breast cancer, when sections of primary breast tumors from patients with the luminalbasal subtype and xenografted tumors were analyzed, the Notch1 transcripts were elevated in basal-like/claudin-low, estrogen receptor 1 negative tumors. This high level of Notch1 activity was suppressed by a GSI. Several GSIs were examined: DAPT, *N*-[*N*-(3,5-difluorophenyl)-L-alanyl]-S-phenylglycine t-butyl ester; compound E, [(2*S*)-2-[(3,5-difluorophenyl)acetyl]amino-*N*-(3*S*)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1*H*-1,4-benzodiazepin-3-yl]propanamide]; XIX, (2*S*,3*R*)-3-(3,4-difluorophenyl)-2-(4-fluorophenyl)-4-hydroxy-*N*-[(3*S*)-2-oxo-5-phenyl-2,3-dihydro-1*H*-benzo[e][1,4]diazepin-3-yl]-butyramide. In addition, treatment with GSIs or Notch1 downregulation by shRNA maintained an ER (estrogen receptor) positive luminal phenotype in breast cell lines even in the absence of estrogen (Haughian et al., 2012).

In mouse models, continuous Notch1 activation in the mammary epithelium induced neoplasms and was correlated with increased levels of cyclin D1, a possible target of Notch signals in the mammary epithelium (Kiaris et al., 2004).

Notch1 was especially implicated in triple-negative breast cancer (TNBC). In 200 human breast cancer samples, higher expression of mRNA levels of Notch1 was statistically significant in TNBC but insignificant in ER-negative samples. By applying a predictive model for TNBC, a 1-U increase in the log of Notch1 led to an increase in the likelihood of having

TNBC by 6.4-fold (95% confidence interval of 2.04–20.11) (Yuan et al., 2015b). Notch1 was also correlated with Ras/MAPK pathways, and hyperactivation of Notch1 and Ras/MAPK was coordinated (Mittal et al., 2014). Signaling pathways upstream of Notch1 were also revealed; c-Jun N-terminal kinase, a member of the MAPK family, promoted cancer stem cell tumorigenesis in TNBC through an upregulation of Notch1 at both transcription and protein levels (Xie et al., 2017).

One meta-analysis examined 3867 patients and revealed that Notch1 in breast cancer tissues compared with normal ones was significantly overexpressed. Furthermore, patients with higher Notch1 expression were more likely to progress from ductal carcinoma in situ to invasive cancer, with an odds ratio of 3.75 (95% CI 1.8–7.78) (Yuan et al., 2015). The involvement of Notch1 in EMT was examined in breast cancer cell lines, and abnormal expression of Notch1 intracellular domain led to an decrease in E-cadherin levels and promoted invasiveness in MCF-7 cells, whereas Notch1 inhibition using DAPT (RO4929097) in MDA-MB-231 caused an increase in E-cadherin levels and reduced invasiveness (Bolós et al., 2013).

Glioblastoma. In glioblastomas (GBMs), a recent study evaluated Notch1 expression profiles using four cohorts of gliomas. Results from this study showed that Notch1 was overexpressed in patients with GBM compared with normal controls, and there was a positive correlation between Notch1 and NF- κ B(p65) levels. In addition, they were both colocalized in the same GBM tissue cells (Hai et al., 2018b).

When 69 glioma tissue specimens were compared with eight normal specimens, Notch1 level was overexpressed in 71% of glioma specimens compared with 13% of eight normal specimens ($P < 0.05$) (Han et al., 2017).

Head and Neck Squamous Cell Carcinoma. Notch1 blockade was associated with a reduction in immunosuppression in this type of carcinoma. Genetically modified head and neck squamous cell carcinoma mouse models that exhibited increased activity of Notch1 were exposed to a γ -secretase inhibitor (DAPT). This led to enhanced tumor immunity mediated by a decrease in the immune checkpoint molecules, myeloid-derived suppressor cells, and tumor-associated macrophages (Mao et al., 2018).

Wieland et al. (2017) investigated the role of Notch1 active intracellular domain protein (N1-ICD) located in vascular endothelial cells in metastasis. The expression of N1-ICD was elevated in several types of cancer, including lung adenocarcinoma, serous ovarian carcinoma, colorectal carcinoma, breast carcinoma, and melanoma. When compared with clinical data, an association between the levels of N1-ICD and metastasis was demonstrated. For example, patients with positive sentinel lymph nodes had higher rates of positive N1-ICD. Similarly, melanoma patients with positive N1-ICD expression had higher rates of metastasis.

Notch1 and Cancer Stem Cells

Notch, Hedgehog, Wnt, and JAK/STAT (Janus kinase/signal transducer and activator of transcription) are signaling pathways that are important for the survival of cancer stem cells (Matsui, 2016). Many studies provided proof that the antitumor and chemosensitization effect of Notch1 downregulation and pharmacologic inhibition was mainly a consequence of a reduction in the cancer stem cell population.

Breast Cancer. In TNBC cell lines, the use of Notch1 monoclonal antibodies (mAbs) has been shown to significantly reduce the cancer stem cell (CD44⁺/CD24⁻/low) population (Qiu et al., 2013). When cancer stem cells with stemness markers were isolated from a renal cell carcinoma cell line, Notch1, among other Notch receptors, was overexpressed in these stem cells. Notch1/2 inhibition with a γ -secretase inhibitor (MRK-003) increased sensitivity to cisplatin and sorafenib and reduced stemness markers. Xenografts from these treated cancer stem cells manifested enhanced apoptosis and decreased proliferation (Xiao et al., 2017). In HER2-positive breast cancer, Notch1 plays an important role in the development of trastuzumab resistance by enhancing stem cell survival. The enrichment of the stem cell population by Notch1 and, consequently, poor survival rates were mediated via inhibition of PTEN (Baker et al., 2018).

Colorectal Cancer. In colon tumor cell line HCT-116, the transduction of intracellular domain of Notch1 produced an increase in CD44, a cell surface glycoprotein that is overexpressed in cancer stem cells. Inhibition of the Notch1 signaling pathway by using pan-Notch inhibitor DAPT led to a 41% decrease in CD44 ($P < 0.001$). Previous results indicate the correlation of stemness and Notch1 expression in colon cancer (Fender et al., 2015).

Ovarian Cancer. Galectin-3 is an extracellular protein that functions as a cross-linker. Galectin-3 protein was correlated with increased stemness of ovarian cancer cells, reflected clinically as poor survival and relapse in patients with ovarian cancer (Kim et al., 2011). Overexpression of Galectin-3 in a human ovarian cancer cell line, A2780, caused increased activity of Notch signaling. Furthermore, a reduction in the expression of this protein decreased NICD1 levels and its target genes, Hes1 and Hey1 (Kang et al., 2016).

Glioblastoma. Protein kinase C ι (PRKCI) gene, a member of the atypical protein kinase C family, in GBM stem-like cells is spatially close to Notch1 gene. Silencing PRKCI gene with shRNAs was correlated with downregulation of the Notch pathway genes (including Notch1) in vitro and reduced tumor growth in vivo. Targeting PRKCI using shRNAs also led to reduced proliferation in GBM stem-like cell lines NCH421k, NCH644, and NCH44 (Phillips et al., 2016).

A recent review of the trials targeting cancer stem cells through the Notch signaling pathway was conducted by Venkatesh et al. (2018). The assembled evidence in this review suggested that Notch inhibition represents a promising opportunity to manage cancer through its strongest, most resistant component, cancer stem cells.

Notch1 as a Prognostic Tool

Extensive efforts were made in recent years to explore the prognostic value of Notch1 in different types of cancer.

Breast Cancer. When correlated with patient follow-up data, high levels of Notch1 expression in breast cancer tissues were associated with lower 5-year survival rates compared with tissues with low levels of Notch1 (49% vs. 64%). Additionally, tumors that expressed high levels of Notch1 either expressed high or low levels of JAG1. Those with high expression levels of both Notch1 and JAG1 have reduced overall survival compared with other tumors (Reedijk et al., 2005). Further evidence for the prognostic value of Notch1 was

provided by a meta-analysis that examined the microarray data of 4463 patients from 17 studies. Increased levels of Notch1 were correlated with a decrease in recurrence-free survival regardless of other prognostic factors or type of breast cancer (Abravanel et al., 2015). In another study that used antibodies for active NICD and pErk1/2 specific antibodies to detect Ras/MAPK activity in grade III ductal carcinoma breast cancer patients, 61.7% of patients exhibited a high Notch1 and high pErk phenotype. Interestingly, 89% patients (71 out of 80) of the high pErk samples had high Notch1 levels, and more than two-thirds of the TNBC cases belonged to this phenotype (Mittal et al., 2014). Another study that investigated the prognostic value of Notch1 examined paraffin-embedded ductal carcinoma in situ tissues. Results from the study revealed that increased levels of NICD were correlated with recurrence at 5 years ($P = 0.012$).

Gallbladder Cancer. The clinicopathologic importance of Notch1 was also investigated in gallbladder cancer. This rare and aggressive cancer includes three subtypes: adenocarcinoma, which is the most common subtype; squamous cell carcinoma; and adenosquamous carcinoma. Notch1 expression was significantly associated with low surgical curability, invasion, and metastasis in all subtypes. Additionally, when compared with patients with negative Notch1/Notch3 expression, those with positive Notch1/Notch3 expression had statistically significant shorter survival times ($P < 0.05$ in all subtypes). Authors concluded that Notch1 expression was one of the factors that independently predicted poor prognosis in [patients with squamous cell/adenosquamous carcinomas and adenocarcinoma (Liu et al., 2016)].

Glioblastoma. Notch1 expression was negatively correlated with overall survival in patients with glioblastoma; those with low Notch1 expression had an overall survival of 26 months, compared with 15 months in patients with high Notch1 expression ($P = 0.002$). Furthermore, Notch1 expression independently predicted overall survival (Han et al., 2017).

In addition, analysis of survival data from numerous databases revealed that high expression levels of Jagged1 (ligand for Notch1 receptor) was positively correlated with poor survival, $P < 0.0001$. Higher expression levels of Notch1 ligand were associated with higher levels of NF- κ B(p65) (Hai et al., 2018a).

T-Cell Acute Lymphoblastic Leukemia. In a study of South Indian pediatric patients with T-cell acute lymphoblastic leukemia, decreased degradation of Notch1 was associated with better clinical outcome. The degradation of Notch1 was controlled by the E3 ubiquitin ligase FBXW7 and the proline, glutamic acid, serine, and threonine-rich domain in Notch1. Consequently, mutations in NOTCH1/FBXW7 led to increased stability of the Notch1. In these pediatric patients, NOTCH1/FBXW7 activating mutations were associated with increased levels of Notch1 protein. When compared with NOTCH1/FBXW7-nonmutated patients, two-thirds (65%) of patients with NOTCH1/FBXW7-mutated T-ALL responded better to prednisolone and had a more favorable clinical outcome (Natarajan et al., 2015).

Adrenocortical Tumors. Aberrations in the Notch pathway are the most frequent abnormality in adrenocortical tumors. When fresh-frozen samples of adrenocortical carcinomas were analyzed, there was an overexpression of Jagged1 (Notch1 ligand) and HEY2 (downstream target of Notch1)

compared with normal adrenal glands or benign adrenocortical lesions. Overexpression of Jagged1 and HEY2 was at both the mRNA and protein level. However, in terms of clinical outcome measured as overall survival, the impact of Jagged1 and HEY2 was not the same. High levels of HEY2 expression were associated with poor prognosis (median overall survival: 50 vs. 100 months, $P = 0.13$, hazard ratio (HR) = 1.68, 95% CI 0.86–3.37), whereas high Jagged1 protein levels were associated with favorable impact on survival, but only in patients who did not receive treatment (median overall survival: 108 vs. 50 months, $P < 0.05$, HR = 0.47, 95% CI 0.24–0.94). Moreover, the positive effect of Jagged1 was especially evident in cancers associated with low nuclear β -catenin expression (Ronchi et al., 2015).

Lung Cancer. In patients with non-small-cell lung cancer (NSCLC), Notch1 levels also had clinical predictive value. A recent meta-analysis that assessed 19 studies and involved 3663 patients with NSCLC revealed that Notch1 and Notch3 overexpression was associated with poor overall survival (Notch1: HR, 1.29; 95% CI 1.06–1.57) (Yuan et al., 2015a).

Other Types of Cancer. Notch1 was also explored for its prognostic value in many other types of cancer. In hepatocellular carcinoma, overexpression was associated with poor survival and a more aggressive disease (Wu et al., 2015). In colorectal cancer (CRC), the extent of expression of Notch1 and Jagged1 was correlated with histologic grading and lymph node metastasis of CRC (Zheng et al., 2015). And in acute myeloid leukemia, high gene expression levels of Notch1 and Jagged1 independently predicted poor prognosis in terms of overall survival and relapse-free survival (Xu et al., 2011).

Resistance to Therapy

Chemotherapy, hormone therapy, and radiation remain the main modalities of clinical management in cancer. Yet successful management of these therapeutic strategies is limited by resistance. Many *in vitro* studies confirmed the association of resistance with Notch1 overexpression in many types of cancer.

Breast Cancer. Further evidence concerning the effect of Notch1 inhibition on chemosensitivity was shown in the TNBC cell line MDA-MB-231. The cytotoxic effect of paclitaxel was enhanced after its combination with Notch1-siRNA, and this was achieved by altering the expression of paclitaxel targets through increased expression of Caspase-3 and Caspase-9 and reduced expression of Bcl-2 (B-cell lymphoma 2) (Zhou et al., 2017). Another study, conducted by Zang et al. (2010), used a similar approach and revealed that Notch1-siRNA caused downregulation of Notch1 and improved chemosensitivity to doxorubicin and docetaxel. Notch1 mAbs were also used in triple-negative breast cancer xenografts to investigate their effect when combined with docetaxel. Notch1 blockade improved the cytotoxic effects of docetaxel through targeting cancer stem cell population (Qiu et al., 2013).

Notch1 inhibition by either siRNA or a γ -secretase inhibitor (LY-411,575 and MRK-003) improved sensitivity to trastuzumab in trastuzumab-resistant cells (Osipo et al., 2008; Pandya et al., 2011). When ATP binding cassette subfamily c member 1-expressing multidrug resistance MCF-7/VP (etoposide-resistant subline) cells were compared with MCF-7/wild-type cells, higher levels of intracellular Notch1, the cleaved form of Notch1, were detected. Furthermore, the use of either

a γ -secretase inhibitor (DAPT) or shRNA resulted in a reduction in intracellular Notch1, which was followed by a decrease in ATP binding cassette subfamily c member 1 (Cho et al., 2011). In MDA-MB-231 and MCF-7 human breast cancer cell lines, treatment of cells with a combination of Notch1 siRNA and numerous doses of doxorubicin/docetaxel led to increased chemosensitivity (Zang et al., 2010).

Chemoresistance of breast cancer-related bone metastasis was also examined. The effect of a monoclonal antibody against Jagged1 (ligand for Notch1 receptor), clone 15D11, was studied in bone metastasis. Mice treated with paclitaxel alone developed resistance to chemotherapy, whereas those that received paclitaxel and Jagged1 antibodies exhibited a significant reduction of bone metastasis and no development of resistance (Zheng et al., 2017).

Chronic Lymphocytic Leukemia. In cells isolated from patients with CLL, the presence of Notch1 activating mutations prompted resistance to fludarabine, and this effect was reversed using Notch1 blockade induced by a γ -secretase inhibitor (GSI-IX) (Arruga et al., 2014).

Lung Cancer. In p53 competent lung cancer cell lines, induction of Notch1 prevented cell death after exposure to Adriamycin. It was proposed that this was mediated through inhibition of the functions of p53 (Mungamuri et al., 2006). Notch1 downregulation using siRNA in small-cell lung carcinoma cell lines H69AR and SBC-3 resulted in increased chemosensitivity that was mediated through a reduction in cell adhesion-mediated drug resistance (Hassan et al., 2016).

In NSCLC, the combination of γ -secretase inhibitor (BMS-906024) with paclitaxel and cisplatin increased sensitivity to these chemotherapies and resulted in improved antitumor effect in both cell lines and NSCLC patient-derived xenografts (PDXs). Interestingly, samples without Kirsten rat sarcoma (KRAS) and BRAF (v-raf murine sarcoma viral oncogene homologue B1) mutations exhibited a higher synergistic effect with paclitaxel compared with samples with KRAS or BRAF (v-raf murine sarcoma viral oncogene homologue B1) activating mutations. However, samples with high Notch1/Notch3 expression were not correlated with increased synergy. This suggests that the effect of γ -secretase inhibitors in a certain subgroup of NSCLC samples is not through Notch inhibition (Morgan et al., 2017).

Radiotherapy. Notch1 is also involved in radiotherapy resistance. The response of cancer-initiating cells [CD24(–/low)/CD44+] and activation of Notch1 signaling pathway to radiation was explored. Cells treated with ionizing radiation had enhanced activation of Notch1 signaling pathway (Phillips et al., 2006). In glioblastoma cell lines (U87MG and U251), downregulation of Notch1 using CRISPR/Cas9 improved sensitivity to radiation through enhancing apoptosis and significantly reduced tumor growth in mouse xenografts ($P < 0.05$) (Han et al., 2017).

The treatment schedule, i.e., whether Notch1 inhibition is administrated before or after chemotherapy/radiation, is an important factor in determining the effect of this addition. The schedule of administration of Notch1 inhibitors is important to achieve an equilibrium between pro- and antiangiogenic effects. This equilibrium will partially normalize the vasculature before treatment with chemotherapy/radiation and enhance tumor oxygenation and blood supply, which will result in improved antitumor effects. The timing of Notch1 treatment is also crucial to counteract the increased levels of

Notch1 after chemotherapy/radiation treatment that is correlated with resistance (Yahyanejad et al., 2016).

Targeting Notch1 in Cancer

Anticancer effects using Notch1 inhibition can be achieved by numerous strategies (Fig. 2). These include Notch1 monoclonal antibodies, Notch1 decoy, Notch1 siRNA, Notch1 microRNA (miRNA), natural products, anti-DLL4 antibodies, α -secretase inhibitors, γ -secretase inhibitors, sarco/endoplasmic reticulum calcium ATPase (SERCA) inhibitors (SERCAs are pumps that are important for Notch1 trafficking), protein *O*-fucosyltransferase-1 (POFUT1) knockdown (transfers fucose to epidermal growth factor-like repeats in Notch receptor), and pan-Notch inhibition mediated through protein-protein interaction (PPI) inhibitors that target the Notch transcription complex in the nucleus (Sorrentino et al., 2019). These Notch1 inhibition approaches can be further developed and may provide promising therapeutic agents (Table 1).

The most studied mode of deactivation of Notch signaling pathway is inhibition that is mediated through the γ -secretase enzyme. The GSIs are functionally different, with variable cleavage effects on the Notch precursors. Hence, they do not possess equivalent biologic effects (Ran et al., 2017).

Several clinical trials are being conducted to explore the potential impact of secretase inhibitors in cancer therapy as single agents (Deangelo et al., 2006; Krop et al., 2012; Strosberg et al., 2012; Tolcher et al., 2012; De Jesus-Acosta et al., 2014; Hughes et al., 2015; Papayannidis et al., 2015; Pant et al., 2016) or in combination with other drugs. An oral GSI combined with docetaxel was assessed for safety and efficacy in a phase 1b,

open-label, multicenter study in patients with advanced triple-negative breast cancer. Results have shown that the pharmacokinetics of docetaxel was not changed by coadministration of GSI (PF-03084014) and demonstrated an acceptable safety profile (Locatelli et al., 2017). In another study, the impact of docetaxel combination of increasing doses of docetaxel with γ -secretase inhibitor (MK-0752) was studied in both a xenograft model and clinically in advanced breast cancer. Results showed that the combination was effective in reducing cancer stem cells, with an acceptable safety profile in patients (Schott et al., 2013). The pharmacodynamic and pharmacokinetic properties of the γ -secretase inhibitor MK-0752 was also explored in refractory or recurrent central nervous system malignancies in children. The study estimated the maximum tolerated dose, investigated the dose-limiting toxicities, and revealed that this γ -secretase inhibitor was tolerated in children (Fouladi et al., 2011). PF-03084014, a γ -secretase inhibitor, was tested in a phase 1 trial in various types of cancer, such as desmoid cancers and advanced solid malignancies, to determine safety, tolerability, and the dose needed for phase 2 clinical studies. Results showed that the γ -secretase inhibitor had an acceptable safety profile (Hughes et al., 2015; Messersmith et al., 2015). LY900009, another γ -secretase inhibitor, was also studied in a phase 1 trial to determine safety and dose needed in advanced cancers to reduce tumor progression (Pant et al., 2016).

In advanced solid cancers, γ -secretase inhibitors were also combined with other anticancer medications in phase 1 trials to investigate toxicities and tolerability in patients. RO4929097, an oral γ -secretase inhibitor, was combined with temsirolimus (Diaz-Padilla et al., 2013) and with gemcitabine (Richter et al., 2014).

Fig. 2. Notch1 inhibition modalities. Figure was created using BioRender.com. C0-A, coactivator; ER, endoplasmic reticulum; MAML, coactivator of the Mastermind-like family; RBPJ, DNA binding factor—recombination signal binding protein for immunoglobulin κ J region.

TABLE 1

Different modalities of Notch1 inhibition with antitumor effects in different types of cancer

Study	Type of Cancer	Notch1 Inhibition	Tissue/Cell Line	Outcome Measured	Results
Qin et al., 2004	Melanoma	γ -Secretase tripeptide inhibitor: z-Leu-Leu-Nle-CHO	Melanoma cell lines Melanoma xenografts	Investigate the effect on apoptosis	Enhanced apoptosis Upregulation of BH3-only members (Bim and NOXA)
Curry et al., 2005	KS	γ -Secretase inhibitors: tripeptide aldehyde inhibitor and a peptidomimetic inhibitor (LY-411575)	KS cell line, SLK Nude mice injected with SLK cells intradermally	Investigate the effect on apoptosis	Enhanced apoptosis mediated through growth inhibition or tumor regression
Paris et al., 2005	Human glioblastoma and human lung Adenocarcinoma	γ -Secretase inhibitor: LK-6	Human glioblastoma (U-87 MG) xenografts	Assess the effect on tumor volume, vascularization, angiogenesis, and proliferation	Inhibition of brain tumor growth
			Human lung adenocarcinoma (A-549) cell lines xenografts		Reduction of vascularization Inhibition of tumor angiogenesis Inhibition of proliferation
Nickoloff et al., 2005	Melanoma	γ -Secretase inhibitor: N-benzyloxycarbonyl-Leu-Leu-Nle-CHO	Melanoma cell lines	Examine the effect on apoptosis	Enhanced apoptosis Induced a proapoptotic BH3-only protein, NOXA
Noguera-Troise et al., 2006	Lung carcinoma	Dll4 fusion protein	Human umbilical vein endothelial cells Lewis lung tumor mice model	Examine the effect of Dll4 inhibition on tumor growth	Reduced tumor growth
Lewis et al., 2007	T-ALL	γ -Secretase Inhibitor: MRK-003	T-ALL cell line	Examine the effect on apoptosis and cell viability	Decreased cell viability Induction of apoptosis
Seehnet et al., 2007	Colon cancer Kaposi sarcoma	Dll4 fusion protein	HT29 (human colon cancer cell line) KS-IMM (human Kaposi sarcoma cancer cell line)	Investigate the antitumor effects of Dll4 inhibition using fusion proteins	Inhibition of tumor growth
Ridgway et al., 2006	Breast cancer	Dll4 antibodies	MDA-MB-435, HM7, Colo205, and Calu6 xenograft tumor models EL4 mouse lymphoma tumor model	Examine the antitumor effect of anti-Dll4 antibodies	Reduced rate of tumor growth
Funahashi et al., 2008	Colon adenocarcinoma Lung adenocarcinoma	Notch1 decoy	Human umbilical vein endothelial cells	Investigate the effect of the decoy on angiogenesis in vitro and in vivo	Inhibited angiogenesis in mouse skin
	Mouse mammary cells Human neuroblastoma cells		Mouse mammary carcinoma Mm5MT and NGP human neuroblastoma cells and xenografts		No effect on tumorigenicity of cells in vitro
Rasul et al., 2009	Breast cancer	γ -Secretase inhibitors:	ER-positive (MCF-7, T47D, and ZR-75-1) cell lines	Examine the effect on apoptosis	Reduced xenograft growth in mice
		DAPT	ER-negative (MDA-MB-231 and CAL-51) cell lines		Enhanced apoptosis independent of ER status
		Compound E ((2S)-2-[(3,5-difluorophenyl)acetyl]amin-N-[(3S)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl]propanamide) Inhibitor 1 (GSI1: z-Leu-Leu-Nle-CHO)			Downregulation of Bcl-2, Bax, and Bcl-XL Proteasome inhibition
Luistro et al., 2009	Pulmonary adenocarcinoma Breast cancer Colorectal adenocarcinoma Pancreatic carcinoma	γ -Secretase inhibitor: RO4929097	Human cancer cell lines and the xenograft models of A549, MDA-MB-468, LOVO, BxPC3, HCT-116, AsPC-1, MiaPaCa-2, and Calu-6	Examine the efficacy and pharmacodynamic properties	Induction of cell cycle arrest at G2/M Downregulation of angiogenic genes Tumor cell differentiation Antitumor activity Active following oral dosing

(continued)

TABLE 1—Continued

Study	Type of Cancer	Notch1 Inhibition	Tissue/Cell Line	Outcome Measured	Results
Moellering et al., 2009	Metastatic site of pancreatic cancer	Notch1 antibody	T-ALL cell lines: CUTLL1, SUPT1, HPB-ALL, TALL-1, DND-41, and KOPT-K1 Mouse model of T-ALL with dual NOTCH1 mutations identified in human T-ALL cells	Explore the effect on cell proliferation	No body weight loss or Notch-related toxicities
	Pulmonary anaplastic adenocarcinoma				Activation of caspase 3 and 7
	T-ALL				Induced apoptosis
					Significant decrease in disease burden and leukemic infiltration in secondary recipient mice that received treatment
Real et al., 2009	T-ALL	γ -Secretase inhibitors plus glucocorticoids γ -Secretase inhibitors: Compound E ((2 <i>S</i>)-2-(((3,5-difluorophenyl)-acetyl-amino)- <i>N</i> -[(3 <i>S</i>)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1 <i>H</i> -1,4-benzodiazepin-3-yl]propenamide and DBZ ((2 <i>S</i>)-2-[2-(3,5-difluorophenyl)-acetyl-amino]- <i>N</i> -(5-methyl-6-oxo-6,7-dihydro-5 <i>H</i> -dibenzo[<i>b</i> , <i>d</i>]azepin-7-yl)-propionamide	Patient-derived xenografts	Investigate the effect of using a combination of GSIs and glucocorticoids on the intestinal toxicity and antitumor efficacy	Enhanced antileukemic effects of GSIs Reduction of intestinal toxicity Improved sensitivity in glucocorticoid-resistant T-ALL
Wu et al., 2010	T-ALL	Notch1 antibody	T-ALL cell line and xenograft models of HPB-ALL Xenograft models of Calu-6 and HM7 cell lines Human colon cancer line, MT-3	Assess the function and intestinal toxicity of Notch1- and Notch2-specific inhibitors	Dysregulation of tumor angiogenesis mediated through a decrease in tumor volume and growth inhibition
	Human colon cancer				
	Anaplastic carcinoma				
Aste-Amézaga et al., 2010	Colon adenocarcinoma	Notch1 EGF-repeat region antibody	Cancer cell lines: LS-1034, BxPC3, Colo205, and TALL-1, T-REX-293 and Flp-In-3T3.	Investigate the inhibitory effect of Notch1 antibodies (wild type)	No significant antiproliferative effect on cell lines NRR antibodies inhibit activation of Notch1 receptors with T-ALL mutations
	Human colon carcinoma				
	Human pancreatic cancer	Notch1 NRR antibody			
Fan et al., 2010	Human colon adenocarcinoma	γ -Secretase inhibitor: [11-endo]- <i>N</i> -(5,6,7,8,9,10-hexahydro-6,9-methanobenzo[<i>a</i>] [8] annulen-11-yl)-thiophene-2-sulfonamide	GBM-derived neurosphere cultures	Estimate the frequency of glioblastoma cancer stem cells by measuring its markers	Depletion of stem-like cells
	T-cell acute lymphoblastic leukemia				
	GBM				
Pandya et al., 2011	Erb-B2–positive breast tumor	γ -Secretase inhibitors: LY 411575 and MRK-003	Orthotopic breast tumor xenografts from trastuzumab- or lapatinib-sensitive and trastuzumab-resistant BT474 cells	Assess the antitumor activity of GSIs	Reduction in tumor recurrence after trastuzumab treatment in sensitive tumors Partial reversal of trastuzumab resistance
Mizugaki et al., 2012	NSCLC	γ -Secretase inhibitors: γ -secretase inhibitor I (in vitro) and γ -secretase inhibitor XX (in vitro, in vivo)	NSCLC cell lines: H460, A549, and H1395 H460 and A549 xenografts in female nude mice	Evaluate the antitumor effect of γ -secretase inhibitors at 24 h after radiation in Notch-expressing lung cancer cell lines	Improved the cytotoxicity of radiation in lung cancer after the administration of GSIs both in vitro and in vivo that is mediated through the activation of apoptosis

(continued)

TABLE 1—Continued

Study	Type of Cancer	Notch1 Inhibition	Tissue/Cell Line	Outcome Measured	Results
Hayashi et al., 2012	Pulmonary adenocarcinoma	Anti- γ -secretase monoclonal antibody (A5226A)	A549 and DND-41 cell lines	Investigate the antitumor effect of the γ -secretase antibody	Decreased proliferation in vivo
Kondratyev et al., 2012	T-acute lymphoblastic leukemia	γ -Secretase inhibitor: MRK-003	DND-41 xenografts in immunodeficient mice	Investigate the effect of γ -secretase inhibitors on cancer stem cells	Eradication of cancer stem cells in vivo and in vitro
	Erb-B2-positive breast tumor		Tumor spheres established from mammary tumors from Neu (N202) transgenic strain		Enhanced apoptosis and differentiation
Sharma et al., 2012	Breast cancer	Notch1 monoclonal antibody	MDA-MB-231 cell line	Study the potential effects of Notch1 antibody	Reduction in CD44(Hi)/CD24(Low) subpopulation Reduced cancer stem cell population Decreased cell proliferation and apoptosis
Floyd et al., 2012	Glioblastoma multiforme	α -Secretase inhibitor compound INCB3619 (methyl(6S,7S)-7-[(hydroxyamino)carbonyl]-6-[(4-phenyl-3,6-dihydropyridin-1(2H)-yl)carbonyl]-5-azaspiro[2.5]octane-5-carboxylate)	Adherent GBM cell lines U87MG, U251MG, T98G, U373MG, and A172	Examine the effect of α -secretase inhibitor in adherent GBM and GBM stem cell lines Study the effect of nanoparticles loaded with these inhibitors	Decreased growth of adherent GBM and GBM stem cell lines Nanoparticles loaded with α secretase inhibitor compared with those loaded with vehicle or γ -secretase inhibitor prolonged survival in mouse model This effect is mediated via a decrease in YKL-40 and LIF receptor
		γ -secretase inhibitor: DAPT	GBM stem cell lines 0308 and 0822		
Bleeker et al., 2013	Acute myeloid leukemia	Ethyl 2-amino-6-(3,5-dimethoxyphenyl)-4-(2-ethoxy-2-oxoethyl)-4H-chromene-3-carboxylate (CXL017)—an inhibitor of SERCA in combination with thapsigargin, cyclopiazonic acid, and 2,5-di-tert-butylhydroquinone.	Human GBM stem cell mouse xenograft (HL60) and multidrug-resistant (HL60/MX2) cell lines	Assess the possible additive effects of the compound with other SERCA inhibitors	Synergistic effect with every SERCA inhibitor Enhanced cytotoxicity for the resistant cell line
Agnusdei et al., 2014	T-ALL	Notch1 monoclonal antibody	Patient-derived xenografts in NOD/SCID mice	Investigate antitumor effects of notch1 antibodies	Enhanced T-ALL cell apoptosis Reduced proliferation Inhibition of Notch target genes expression Ameliorated leukemia-initiating cell functions
Dai et al., 2014	CRC	Jagged1-shRNA	Xenograft mouse model colorectal cancer cell lines, HCT15, HT29, DLD1, and HCT116 cells	Examine the effects of targeting Jagged1 (a ligand to all four notch1 receptors)	Decreased cell viability in vitro G0/G1 phase cell cycle arrest Decreased migration and invasiveness in vitro Decreased cyclin D1, cyclin E, and c-Myc expression. Reduced cell proliferation, growth rate, and metastasis in vivo
Koyama et al., 2014	T-ALL	Bortezomib (proteasome inhibitor) Combination of bortezomib and dexamethasone, doxorubicin, vincristine, doxorubicin, mithramycin, dexamethasone, cytosine	Jurkat, CEM, MOLT4 and KOPT-K1, KMS12-BM, U266, RPMI8226 (MM), KOPM30 (B-ALL), HBL-2 (mantle cell lymphoma),	Investigate the mechanism of anticancer effects of bortezomib and its influence on Notch1 signal	Reduced transcription of Notch1 and decreased levels of NICD Reduction in downstream Notch1 targets: Hes1, GATA3, RUNX3, and NF- κ B

(continued)

TABLE 1—Continued

Study	Type of Cancer	Notch1 Inhibition	Tissue/Cell Line	Outcome Measured	Results
		arabinoside, and 4-hydroxycyclophosphamide	NAMALWA (Burkitt lymphoma), HL-60 and K562 (acute myeloid leukemia) Murine xenografts		Increased levels of Notch1 ICD led to resistance to bortezomib, dexamethasone, and cytosine arabinoside Combination of doxorubicin or dexamethasone and bortezomib improved the downregulation of bortezomib on Notch1 Treatment with either dexamethasone or bortezomib reduced tumor growth Reduced proliferation
Sharma et al., 2015	Breast cancer	mAb for the NRR of Notch1	HEK293, MCF-7, BT-474, MDA-MB-231, HCC-1806, HCT-116, Jurkat and CCRF-CEM cell lines	Investigate the antitumor effect of Notch1 antibodies	Reduction in CD34/CD44 high population
	Squamous carcinoma		MDA-MB-231, HCC-1806, BT-474, and HCT-116 xenografts in female nude mice		Enhanced sensitivity to doxorubicin
	Human colon cancer				
So et al., 2015	ALL				
So et al., 2015	Breast cancer	1,25-Dihydroxy-20R-21(3-hydroxy-3-deuteromethyl-4,4,4-trideuterobutyl)-23-yne-26,27-hexafluoro-cholecalciferol (BXL0124), Gemini vitamin D analog	MCF10DCIS cell line	Explore the effect of Gemini vitamin D on Notch1 inhibition and cancer stem cell population	Reduction in Notch1 receptors and ligands Reduction in the CD44(+)/CD24(–/low) cancer stem cell population
Yu et al., 2015	ATL	Combination of γ -secretase inhibitor, bortezomib, and romidepsin	Murine model of human ATL: MT-1 model	Explore the antitumor effects of a combination of γ -secretase inhibitor, bortezomib, and romidepsin	Improved antitumor effect in terms of tumor size and tumor markers in the serum Enhanced survival of the MT-1 tumor-bearing mice
		γ -Secretase inhibitors: RO4929097 and Compound E			
Tanaka et al., 2015	GICs	γ -Secretase inhibitor: MRK003	Patient-derived GICs	Examine the effect of Notch1 inhibition on cancer stem cells	Reduced viability and sphere formation ability Enhanced apoptosis
Ambrogio et al., 2016	(KRAS)-mutant lung adenocarcinoma	γ -Secretase inhibitor: LY-411575	Kras mutated mice Data set of lung adenocarcinomas	Explore the antitumor effect of combined inhibition of DDR1 and Notch signaling	Induced apoptosis Higher apoptotic response than cisplatin/paclitaxel, irrespective of Trp53 status
			KRAS-mutant lung adenocarcinoma cell lines expressing variable DDR1 level PDX model using human KRAS-mutant adenocarcinomas (PDX1–3)		
Shang et al., 2015	DTs	γ -Secretase inhibitor: PF-03084014	DT cell strains	Examine the antitumor effects of PF-03084014	Reduced cell invasion Induced growth inhibition Induced cell cycle arrest
Shan et al., 2015	T-ALL triple-negative breast cancer	γ -Secretase inhibitor: BMS-871	MDA-MB-157 triple-negative breast cancer xenograft model	Examine the anticancer effects of BMS-871	Inhibition of tumor growth
Ono et al., 2016	AML	L-fucose-bound liposomes loaded with daunorubicin	TALL-1 tumor model Patient-derived acute leukemia cells Cell lines: HL60, RPMI8226, KG1, and MOLT4 cells	Consequences of targeting Notch1-positive AML cells by fucose-bound liposomes	Inhibition of tumor growth in mouse models Reduced proliferation of AML patient-derived leukemia cells
Jiang et al., 2016	GC	miR-124	GES-1 cell line and GC cell lines: SGC-7901, BGC-823, MGC-803, KATO-3, and AGS Gastric cancer	Investigate the connection between miRNAs and the Notch signaling pathway in gastric cancer	Inhibition of cell growth, migration and invasion Arrested cell cycle

(continued)

TABLE 1—Continued

Study	Type of Cancer	Notch1 Inhibition	Tissue/Cell Line	Outcome Measured	Results
Tanaka et al., 2015	GBM	γ -Secretase inhibitor: MRK003	Patient-derived GICs	Examine the effectiveness of MRK003 on GICs	Regulated Notch1 ligand (JAG1) Reduced viability of GICs Reduced sphere formation
Bettinsoli et al., 2017	Neuroblastoma	miRNA-34 siRNAs targeting the DLL1 gene	Neuroblastoma cell lines (DSMZ), SH-SY5Y, IMR-32, KELLY	Assess the antitumor effect of DLL1 inhibition	Induced apoptosis Halted cell proliferation Initiated neuronal differentiation
Nasrin et al., 2018	Oral squamous cell carcinoma	γ -Secretase inhibitor: DAPT with Triton X-100 (0.001%)	Human OSCC cell line H413	Study the effect of using a cell permeabilization agent (Triton X-100) to deliver GSIs in cancer	Decrease in Notch1 gene expression Inhibition of cell growth
McMillan et al., 2017	T-ALL	CRISPR-mediated knockout of POFUT1 (an enzyme that transfers fucose to epidermal growth factor-like repeats in Notch receptors)	U2OS and 293T cells	Investigate the effect of POFUT1 knockout on Notch1 signaling	Suppression of normal and certain mutated Notch1 signaling
Han et al., 2017	GBM	Notch1 downregulation via CRISPR/Cas9	Glioblastoma cell lines: U87MG and U251 Mouse xenografts	Examine the effect of Notch1 downregulation on sensitivity to radiation, the ability of glioblastoma cell lines to clone itself and grow, and the growth of xenografts	Reduced radioresistance Enhanced apoptosis Reduced xenograft growth
Liao et al., 2018	Colorectal cancer	Notch1-siRNA γ -Secretase inhibitor: DAPT	Colorectal cancer cell lines (COLO205, HT29, SW480, SW1116, and LoVo) Colorectal carcinoma, colorectal adenoma, and paracancerous tissues and normal colorectal tissues	Examine the role of Notch1 in colorectal cancer	Reduced growth and proliferation of colorectal cancer cells Induced cell apoptosis
Akbarzadeh et al., 2018	Ovarian cancer	γ -Secretase inhibitor: DAPT (<i>N</i> -[<i>N</i> -(3,5-difluorophenacetyl)-l-alanyl]- <i>S</i> -phenylglycine <i>t</i> -butyl ester)	OVCAR-3 cells	Investigate the effect on proliferation, metastasis, and activity of metalloproteinases 2 and 9	Decreased proliferation and metastasis Reduced activity of metalloproteinases 2 and 9
Baldoni et al., 2018	CLL	Bepridil (calcium channel blocker)	Culture of primary cells from patients with CLL	Investigate the antitumor effects of bepridil	Reduction of leukemic cells infiltration Reduced apoptosis Reduced activation of Notch1
Di Bari et al., 2018	GBM	mir-34a-5p	U87MG and U251MG cell lines	Examine the relationship between M2 receptors and Notch1 and EGFR pathways	M2 activation negatively controlled Notch1 through increased levels of mir-34a-5p in wild-type cells M2 activation led to inhibition of proliferation and induction of apoptosis
Habets et al., 2019	T-ALL	Selective inhibitor of PSEN1 (catalytic center of secretase complexes): MRK-560	Psen1 (catalytic center of secretase complexes) knockout mouse Patient-derived NOTCH1-driven leukemia mouse models HPB-ALL, DND-41, and Jurkat cell lines	Explore the antitumor effects	Decreased the involvement of mutant NOTCH1-driven leukemia in mice in vivo Cell cycle arrest in T-ALL cell lines
He and Zou 2019	Osteosarcoma	Notch1 siRNA γ -Secretase inhibitor: DAPT	Bone marrow mesenchymal stem cell (BMSC) cell line	Investigate the influence of Notch1 inhibition on the differentiation and tumorigenesis of BMSC	Reduced proliferation Enhanced osteogenic differentiation of BMSCs
Shiraishi et al., 2019	Breast cancer neuroblastoma	NSI-1 that targets the intracellular domain (NICD)	Human breast adenocarcinoma cell lines (MCF-7 and MDA-MB-231)	Investigate the effect of an inhibitor that targets NICD of Notch	Reduced expression of HES1

(continued)

TABLE 1—Continued

Study	Type of Cancer	Notch1 Inhibition	Tissue/Cell Line	Outcome Measured	Results
			Human neuroblastoma cell line (SH-SY5Y)		Inhibited the translocation of NICD into the nucleus Reduced cell viability of MCF-7 and MDA-231 cells
Silkenstedt et al., 2019	MCL	Notch1 monoclonal antibody: OMP-52M51	MCL mouse model	Explore the effects of notch1 antibodies	Reduction in Notch target genes in Notch1-mutated cells
			NOTCH1-mutated Mino cells		Suppression of lymphomagenesis and disease progression in Notch1-mutated cells
			JeKo-1 cell line		

AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; ATL, adult T-cell leukemia; DT, desmoid tumor; GC, gastric cancer; GIC, glioblastoma initiating cell; KS, Kaposi sarcoma; MCL, mantle cell lymphoma; NRR, negative regulatory region; NSI-1, Notch signaling inhibitor-1; OSCC, oral squamous cell carcinoma; Bax, an effector that is essential in apoptosis and is activated by the BH3-only proteins; Bim, a member of BH3 (Bcl-2 homology domain 3)-only proteins that promote cell death; DDR1, discoidin domain receptor 1; GATA3, a gene that encodes a protein that belongs to the GATA family transcription factors; LIF, leukemia inhibitory factor; NOXA, a member of BH3-only proteins that promote cell death; PSEN1, Presenilin 1 gene; RUNX3, Runt-related transcription factor 3; Trp53, transformation related protein 53 gene; YKL-40, Chitinase 3-like 1 glycoprotein.

Despite the promising role that γ -secretase inhibitors may hold, their use in the clinical management of cancer faces many challenges. A major obstacle to their clinical use seems to be their gastrointestinal adverse effects (Imbimbo, 2008). These adverse effects are a consequence of simultaneous inhibition of both notch1 and Notch2, and blockade of either one individually ameliorates the gastrointestinal toxicity (Wu et al., 2010). This suggests that alternative approaches would be more appropriate, such as targeting Notch receptors independently, using combination therapies that use lower doses, or targeting only cells with mutant Notch while sparing the wild-type cells. Other strategies that manipulate dosing regimens were also investigated to reduce or avoid gastrointestinal toxicity of γ -secretase inhibitors. One strategy was to use a combination of Notch1 inhibition with glucocorticoids; this regimen reduced intestinal toxicity and improved the response of the glucocorticoid-resistant T-ALL (Real et al., 2009). The study tested two γ -secretase inhibitors: compound E ([[(2S)-2-[[[(3,5-difluorophenyl)-acetyl-amino]-N-[(3S)-1-methyl-2-oxo-5-phenyl-2,3-dihydro-1H-1,4-benzodiazepin-3-yl] propanamide) and DBZ ((2S)-2-[2-(3,5-difluorophenyl)-acetyl-amino]-N-(5-methyl-6-oxo-6,7-dihydro-5H-dibenzo[b,d]azepin-7-yl)-propionamide. Another approach was to employ intermittent dosing schedules; dosage regimens using the γ -secretase inhibitor RO4929097 with frequent drug holidays were shown to be well tolerated and effective (Tolcher et al., 2012).

Another important aspect of utilizing the γ -secretase inhibitors or any other pharmacologic Notch1 inhibition is the issue of resistance to Notch1 inhibition. The detection of Notch1 resistance dictates the need to understand the mechanisms and signaling pathways that are involved. Furthermore, it is crucial to tease out the subgroup of patients that possess this resistance and develop methods and approaches to overcome it and improve response to Notch1 inhibition. In T-cell acute lymphoblastic leukemia mouse models, resistance to Notch1 inhibition was induced by loss-of-function mutations of PTEN (Mendes et al., 2016). Similar results were discovered in *D. melanogaster*, in which loss of PTEN function was associated with resistance to Notch1 inhibition (Palomero et al., 2007) produced by the γ -secretase inhibitor, Compound E (Seiffert

et al., 2000). In T-ALL, PTEN downregulation was not a result of PTEN gene mutations and subsequent decrease in protein level but, rather, a decrease in PTEN lipid phosphatase activity. This diminished post-translational activity is caused by hyperactivity of casein kinase 2 (CK2) and high levels of reactive oxygen species (Silva et al., 2008). Based on the previous findings, targeting both the CK2 and Notch inhibition through a combination of CK2 inhibitors and γ -secretase inhibitors in vitro resulted in improved antitumor effect and reduced proliferation (Silva et al., 2010).

Resistance to γ -secretase inhibitors can also be mediated through an epigenetic mechanism in which the withdrawal of the γ -secretase inhibitor (Compound E) can lead to the reversal of resistance (Knoechel et al., 2014).

Another challenge that faces the clinical use of γ -secretase inhibitors is that the secretase enzyme catalyzes many substrates, such as CD44, Notch receptors (all four receptors), many Notch ligands, epidermal growth factor receptor B2 (Erb-B2) receptor tyrosine kinase 4, E-cadherin, and many others (Nickoloff et al., 2003; Haapasalo and Kovacs, 2011). An inhibitor of γ -secretase may affect the previously mentioned substrates and might also inhibit other proteases involved in cell function (Shih and Wang, 2007).

Another interesting approach to inhibit Notch1 in pancreatic cancer is through targeting aspartate β -hydroxylase, which catalyzes the hydroxylation of EGF-like repeats in Notch receptors and ligands. Treatment of pancreatic cancer cell lines with MO-I-1100, an inhibitor of B-hydroxylase, led to reduced cell growth that is mediated through downregulation of Notch signaling pathways (including Notch1) (Dong et al., 2015).

Pan-Notch inhibition can also be achieved through small molecules that inhibit PPI and impair the Notch transcription complex in the nucleus. Recently, a phase 1-2A study was launched to examine the pharmacokinetics and optimum dose and dosing schedule of CB-103 (PPI inhibitor) in patients with advanced solid tumors and blood malignancies (Perez Garcia et al., 2018).

Dll4 blockade is another approach by which Notch1 inhibition can be achieved; Dll4 is one of the five Notch membrane-bound ligands [Jagged1, Jagged2, Dll1, Dll3, and

Dll4]. Dll4 protein binds to Notch1 receptor and initiates the Notch1 signaling reactions. Blockade of DLL4/Notch1 signaling pathway allows for a more specific inhibition that targets angiogenesis rather than a more general approach, such as using γ -secretase inhibitors.

Dll4 protein is specific to the endothelium, especially arterial vessels, and plays a vital role in the development of blood vessels during the embryonic stage and growth of new blood vessels (angiogenesis) throughout life. Additionally, it is highly expressed throughout tumor angiogenesis and induced by hypoxia (Mailhos et al., 2001).

Selective blockade of Dll4 exhibited antitumor effects; this inhibition was achieved by selective Dll4 antibodies. The treatment of MDA-MB-435, HM7, Colo205, and Calu6, well established xenograft tumor models, caused a reduction in the tumor growth rate compared with the control (Ridgway et al., 2006). Another method for Dll4 blockade was to use a soluble Dll4 fusion protein that binds to receptors but does not produce a signal. The inhibition of Dll4/Notch resulted in a disturbance of the balance that is needed to produce healthy productive vessels, which in turn led to nonefficient angiogenesis, inhibiting tumor growth (Noguera-Troise et al., 2006). Similar results were obtained using the same approach in colon cancer and Kaposi sarcoma xenografts (Scehnet et al., 2007).

Clinical trials were initiated to investigate the therapeutic advantages of anti-DLL4 antibodies as a single agent (Chiorean et al., 2015; Casulo et al., 2016) (NCT01952249) and (NCT01577745) or in combination with gemcitabine with or without (+/–) Abraxane (NCT01189929) (which was discontinued), in combination with FOLFIRI (leucovorin calcium (calcium folinate), 5-fluorouracil, and irinotecan combination) (NCT01189942), and in combination with carboplatin and pemetrexed (NCT01189968).

Brontictuzumab is an anti-Notch1 monoclonal antibody that binds to the extracellular domain of the receptor. The drug was assessed in a phase 1 study to determine the safety, pharmacokinetics, and clinical benefit in patients with solid tumors. At the maximum tolerated dose (1.5 mg/kg every 3 weeks), the drug was well tolerated by the patients and was accompanied by the usual gastrointestinal toxicity that is seen in Notch inhibition, which was mainly manifested as diarrhea (Ferrarotto et al., 2018).

α -Secretases are a family of metalloproteinases [also called a disintegrin and metalloproteinase (ADAM)] that cleave the Notch extracellular domain. This cleavage facilitates the release of intracellular domain by the γ -secretase enzyme, especially ADAM10 for Notch1 signaling (Bozkulak and Weinmaster, 2009). Although inhibitors of this family of enzymes are not as widely studied as the γ -secretase inhibitors, in glioblastoma (GBM), α -secretase inhibitors decreased the growth of adherent GBM and GBM stem cell lines and prolonged survival in mouse models (Floyd et al., 2012).

Natural products were always a valuable source of possible anticancer therapy. Many exert an antitumor effect through Notch1 inhibition. These can be explored as single treatments or adjuvant therapy with other Notch1 inhibitors for possible additive or synergistic effect (Table 2). Thapsigargin, which is obtained from the roots of *Thapsia garganica*, has been linked to an inhibition of SARCA channels, which consequently led to aberrant Notch1 trafficking and subsequent reduction in Notch1 cell surface receptors. A phase 1 clinical trial was

launched to assess the safety of mipsagargin, a prodrug of thapsigargin, and establish the dose that is needed for phase 2 studies in patients with advanced solid tumors. Mipsagargin had acceptable pharmacokinetic properties and was well tolerated by patients (Mahalingam et al., 2016).

Long noncoding RNAs (lncRNAs) and miRNAs are important members of the noncoding RNAs. Although they do not code for proteins, they serve as important regulators of gene expression. Mutations and aberrant expression of these noncoding RNAs were strongly associated with tumorigenesis and resistance of cancer (Hayes and Lewis-Wambi, 2015; Khurana et al., 2016).

In a study that examined the role of Notch1 in cisplatin-resistant gastric cancer cell lines SGC7901/DDP and BGC823/DDP, results showed that Notch1 was overexpressed in these resistant cells. Further examination of the expression of lncRNA, AK022798, suggested that the effect of Notch1 on the evolution of these resistant cells might be mediated through this lncRNA. Moreover, the use of siRNA to down-regulate lncRNA AK022798 expression reduced the expression of drug resistance genes (Hang et al., 2015).

miRNAs consist of 19–24 nucleotides and can negatively or positively regulate different genes and signaling pathways (Chen et al., 2016). miRNAs can lead to increased or decreased levels of Notch1 depending on the type of miRNA and cancer.

miR-223 is a noncoding RNA that was overexpressed in gemcitabine-resistant pancreatic cancer cells. miR-223 exerted an important role in the generation of the EMT phenotype through the downregulation of Fbw7 (F-box/WD repeat-containing protein 7), which led to increased stability of Notch1 (Ma et al., 2015).

In breast cancer, miR-34a inhibited proliferation, migration, and cancer stem cell population through downregulation of Notch1. In addition, overexpression of miR-34a enhanced chemosensitivity toward paclitaxel via negative regulation of Notch1. In TNBC cell line MDA-MB-231, a negative correlation was established between miR-9 and Notch1, and increased expression of miR-9 abolished Notch1 intracellular domain.

In breast cancer, when human tissue specimens were examined, miR-34a was downregulated in tumor tissues in comparison with the adjacent normal tissues. In addition, overexpression of miR-34a in an MCF-7 cell line led to an inhibition of viability and invasion of cancer cells. These effects were induced through targeting Notch1 (Rui et al., 2018). In TNBC cells, miR-34a was codelivered with doxorubicin to MDA-MB-231 cells using nanoparticles. The use of the combination enhanced the antitumor effects of doxorubicin. In addition, recovery of normal levels of miR-34a in cancer cells led to reduced migration that was mediated through lowering the expression levels of Notch1 (Deng et al., 2014).

In glioblastoma, miRNAs that are specific to the Notch signaling pathway (including Notch1) may reach up to 32 miRNAs. These include miR-9, miR-34a, miR-92b, miR-124, miR-137, and miR-219-5p (Sun et al., 2012). In glioma cell lines, overexpression of miR-145 reduced Notch1 activation through decreased levels of Notch1 protein and its gene targets (Du et al., 2017). miR-34a was also involved in tumorigenesis of glioblastoma via Notch1, and overexpression of this miRNA in glioma cells reduced Notch1 protein levels and inhibited tumor growth in xenografts (Li et al., 2009). In the glioblastoma cell line that represents p53 wild type

TABLE 2

Natural products with antitumor effects mediated through Notch1 inhibition

Study	Type of cancer	Notch1 Inhibition	Tissue/Cell Line	Outcome Measured	Results
Chen et al., 2007	Raji cell line	Curcumin	Raji cell line	Explore the mechanisms by which curcumin affects Raji cells	Inhibition of proliferation Attenuation of Notch1 and NF- κ B signaling pathways Induction of apoptosis
Koduru et al., 2010	Colon cancer	WA is a bioactive compound derived from <i>Withania somnifera</i>	Colon cancer cell lines: HCT-116, SW-480, and SW-620	Examine the effect of WA on Notch1 signal inhibition	
Li et al., 2012	Osteosarcoma	Curcumin Notch1 siRNA	Osteosarcoma cell lines: U2OS, SaOS-2, and MG-63	Investigate the antitumor effect of curcumin in osteosarcoma cell lines	Suppression of Notch1 expression and its downstream genes Cell cycle arrest at the G2/M phase Prevent proliferation and invasion of osteosarcoma cells
Zhen-Jun et al., 2012	Gastric cancer	DA, a component of Radix <i>Lithospermum erythrorhizon</i>	Human gastric cancer cell line SGC-7901 Murine gastric cancer cell line MFC	Examine effects of DA on cell growth in human gastric cancer cell line	Reduced activation of Notch1 signaling pathway Inhibition of progression of gastric cancer cells
Ponnurangam et al., 2012	Colon cancer	Honokiol in combination with IR	Colon cancer cell lines and xenografts: HCT116 and SW480	Examine the effect of combination on sensitivity of cancer stem cells to IR	Inhibition of cancer growth Inhibition of notch1 signaling pathway Decreased levels of Notch1 receptors in cell lines
Roti et al., 2013	T-ALL	Thapsigargin (small-molecule SERCA inhibitor that is found in the roots of <i>T. garganica</i>).	DND41 cell line	Investigate the effect of inhibition of calcium channels responsible for Notch1 trafficking inhibitors	Aberrated Notch1 maturation in cultured cells Tumor growth suppression in T-ALL xenograft mode
Koprowski et al., 2015	CCA	Curcumin	CCA cell lines: CCLP-1 and SG-231	Explore the antitumor effects of curcumin on CCA	Decreased levels of Notch1, HES-1 Enhanced apoptosis Inhibition of growth
Wang et al., 2016	Glioma	Eupatilin, a flavonoid in <i>Artemisia asiatica</i> Nakai (Asteraceae) Notch-1 siRNA	Human glioma cell lines: LN229 and U87MG	Investigate the molecular mechanism and effects of eupatilin	Inhibition of proliferation Decreased cell invasion and migration Enhanced apoptosis Suppression of Notch-1 expression
Zhang et al., 2016	Breast cancer	PF, an ingredient of Chinese peony	Human breast cancer cell lines: MDA-MB-231 and MCF-7	Examine the antitumor effects of PF in breast cancer	Reduced proliferation and invasion of breast cancer cells Reduced expression of Notch-1
De Ford et al., 2016	T-ALL	Casearin J (tricyclic clerodane diterpene found in <i>Casearia sylvestris</i>)—inhibitor of the SERCA pump	CCRF-CEM, CEM-ADR5000, and Jurkat cell lines CD3 ⁺ cells from human blood	Investigate the antitumor effects of the SERCA inhibitor	Induced cell death Inhibition of HD mutant Notch exocytosis
Roti et al., 2018	T-ALL	Folate conjugated to an alcohol derivative of thapsigargin (inhibitor of mutant notch1 receptor trafficking)	17 T-ALL cell lines	Examine the selective antitumor effects	Enhanced apoptosis Reduced T-ALL cell viability Selective effects on mutant cells compared with wild type Decreased intracellular domain of notch1 levels in T-ALL cells

CCA, cholangiocarcinoma; DA, β,β -dimethylacrylshikonin; IR, ionizing radiation; PF, paeoniflorin; WA, Withaferin-A.

(U87MG), mir-34a-5p negatively controlled the Notch-1/EGFR axis and led to inhibition of cell proliferation. This cross talk was achieved by activation of the M2 acetylcholine muscarinic receptors (Di Bari et al., 2018). Numerous miRNAs were studied and were successfully used to inhibit Notch1 activity, which makes them a promising therapeutic approach for numerous types of cancer.

Conclusion

Signaling pathways in cancer are not equal; some are more important than others and constitute key nodes in the web of tumorigenesis. The Notch family is one of these important pathways, and Notch1 aberrant activity is detected in many cancers. In some cancers the role of Notch1 is clear, but in others, many future studies are needed to provide a more defined answer. The role of Notch1 is as heterogeneous as cancer; its role in terms of magnitude, cross talk with other signaling pathways, and whether it behaves as a tumor suppressor or an oncogene depends on the type of cancer, stage, and mutations in other relevant genes. Yet despite its multifaceted role, it remains a valuable and legitimate target that must be explored in the pursuit of new and safer therapeutic modalities.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Gharaibeh, Elmadany, Alwosaibai, Alshaer.

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