The many facets of Notch signaling in breast cancer: toward overcoming therapeutic resistance

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Breast cancer is the second leading cause of cancer-related death in women and is a complex disease with high intratumoral and intertumoral heterogeneity. Such heterogeneity is a major driving force behind failure of current therapies and development of resistance. Due to the limitations of conventional therapies and inevitable emergence of acquired drug resistance (chemo and endocrine) as well as radio resistance, it is essential to design novel therapeutic strategies to improve the prognosis for breast cancer patients. Deregulated Notch signaling within the breast tumor and its tumor microenvironment (TME) is linked to poor clinical outcomes in treatment of resistant breast cancer. Notch receptors and ligands are also important for normal mammary development, suggesting the potential for conserved signaling pathways between normal mammary gland development and breast cancer. In this review, we focus on mechanisms by which Notch receptors and ligands contribute to normal mammary gland development and breast tumor progression. We also discuss how complex interactions between cancer cells and the TME may reduce treatment efficacy and ultimately lead to acquired drug or radio resistance. Potential combinatorial approaches aimed at disrupting Notch- and TME-mediated resistance that may aid in achieving in an improved patient prognosis are also highlighted.

Breast cancer is the most prevalent cancer among women worldwide (ShahidSales et al. 2018; Ghasemi et al. 2019). Breast cancer is a highly heterogeneous disease with many subtypes, and treatment choice is based on the presence or absence of different hormone receptors, such as estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), as well as tumor grade and age of the patient (Desmedt et al. 2008). Unfortunately, traditional treatment methods, including chemotherapy, endocrine therapy, and radiation therapy are often not curative, and only im-

prove clinical outcome (Schmidberger et al. 2003; Yang et al. 2020) in a subset of patients. In addition, while some patients respond to therapies initially, de novo or acquired therapeutic resistance further compromises clinical response, resulting in worsened patient outcome. While therapeutic resistance is often associated with tumor cells themselves, signaling ligands and soluble factors within the tumor microenvironment (TME) may contribute to this process through aberrant activation of various signaling pathways in tumor cells, which may assist them in escaping the immune response (Quail and Joyce 2013; Zahreddine and Borden 2013).

The highly conserved Notch signaling pathway is one of the key regulators including cell fate and cell differentiation decisions in the developing mammary gland (Dontu et al. 2004; Bouras et al. 2008; Chakrabarti et al. 2018). Recent studies established that Notch signaling is frequently deregulated in the progression of different breast cancer subtypes as well as in acquisition of therapeutic resistances (Shi and Harris 2006; Guo et al. 2011; Takebe et al. 2015; Brzozowa-Zasada et al. 2017; Lamy et al. 2017; Krishna et al. 2019). The Notch signaling pathway is mediated through one of the four Notch receptors and one of five Notch ligands. Due to its ubiquitous nature, global targeting of Notch signaling through all receptors is likely to have adverse effects. However, preclinical research reveals that therapeutic targeting of selective Notch receptors/ligands and components of the TME enhance the effectiveness of modern clinical therapies for breast cancer (Mollen et al. 2018). As such, further study is required to determine the safety and efficacy of therapeutic strategies in the treatment of breast cancer.

In this review, we briefly highlight context- and subtype-dependent intriguing pleiotropic functions of Notch signaling as an oncogene or suppressor. We then describe the changes in the expression of different Notch receptors and ligands in the context of normal mammary gland

development followed by breast carcinoma development, and then discuss their involvement in the interplay between tumor cells, cancer stem cells (CSCs), and immune cells (T-cells, tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs), and cancer-associated fibroblasts (CAFs) present in the TME of breast cancer. We also summarize recent research describing the involvement of Notch signaling in acquisition of breast cancer chemoresistance, endocrine resistance, and radiation resistance, thereby supporting Notch signaling as a promising therapeutic target. Furthermore, we address current knowledge on combinatorial therapeutic targeting of both Notch and the TME to enhance current treatment efficacy for treatment-resistant breast cancer patients.

Notch signaling overview

Notch signaling is an evolutionarily conserved cell to cell communication pathway that reiteratively regulates a diverse array of cellular processes including proliferation, cell fate decisions, embryonic development, and renewal and maintenance of adult tissue (Artavanis-Tsakonas et al. 1999; Lai 2004; Bray 2006; Fortini 2009). The basic molecular players in this pathway are four receptors (NOTCH1–4) and five ligands (Delta-like ligand 1 [DLL1], Delta-like ligand 3 [DLL3], Delta-like ligand 4 [DLL4], Jagged 1 [JAG1], and Jagged 2 [JAG2]) in mammals (Nickoloff et al. 2003; Hori et al. 2013; Bray 2016). Under normal physiological conditions, binding of a Notch ligand to its receptor initiates Notch signaling by releasing the intracellular domain of the Notch receptor (NICD) following cleavage by a member of the disintegrin and metalloproteinase family (ADAM17 or ADAM10) of proteases and a presenilin-dependent γ-secretase complex (Brou et al. 2000; Fortini 2001; Hori et al. 2013). The released NICD then translocates into the nucleus where it modulates gene expression primarily by binding to the ubiquitous transcription factor CSL (CBF1/suppressor of hairless/Lag-1, also known as RBPJ). NICD binding to CSL recruits additional transcription factors, converting the complex from a transcriptional repressor into an activator in conjunction with cofactors such as master mind-like (MAML) proteins. This complex then induces transcription of downstream targets including several Hairy/Enhancer of Split related genes (HES and HEY) (Brou et al. 2000; Fortini 2001; Bray 2016). Both HES and HEY proteins are comprised of two domains, one that regulates DNA-binding specificity and a second helix-loop-helix domain that determines formation of either homo or heterodimers. Through either interaction with corepressors or by sequestration of different transcriptional factors, dimers of HES and HEY proteins regulate cell growth, differentiation, angiogenesis, and apoptosis (Kageyama et al. 2007).

Notch as oncogene

Early Notch signaling studies focused on its pivotal role in development and tissue homoeostasis (Artavanis-Tsakonas et al. 1999; Shih Ie and Wang 2007; Chakrabarti et al. 2018). However, an oncogenic potential for Notch

signaling was first discovered in a subset of human T-cell acute lymphoblastic leukemia (T-ALL) patients with chromosomal translocation (Reynolds et al. 1987; Ellisen et al. 1991; Radtke et al. 1999; Aster et al. 2008; Zou et al. 2013). This translocation occurs due to the fusion of the 3′ end of NOTCH1 with the T-cell receptor β (TCRB) promoter enhancer region, leading to the formation of truncated and constitutively active NOTCH1 protein. The oncogenic role of this truncated form of NOTCH1 was identified by Pear et al. (1996) in a mouse bone marrow transplantation study in which higher expression of truncated NOTCH1 promoted T-ALL. Furthermore, a gene sequence study of primary human T-ALL tumors of all molecular subtypes revealed that 50%–60% of tumor samples showed activating NOTCH1 mutations (Weng et al. 2004). Subsequent identification of activating mutations in Notch genes have been reported in studies of other hematopoietic malignancies, including B-cell chronic lymphocytic leukemia (Di Ianni et al. 2009), mantle cell lymphoma (Kridel et al. 2012), and splenic marginal zone lymphoma (Kiel et al. 2012) and are often correlated with poor patient outcome and therapeutic resistance.

A potential oncogenic function for Notch has also been reported in solid tumors, such as lung (Dang et al. 2000; Allen et al. 2011; Yuan et al. 2015), breast (Gallahan and Callahan 1997; Reedijk et al. 2005; Hu et al. 2006; Stylianou et al. 2006), ovarian (Park et al. 2006; Zhu et al. 2019), and squamous cell carcinomas (Fukusumi and Califano 2018; Loganathan et al. 2020). The first evidence of an oncogenic role for Notch in solid tumors was noted in murine breast tumors, in which MMTV (mouse mammary tumor virus) insertion into the Notch4 locus results in formation of a truncated and active form of Notch4 capable of driving the formation of mammary tumors. A similarly truncated and activated form of NOTCH4 has been found in human breast cancer BT474 (ER+ HER2+) and HS578 (TNBC) cell lines (Imatani and Callahan 2000), and an activated NOTCH1 ICD domain is observed in certain human mammary cancer epithelial cell lines (Stylianou et al. 2006). NOTCH3 seems to play a role specifically in the proliferation of ERBB2 (HER2)-negative breast cancer cell lines (Yamaguchi et al. 2008). Studies in primary human breast cancers have shown that high-level expression of JAG1 and/or NOTCH1 in tumors correlates with poor overall survival of patients with advanced breast cancer (Reedijk et al. 2005; Dickson et al. 2007). It has also been shown that NUMB, a key negative regulator of the Notch pathway, is lost in >50% of tumors due to ubiquitination and proteosomal degradation, and this loss is also correlated with higher-grade tumors (Pece et al. 2004). Besides breast cancer, oncogenic Notch function was further validated in several additional cancers, including nonsmall cell lung carcinoma, colorectal cancer, melanoma, and medulablastoma (Radtke and Raj 2003; Nowell and Radtke 2017).

Notch as tumor suppressor

While the studies described above suggest that Notch signaling plays a pro-tumorigenic role, growing evidence also

suggests that these same pathways can have a potent tumor suppressor function in both solid tumors and hematological malignancies (Dotto 2008; Nowell and Radtke 2017). For example, two studies identified predominant *NOTCH1*, *NOTCH2*, and *NOTCH3* mutations in head and neck squamous cell carcinoma (HNSCC) patients. Mutations identified from these studies were missense, nonsense, or insertion/deletions within the extracellular domain of the Notch receptors, and therefore are predicted to result in loss of function (Agrawal et al. 2011; Ock et al. 2016). Subsequently, loss-of-function mutations (both missense and nonsense) in Notch components were evident in various squamous cell carcinomas (SCCs), including cutaneous SCCs (Wang et al. 2011; Pickering et al. 2014), esophageal SCCs (Song et al. 2014), lung SCCs (Wang et al. 2011), and bladder SCCs (Rampias et al. 2014). In addition to SCCs, Notch also behaves as a tumor suppressor in other solid cancers such as PDAC (pancreatic ductal carcinoma) (Avila and Kissil 2013), HCC (hepatocellular carcinoma) (Viatour et al. 2011), and NSCLC (nonsmall cell lung cancer) (Zou et al. 2018). Most of these studies focused on Notch receptors; however, it remains unclear whether Notch ligands have any tumor-suppressive function. The predominant oncogenic function of Notch signaling in breast cancer and lymphocytes versus the tumor-suppressive role in SCCs and other cancers highlights the intriguing dual role of a single signaling pathway. In this review, we focus primarily on the oncogenic function of Notch signaling in subtypes of breast cancer, highlighting areas where therapeutic targeting of this pathway may improve patient outcome.

Role of notch signaling in mammary gland development

Unlike other organs, the majority of mammary gland development occurs postnatally at the onset of puberty. The mammary gland comprises two primary lineages, the inner luminal and the outer basal/myoepithelial cell layers. The mammary gland undergoes cycles of major modeling and remodeling during pregnancy and involution, suggesting the presence of adult stem cells. While these adult stem cells were originally believed to reside in the basal/myoepithelial cell layer (Shackleton et al. 2006; Rios et al. 2014), recent studies also support the presence of lineage-specific stem cells in both the luminal and basal lineages (Van Keymeulen et al. 2011, 2017). For example, lineage tracing experiments using Notch1-Cre-ERT2 mice demonstrate that Notch1 receptor exclusively labels ERα^- luminal progenitors. Notch1 expressing luminal mammary cells are strictly unipotent in adult mice, but surprisingly can give rise to both basal and luminal progeny in transplantation experiments or when tracing is initiated in embryos, demonstrating cell plasticity (Rodilla et al. 2015). Similarly, using a Notch3-Cre-ERT2 transgenic mouse, it was shown that Notch3 is expressed in luminal progenitor population that gives rise to ductal lineage cells capable of surviving multiple successive pregnancies, suggesting a capacity to self-renew (Lafkas et al. 2013). Another study based on short- and long-term line-

age tracing using Notch2-Cre mice show that Notch2 labels two luminal progenitor populations, S and L, that give rise to respective lineages during puberty (Šale et al. 2013). In this study it was shown that Notch2 paralogue is particularly important for tertiary branches and alveolar clusters. However, the function of Notch2 in pregnancy is not clear and needs further studies. In contrast to Notch1, Notch2, and Notch3 receptors, lineage studies with Notch4 are not yet available, most likely due to lack of good lineage tracing mouse model. However, interesting studies show that Notch4 is restricted to both basal and myoepithelial compartments containing the mammary stem cell population (Raouf et al. 2008; Harrison et al. 2010). Inhibition of Notch4 reduces mammary stem cell and bipotent lineage cell number as well as branching morphogenesis in vitro (Soriano et al. 2000; Dontu et al. 2004). It was also suggested that Notch4 can inhibit lumen formation of alveolar structures, suggesting its role in cell polarity of mammary epithelial cells. Taken together, these studies have clearly demonstrated a critical role for Notch receptors during normal mammary gland development and cell fate determination.

Compared with the function of Notch receptors, the function of Notch ligands in basal and luminal cells in normal mammary gland development are less established. Gene expression studies have shown that *Dll1* is expressed at higher levels in basal cells, whereas *Jag1* is higher in luminal progenitor cells (Bouras et al. 2008). *Dll4* and *Jag1* expression are higher in mammary glands during pregnancy (Raafat et al. 2011), as are Notch target genes such as *Hey2*, but their function is not yet understood. Recent studies from our group using Dll1 conditional knockout mice and reporter mouse models (Dll1^{mCherry} and Dll1$^{GFP-Cre-ERT2}$) demonstrated that Dll1 is predominantly expressed in basal cells and promotes virgin mammary gland development by recruiting neighboring mammary gland macrophages (Chakrabarti et al. 2018). Our results indicate that cross-talk between Dll1$^+$ mammary stem cells and macrophages maintain the local stem cell niche in virgin mammary glands. Few interesting questions still remain such as if a similar cross-talk between Dll1$^+$ stem cells/progenitors and macrophages or other stromal cells also occurs during pregnancy and/or involution.

Role of Notch signaling in different subtypes of breast cancer

Breast cancer remains a major clinical challenge with high rates of mortality and recurrence. It is a highly heterogeneous disease, as tumors display diverse morphological, immunohistochemical, and phenotypic features. Currently, breast cancers are classified into five major subtypes: luminal A and B (60%–70%), HER2 (10%), basal-like, and claudin-low (15%–20%) (Prat and Perou 2011; Hon et al. 2016). Both luminal A and B breast cancers are positive for estrogen (ER) and progesterone (PR) receptors. Luminal A tends to be less aggressive, with a low proliferation rate (low Ki67) and higher survival rates with fewer recurrences relative to luminal B (ER$^+$ and/or PR$^+$,

HER2$^+$ or HER2$^-$) tumors, which are more aggressive and highly proliferative (high Ki67) (Ellis et al. 2008; Creighton 2012). Most HER2-positive tumors are high-graded aggressive tumors with poor survival rates (Li and Li 2013). Basal-like subtypes express high levels of basal cell markers and basal cytokeratins (Yehiely et al. 2006; Rakha et al. 2008). Finally, claudin-low breast tumors are associated with stem cell and epithelial-to-mesenchymal transition (EMT)-mediated processes (Hennessy et al. 2009; Dias et al. 2017). Both basal-like and claudin-low subtypes are characterized by the absence of all hormone receptors (ER$^-$PR$^-$HER2$^-$) and thus are categorized as triple-negative breast cancer (TNBC) (Lehmann et al. 2011). These are highly aggressive with high rates of distant metastasis and poor prognosis due to lack of effective targeted therapies.

The importance of Notch signaling in human breast cancer development has been well documented (Reedijk 2012); however, understanding of the role of Notch receptors and ligands in different breast cancer subtypes remains incompletely understood. In the following subsections, we discuss the function of different Notch receptors and ligands in different subsets of breast cancer.

Notch receptors in different subtypes of breast cancer

The functional outcome of Notch signaling is highly dependent on the cellular context as well as on the temporal and spatial expression of each of its receptors and ligands (Bray 2006; Capaccione and Pine 2013). Therefore, it is not surprising that aberrant expression or function of Notch signaling components can promote or suppress different subtypes of breast cancer. A brief account of this intriguing dual function of Notch signaling component in the context of breast cancer subtypes is presented in Table 1.

Notch1 An oncogenic function for Notch1 is well established in breast cancer. Hu et al. (2006) demonstrated that overexpression of activated murine Notch1 in transgenic mice blocks mammary gland development and induces mammary tumors. Earlier studies also show NOTCH1 is activated and associated with metastatic breast cancer cells (Mohammadi-Yeganeh et al. 2015). High-level JAG1/NOTCH1 expression is connected to poor overall patient survival in human breast cancer, suggesting that a JAG1/NOTCH1 activation loop may provide oncogenic function in promoting tumor formation (Reedijk et al. 2005). In addition, transcriptional profiling of patient tumors and 14 breast cancer cell lines linked NOTCH1 and survivin, both downstream targets of NUMB, to the TNBC subgroup in an inverse manner. Mechanistically, the oncogenic effect of Notch signaling in TNBC was thought to occur within CD24^{low}CD44^{high} CSCs (Rennstam et al. 2010). Notably, another study revealed that the increased NOTCH1 activity observed in TNBC was due to a PEST (rich in proline [P], glutamic acid [E], serine [S], and threonine [T]) domain mutation in *NOTCH1* (Wang et al. 2015), highlighting the importance of this domain in Notch signaling. A recent study in the BRCA1 transgenic mouse model identified *Notch1* as a top putative oncogene able to overcome the apoptosis caused by BRCA1 deficiency and to promote TNBC formation by activating the epithelial–mesenchymal transition (EMT) signaling pathway (Miao et al. 2020). Several studies also highlight the strong connection of NOTCH1 signaling with EMT, migration, and invasion of TNBC

Table 1. *Function of Notch receptors/ligands in different subtypes of breast cancer*

Components of Notch pathway	Breast cancer subtypes	Function
NOTCH1	MMTV/neu transgenic mice (Hu et al. 2006), BRCA1 GEMM (Shao et al. 2015), ER$^+$ luminal cell lines (Bolós et al. 2013), TNBC cell lines (Mohammadi-Yeganeh et al. 2015), TNBC human patients (Rennstam et al. 2010; Wang et al. 2015), invasive ductal carcinoma (IDC) human patients (Reedijk et al. 2005)	Oncogenic
NOTCH2	HER2$^+$ human patients (Florena et al. 2007), basal subtype of IDC human patients (Lee et al. 2018)	Oncogenic
	ER$^+$ luminal cell lines (Fu et al. 2010), TNBC cell lines (O'Neill et al. 2007)	Tumor-suppressive
NOTCH3	MMTV/neu transgenic mice(Hu et al. 2006), ER$^+$ and HER2$^+$ human patients (Hirose et al. 2010; Xu et al. 2016)	Oncogenic
	TNBC cell lines (MDA-MB-231 and T98G) (Zhang et al. 2016), ERBB2$^-$ basal tumor cells (Choy et al. 2017)	Tumor suppressive
NOTCH4	MMTV/neu transgenic mice (Hu et al. 2006), WAP-h-Int3sh transgenic mice (Raafat et al. 2004), TNBC human patients (Yao et al. 2011; Wang et al. 2018)	Oncogenic
JAG1	TNBC and basal-like human patients (Li et al. 2014; Chen et al. 2016), TNBC cell lines (Sethi et al. 2011; Tao et al. 2011), luminal cell lines (Li et al. 2014)	Oncogenic
JAG2	Luminal and TNBC cell line (Xing et al. 2011; Kontomanolis et al. 2018)	Oncogenic
DLL1	ER$^+$ luminal and TNBC mammary tumors (Kumar et al. 2019), TNBC cell lines (Shui et al. 2017)	Oncogenic
DLL4	Luminal A and B, TNBC, and HER2 human patients and cell lines (Kontomanolis et al. 2014)	Oncogenic
DLL3	Less evident in breast cancer	Not known

cells (Shao et al. 2015) and ER$^+$ MCF-7 cells (Bolós et al. 2013), suggesting that Notch1 is a key regulator of EMT in breast cancer (Espinoza and Miele 2013). Based on these findings, a better understanding of the connection between CSCs, EMT, and Notch1 activity is needed to appreciate the predominant oncogenic function of this receptor in breast cancer.

Notch2   Unlike Notch1, the specific role of Notch2 in breast cancer remains ambiguous. In a study, increased NOTCH2 receptor protein and mRNA expression correlated with better survival in ER$^+$ luminal breast cancer patients (Fu et al. 2010). Similarly, a recent human breast cancer MDA-MB-231 (TNBC) xenograft study revealed anti-tumorigenic and apoptotic activity for NOTCH2 (O'Neill et al. 2007). On the contrary, a report shows that NOTCH2 is positively correlated with HER2 (Florena et al. 2007) and NOTCH2 activation maintains the mesenchymal phenotype in basal subtype of invasive breast cancer patients (Lee et al. 2018). Thus, better understanding of the context-dependent Notch2 activity is imperative in breast cancer subtypes for successful targeted therapy against Notch2.

Notch3   Similar to Notch2, Notch3 has been reported to have a dual function in breast cancer. While constitutive Notch3 signaling exhibits oncogenic potential in a murine breast cancer model (Hu et al. 2006), NOTCH3 amplification and overexpression at the protein and mRNA level were associated with better survival in HER2$^-$ and ER$^+$ human breast cancer patients (Hirose et al. 2010; Xu et al. 2016). In TNBC, ectopic NICD3 overexpression facilitated the inhibition of EMT through up-regulation of the HIPPO pathway and E-cadherin in an RBPJ-dependent manner, whereby knockdown of *NOTCH3* abrogated this effect (Zhang et al. 2016). Additionally, nonsense and missense *NOTCH3* mutations in breast cancer support its tumor suppressor capabilities through control of the cellular senescence pathway (Cui et al. 2013). In contrast, in some recent studies NOTCH3 signaling was shown to promote the growth of basal breast cancers in functional studies (Choy et al. 2017). The dual function of Notch3 may either be subtype-dependent or may relate to the choice of model and/or cell lines used. Future experiments involving mouse models such as conditional knockout and reporter model in the context of breast cancer will clarify the molecular mechanism of signaling through Notch3 in breast cancer.

Notch4   The oncogenic function of Notch signaling in breast cancer was first shown using MMTV-In3 (Notch4) transgenic mice. Both *Notch1* and *Notch4* are common sites of proviral integration in murine mammary tumors and induce mammary (MMTV) tumors when overexpressed in transgenic mice (Raafat et al. 2004; Hu et al. 2006). NOTCH4 has recently been shown to be overexpressed in TNBC carcinoma patient samples (Yao et al. 2011; Wang et al. 2018). Activation of NOTCH4 signaling is also associated with cancer progression and regulation of breast CSC activity (Harrison et al. 2010), which implicates NOTCH4 in recurrence of breast carcinoma. Anoth-

er interesting recent study demonstrates that NOTCH4 maintains quiescent mesenchymal-like breast CSC via transcriptionally activating *SLUG* and *GAS1* in TNBC (Zhou et al. 2020). Thus, similar to Notch1, Notch4 functions predominantly as an oncogene in breast cancer.

As is clear, Notch receptor-mediated signaling can play either an oncogenic or tumor suppressor role depending on breast cancer subtype and/or context; therefore, specific and judicial targeting of Notch receptors in the appropriate context will clearly be required to optimize therapeutic safety and efficacy. In addition, the functional role of individual Notch receptors in mediating breast cancer resistance to therapy will be required. Moreover, as the majority of Notch signaling studies are performed using human breast cancer cell lines engrafted into immunocompromised mice, further validation of these studies using mouse models with intact immune background is critically required.

Notch ligands in different subtypes of breast cancer

While Notch receptors in breast cancer progression have received the lion's share of attention to date, the role of specific ligands in breast cancer biology is now being appreciated. For example, JAG1-induced Notch signaling has been implicated in multiple forms of cancer and multiple aspects of cancer biology including tumor recurrence, metastatic process, and drug resistance (Dickson et al. 2007; Li et al. 2014; Andrieu et al. 2016). One of the first evidence that JAG1 was involved in a human cancer was the recognition that *JAG1* mRNA expression is up-regulated in breast cancer and correlates with a poor overall breast cancer survival in a dose-dependent fashion (Reedijk et al. 2005; Dickson et al. 2007). Accumulating evidence has also revealed that JAG1-mediated Notch activation has crucial roles in maintaining CSC populations, augmenting cell survival and proliferation, metastasis, and promoting tumor angiogenesis (Li et al. 2014; Bednarz-Knoll et al. 2016; Chen et al. 2016). Interestingly, two overlapping subtypes, TNBC and basal-like breast cancer, which are normally more aggressive and have poorer prognoses and reduced disease-free survival showed higher levels of JAG1 expression. In contrast, lower JAG1 expression was evident in less aggressive T47D and MCF-7 luminal subtype breast cancer cell lines (Li et al. 2014). Further research studies in mice have demonstrated that JAG1 expression in TNBC cells favors osteolytic bone metastasis by activating the Notch signaling pathway in the bone stromal microenvironment (Sethi et al. 2011; Tao et al. 2011). The up-regulation of another Notch ligand, DLL4 in tumor, suggests that it has a prime role in tumor angiogenesis. Zohny et al. (2020) and Jia et al. (2016) demonstrated that inhibition of DLL4 impaired vasculature development and reduced breast tumor growth along with tumor angiogenesis in DLL4 heterozygous knockout mice and in a human patient-derived xenograft (PDX) model, respectively. DLL4 is also overexpressed in breast cancer cells and is linked to nodal and distant metastasis (Kontomanolis et al. 2014).

The role of the other Notch ligands, namely, JAG2, DLL1, and DLL3 in breast cancer is less evident, but several recent studies indicate that they may contribute to

pathology. For example, hypoxia-induced JAG2-driven Notch activation can promote breast cancer metastasis and self-renewal of cancer stem-like cells (Xing et al. 2011). Reduced JAG2 in two luminal breast cancer cell lines, MCF-7 and T47D, promoted caspase activity, suggesting that Notch signaling prevents these cells from undergoing apoptosis (Kontomanolis et al. 2018). Moreover, in vitro coculture studies show that JAG2 is likely to play an important role in breast tumor angiogenesis, since its inhibition in breast cancer cells reduces endothelial tube formation (Pietras et al. 2011).

Previous work from our laboratory has shown that DLL1 protein is significantly up-regulated in ER⁺ breast tumors compared with either normal breast tissue or TNBC tumors. In addition, our study also suggested for the first time that overexpression of DLL1 protein is highly correlated with poor survival of ER⁺ breast cancer patients. Functional studies using human and mouse breast cancer cells further demonstrate that DLL1 enhances tumor growth and metastasis of ER⁺ luminal cells by promoting cell proliferation, maintenance of breast CSCs, and angiogenesis (Kumar et al. 2019). Corroborating our data, another in vitro study showed that miRNA-mediated dysregulation of DLL1 inhibits migration and invasion in luminal and TNBC breast cancer cell lines (Shui et al. 2017). Further in vivo studies addressing the mechanisms of action of DLL1 in TNBC development are required to understand the complex role of this ligand in TNBCs. In this regard, our laboratory is currently focused on identifying possible mechanisms underlying the carcinogenic role of Dll1 in breast cancer subtypes by utilizing multiple transgenic reporter and knockout mouse models. We are also currently determining whether DLL1-mediated Notch signaling represents a novel effective therapeutic target for treatment of patients with aggressive breast cancer (S Kumar, A Nandi, S Singh, et al., unpubl.).

In both preclinical and clinical studies, the role of the Notch ligand DLL3 in breast cancer remains the least clear. While studies suggest that expression of DLL3 is directly related to high-grade patient samples of lung and cervical carcinomas, this does not appear to be the case in breast cancer patients (Furuta et al. 2019; Regzedmaa et al. 2019). These data suggest Notch ligands provide attractive options for therapy in cancer treatment due to their more restricted expression patterns and better-defined functions. Since high expression levels of most Notch ligands result in aberrant Notch activity and correlates with poor clinical outcome, it is possible that differential Notch ligand activation in breast cancer subtypes may be associated with oncogenesis and progression of a particular breast cancer subtype, leading to different clinical outcomes.

Notch signaling within the TME of breast cancer

Initial research exploring the role of Notch signaling in breast cancer progression solely focused on tumor cells. However, in recent years, the involvement of juxtacrine Notch signaling between tumor cells and unique cell types within the TME has been recognized (Meurette and Mehlen 2018). The cross-talk between the cancer cells and their environment involving juxtacrine and paracrine signaling is important for new targeted therapy in breast cancer progression and drug resistance (Hanahan and Coussens 2012). Indeed, Notch activation in tumor cells promotes secretion of numerous soluble factors that can have paracrine effects on cells within the TME (Shen et al. 2017), including immune and stromal cells such as CAFs (cancer associated fibroblasts) and endothelial cells. However, CAFs can also augment Notch signaling and induce resistance by promoting secretion of several cytokines and chemokines (Tsuyada et al. 2012; Boelens et al. 2014). Indeed, breast cancer CAFs can induce NOTCH3/JAG1 activation through secretion of IL-6 (Studebaker et al. 2008). Tsuyada et al. (2012) showed that CAFs can produce high levels of chemokine CCL-2, which can regulate the CSC phenotype and NOTCH1 expression in breast cancer cell lines. Consistently, in a xenograft model in which fibroblasts and breast cancer cells were cotransplanted in NOD/SCID/IL-2Rg-null mice, loss of CCL-2 significantly reduced tumorigenesis and NOTCH1 expression, therefore suggesting that CAFs can cross-talk with the cancer cells via a CCL-2/NOTCH1 axis (Huang et al. 2019). As CAFs are a crucial regulator of Notch-dependent interactions between CSCs and TME cells, they may also contribute to the development of breast cancer resistance, which depends on CSCs. Although CAFs regulate Notch activity, it is currently unclear whether Notch receptors or ligands are responsible for CAF recruitment and activation and will require further study.

Another, very well-established immune component of breast cancer is the tumor-associated macrophages (TAMs) (Lewis and Pollard 2006; Qian and Pollard 2010). Jagged-mediated NOTCH signaling in breast cancer is associated with macrophage differentiation toward TAMs in the TME of luminal breast cancer patient samples (Liu et al. 2017). Furthermore, in this study authors also show that coculture of tumor cells and TAMs result in macrophage differentiation toward more M2 type. Although TAMs can behave both in an inflammatory and an immune-suppressive way, in breast cancer patient samples, TAMs are mostly associated with immune suppressive M2 type, because tumor cells secrete high levels of M-CSF (macrophage colony-stimulating factor) and thus skew macrophages toward the more immunosuppressive M2 subtype (Sousa et al. 2015). Similarly, in the basal subtype of breast cancer, Notch signaling through JAG1 in breast tumor cells activates key macrophage-activating cytokines such as IL-β and CCL-2, which helps in recruitment of M2 type macrophages, which in turn secretes TGF-β and activates TGF-β signaling in tumor cells (Shen et al. 2017). Such cross-talk between tumor cells and TAMs through Notch signaling may also be important for drug-resistant conditions, which needs further exploration (Shen et al. 2017).

Similar to TAMs, myeloid-derived suppressor cells (MDSCs) are also over-represented in the TME of breast cancer (Kumar et al. 2018) and can promote tumor

progression through a wide array of mechanisms including immune suppression (Gabrilovich 2017). Peng et al. (2016) showed that MDSCs can induce Notch signaling in breast cancer cells and endow CSC activity through IL-6/STAT-3 and nitric oxide/Notch cross-talk signaling. However, it is unclear which Notch receptor is involved in this study. Similarly, Welte et al. (2016) showed that addition of MDSCs to breast cancer cells increases CSCs and Notch target genes such as *Hes* and *Hey*. Notch reporter activity was used to confirm activation of Notch upon addition of MDSCs in vitro; however, it is not clear which Notch receptor or ligand was involved besides the mediator RBPJ.

Tregs (regulatory T-cells), another strong immune component for immune suppression have been reported to be increased by both JAG1 and JAG2 through mesenchymal stem cells in an animal model of allergic airway inflammation (Cahill et al. 2015). Both JAG1 and JAG2 are highly expressed in TNBC (Dickson et al. 2007; Xing et al. 2011; Lamy et al. 2017). As Tregs promote evasion of immune surveillance and are linked to tumor invasiveness and poor prognosis, Notch-mediated impairment of Treg function may have prodigious impact on breast cancer therapy. However, no study has directly addressed Notch signaling in Tregs in the context of breast cancer. Thus, it will be important to understand the regulation of different Notch ligands and receptors in the recruitment of Tregs. It is interesting to note that Notch signaling can also impair Treg function, which may limit their immunosuppressive function (Charbonnier et al. 2015). Connection of Notch signaling to other immune cells such a B cells, Dendritic cells (DC) in TME of breast cancer is not yet understood. Considering the limited success of immunotherapy targeting tumor cells in breast cancer, it will be important to understand the regulatory role of Notch signaling between cancer cells and immune cells within the TME of different subsets of breast cancer for better drug targets involving Notch signaling and immune cells. A brief schematic representation of the immune and stromal cells under the regulatory role of Notch signaling in breast TME is presented in Figure 1.

Role of Notch signaling in emergence of resistance to current breast cancer therapies

Considering the aberrant activation of both Notch receptors and ligands and their cross-talk with other oncogenic signaling pathways in breast cancer development, metastasis, recurrence, and resistance, the Notch pathway has been identified as a potential therapeutic target for adjunctive strategies to currently available treatment modalities (chemotherapy, endocrine therapy, and radiotherapy) for breast cancer patients. A brief account of different Notch targeting agents used in the context of breast carcinoma is presented in Table 2. Clinically, progression of breast cancer treatment has improved over the past decades, but challenges remain due to the emergence of therapeutic resistance among a subset of breast cancer patients. The possible mechanisms underlying different kinds of thera-
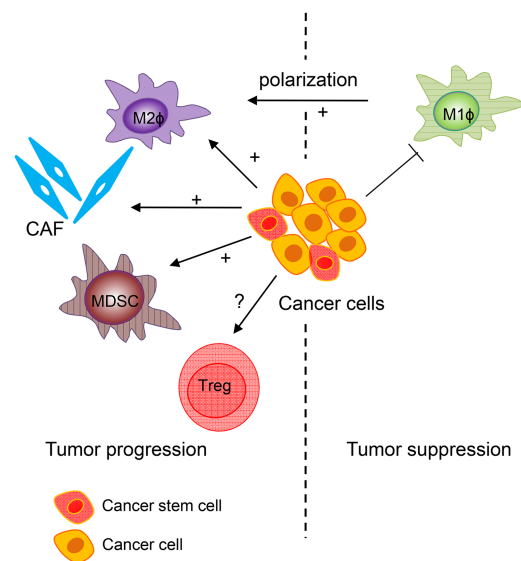

Figure 1. Pleiotropic function of Notch signaling between cancer cells and immune cells within breast tumor microenvironment (TME). Notch signaling is implicated in cross-talk between cancer and immune cells. Notch activation in cancer cells may affect immune cell recruitments. The oncogenic and tumor-suppressive functions of Notch signaling through different subsets of immune cells in context of breast cancer are represented. (CAF) Cancer-associated fibroblast, (M1Φ) macrophage type-1, (M2Φ) macrophage type-2, (MDSC) myeloid-derived stem cell, (Treg) regulatory T cell.

peutic resistance in breast cancers are thought to be driven in part by the interplay of the Notch pathway with CSCs, the heterogeneity of the TME and activation of other signaling pathways (Meurette and Mehlen 2018). The next section of the review will elucidate the role of different Notch receptors and ligands in the development of treatment-resistant breast cancer, which has also been illustrated in Figure 2.

Chemoresistance

Chemotherapies, including anthracyclines/cyclophosphamide and taxanes, are an important component of standard-of-care breast cancer treatment. However, chemoresistance is a major clinical challenge for certain subsets of breast cancer patients. Although, some reports suggest that HER2$^+$ patients do better with chemotherapy, the insensitivity to chemotherapy in another subset of patients such as in TNBC is associated with a 40%–80% risk of recurrence, resulting in distant metastasis and death for most patients (Echeverria et al. 2019). Interestingly, these TNBC patients do better with chemotherapy initially; however, many of these patients develop chemoresistance over time. For metastatic stage IV TNBC patients, chemotherapy resistance accounts for 90% of therapy failure (Nedeljković and Damjanović 2019). Therefore, there is an urgent need to identify the mechanistic basis for breast cancer chemoresistance. Li et al. (2015) showed that the inhibition of Notch signaling

Table 2. *Different Notch pathway targeting agents used in breast cancers*

Agents	Mechanism	Biological target	Developmental stage
GSIs (individual therapy)			
MK0752, RO4929097, PF-03084014 (NCT00106145, NCT02338531, NCT01151449)	Inhibition of final Notch Cleavage by γ-secretase	Metastatic or locally advanced breast cancer or recurred triple-negative breast cancer	Phase I/II clinical trial
Combined therapy			
MK0752 + docetaxel (NCT00645333)		Locally advanced or metastatic breast cancer	Phase I/II clinical trial
MK0752 + tamoxifen or letrozole (NCT00756717)	Notch homologs, Notch ligands, multiple other γ-secretase substrates	Early stage breast cancer	Pilot study clinical trial
RO4929097 + letrozole (NCT01208441)		Postmenopausal ER⁺/PR⁺ stage I or II breast cancer	Phase Ib clinical trial
RO4929097 + vismodegib (NCT01071564)		Metastatic breast cancer	Phase I clinical trial
RO4929097 + paclitaxel + carboplatin (NCT01238133)		Stage II or II triple-negative breast cancer	Phase I clinical trial
Notch receptor monoclonal antibodies (Choy et al. 2017)	Interference with Notch receptors	Notch3	Preclinical
Notch ligand DLL4 monoclonal antibodies (21M18, DLL4 antibody) (Hoey et al. 2009)	Interference with ligand-receptor interactions	Specific for DLL4	Preclinical
Notch-soluble receptor decoys (Kangsamaksin et al. 2015; Liu et al. 2016; Colombo et al. 2018)	Interference with ligand-receptor interactions	Relatively specific for Notch paralogs potential pan-Notch inhibition	Preclinical
siRNA, miRNA- based therapeutics (Shen et al. 2013; Ahmadzada et al. 2018; Loh et al. 2019)	Interference with expression of Notch signaling component	Specific for target mRNAs	Preclinical

pathway (NOTCH1 primarily) with γ-secretase inhibitor (GSI) could enhance the sensitivity to doxorubicin treatment in MDA-MB-231 TNBC cells. Another recent study demonstrated antibody-specific inhibition of JAG1 sensitizes bone metastases to chemotherapy in TNBC cells in vivo in mice, showcasing an important role for JAG1 and the Notch pathway in promoting chemoresistance in breast cancer metastasizing to bone (Zheng et al. 2017). Thus, chemotherapeutic targeting JAG1 may improve the efficacy of chemotherapy in that substantial portion of TNBC breast cancer patients who eventually develop metastatic relapse in bone and other organs. These studies along with others suggest Notch signaling activation is a hallmark for TNBC. Interestingly, many of these Notch signaling work through CSCs. For example, it has been shown that specific inhibition of Notch1 signaling enhances the antitumor efficacy of chemotherapy in TNBC through reduction of CSCs (Qiu et al. 2013). Another study demonstrates that JNK regulates TNBC tumorigenesis by promoting CSC phenotype through Notch1 signaling via activation of c-Jun (Xie et al. 2017) and indicates that JNK/c-Jun/Notch1 signaling is a potential therapeutic target for TNBC.

Similar to receptors, higher expression of Notch signaling ligands such as DLL4 was detected in a docetaxal-resistant luminal ER⁺ luminal MCF-7 breast cancer cell line with increased CSC activity (Wang et al. 2017). Moreover, DLL4 is an important biomarker of chemoresistant breast cancer subtypes (Hoey et al. 2009; Wang et al. 2017). Hoey et al. (2009) have demonstrated that targeting of the DLL4–NOTCH1 axis with humanized anti-DLL4 monoclonal antibody alone or in combination with the chemotherapeutic agent, irinotecan, decreased CSC frequency in a human patients derived xenograft model. Recent studies from our group identified function of Dll1 in chemoresistance of breast cancer through NF-κB signaling (S Kumar, A Nandi, S Singh, et al., unpubl.). Compared with the predominantly chemoresistant functions of Notch ligands and receptors, a few studies also highlight the inhibitory role of Notch signaling in chemoresistance. For example, a recent study demonstrated that down-regulation of NOTCH3 correlated with low-grade chemoresistance in the luminal ER⁺ MCF-7/ADAM breast cancer cell line (Gu et al. 2016), potentially due to its negative impact on transcription factor Fra1 (fos-related antigen-1), which is required for the EMT that promotes chemoresistance in breast cancer (Gu et al. 2016). Together, these findings suggest that effective targeting of specific Notch signaling pathway should have a major impact on chemoresistant cancer patient survival.

In addition to Notch signaling in CSCs in tumor cells, a growing body of evidence supports the notion that components of the TME play specific roles in the development of chemoresistance through Notch signaling. As such, therapeutic strategies that target Notch signaling interactions between cancer cells and cells within the TME could pave
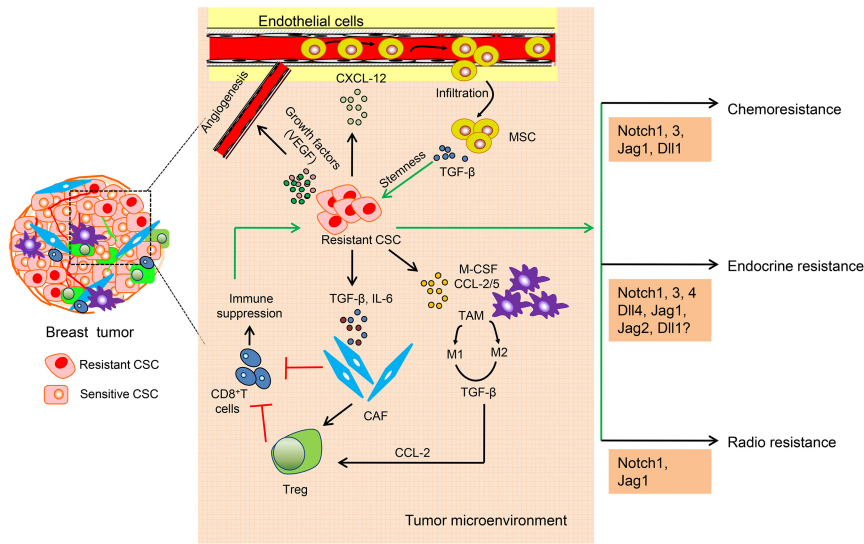
Figure 2. Interaction between cellular components of tumor microenvironment and breast cancer cells regulates Notch signaling driven therapeutic resistances in breast tumors. Different cellular component of tumor microenvironment can induce cancer stem cell survival, stemness, and resistance through either TGF-β-dependent mechanism and or by releasing soluble factors such as cytokines, chemokines, and growth factors that favor angiogenesis and immunosuppressive environment. All of these factors in turn augment Notch ligand- and receptor-mediated chemoresistance, endocrine resistance, and radio resistance in breast tumors. (CAF) Cancer-associated fibroblast, (CSC) cancer stem cell, (CXCL) chemokine (C-X-C motif) ligand, (CCL) chemokine (C-C motif) ligand, (IL) interleukin, (M-CSF) macrophage colony-stimulating factor, (MSC) mesenchymal stem cell, (TAM) tumor-associated macrophage, (TGF) transforming growth factor, (Treg) regulatory T cell, (VEGF) vascular endothelial growth factor.

the way for a new generation of therapies. However, progress has been impeded by limited understanding of the mechanisms of acquired chemoresistance through Notch-dependent tumor–stromal interactions and the absence of predictive biomarkers for response to such TME directed chemoresistance therapies. An interesting study by Sethi et al. (2011) demonstrated that JAG1 in TNBC cells promotes bone metastasis by activating the Notch pathway in supporting bone cells (osteoblasts). It would be interesting to see whether this could contribute to chemoresistance in future studies. Expression of Jag1 may also be important when expressed on stromal cells. For example, a recent study demonstrated that fibroblasts expressing Jag1 on their surface activate Notch3 signaling in TNBC cells, thus promoting the expansion of TNBC cells resistant to chemotherapy and reinitiating tumor growth (Boelens et al. 2014). These studies highlight the functional significance of cross-talk of Notch signaling between tumor cells and TME through juxtracrine signaling. Thus, it is important to fully elucidate the subcellular distribution of different Notch components within the TME and to determine their function in breast cancer chemoresistance to develop targeted strategies that may address chemotherapy resistance.

Endocrine resistance

Endocrine therapy is mostly effective in ER$^+$/PR$^+$ breast cancers that are initially dependent on the ER signaling pathway (Santiago-Gómez et al. 2019). Tamoxifen, a selective ER modulator (SERM) is the current endocrine therapy of choice (An 2016). Other anti-estrogens, such as SERDs (fulvestrant) or aromatase inhibitor (AI), are most often utilized when tamoxifen treatment fails (Howell et al. 2004). Unfortunately, a serious limitation of endocrine therapies is the development of either de

novo resistance, an inability of patients to respond to any ER modulators, or acquired resistance in which a subset of patients gains resistance during the course of treatment (Osborne 1998; Early Breast Cancer Trialists' Collaborative Group 2005; Early Breast Cancer Trialists' Collaborative Group et al. 2011b). As a result, the 5-yr survival rate of the resistant patients is only 20% (Gonzalez-Angulo et al. 2007; Clarke et al. 2015).

The Notch pathway plays an important role in estrogen therapy-resistant breast cancer in luminal A type and luminal B breast cancer (Haughian et al. 2012; Yun et al. 2013; Gelsomino et al. 2018). NOTCH3-mediated signaling is increased by tamoxifen in tamoxifen- and fulvestrant-resistant MCF-7 cell lines and the subsequent interplay between NOTCH3 and PBX1 controls the expression of a large number of genes associated with endocrine resistance (Magnani et al. 2013). In addition, another Notch receptor, NOTCH4 has been shown to confer endocrine resistance and stemness in a tamoxifen resistant MCF-7 breast cancer cell line by sustaining CSCs (Haughian et al. 2012; Lombardo et al. 2014). In another study, NOTCH1 was suggested to be sufficient to activate ER-mediated transcription even in the absence of estrogen delineating its possible function in endocrine resistant breast cancer cell line (Hao et al. 2010). Several other studies provided evidence that other Notch components such as JAG1 and JAG2 are also elevated in endocrine-resistant luminal breast cancers, leading to an increase in CSC activity (Simões et al. 2015). Magnani et al. (2013) recently published that pharmacological inhibition or genetic ablation of Notch signaling could block growth of endocrine-resistant cancer cells, suggesting that combining current treatment options with a blockade of Notch signaling might be therapeutically beneficial. Furthermore, accumulating evidence indicates that CSCs are key drivers of acquired endocrine resistance in ERα$^+$ breast tumors

(Simões et al. 2011, 2015; Piva et al. 2014). Therefore, it is imperative to identify components of Notch signaling pathways that can be targeted to eradicate breast CSCs and hence provide long-term disease-free survival.

Notably, a recent study from our group has demonstrated that progression of ER[+] breast cancer depends on DLL1-mediated Notch signaling and its effects on CSCs (Kumar et al. 2019). However, it is not yet clear whether the tumor-promoting function of DLL1 on CSCs is also responsible for endocrine resistance. As cross-talk between DLL1-mediated Notch signaling and Wnt signaling between mammary stem cells and macrophages have been established in normal mammary gland (Chakrabarti et al. 2018), it is possible that the effect of DLL1 on endocrine resistance may function independent of ER signaling by regulating other signaling pathways in CSCs such as Wnt and SHH pathways (Takebe et al. 2015; Koury et al. 2017). These studies are currently under investigation in our laboratory.

Besides tumor cell intrinsic function, a myriad of evidence has revealed that heterogeneous cell types within the TME can also actively influence endocrine resistance (Junttila and de Sauvage 2013). A recent study has demonstrated that infiltration of macrophages in tamoxifen-resistant breast cancer TME is driven by up-regulation of JAG1 expression in tumor cells and found that Notch activation of these tumor-infiltrating macrophages directly suppresses CD8^{+} T-cell responses in vitro (Ruffell and Coussens 2015), providing a potential mechanistic basis for Notch-dependent immune evasion by resistant tumors cells. Another convincing link between endocrine resistance and tumor stroma was demonstrated by Simian's group (Pontiggia et al. 2012), who showed that cancer-associated fibroblast-derived soluble factors induce tamoxifen resistance in ER^{+} murine breast tumors. Similarly, other preclinical and clinical studies revealed that CD146^{−} CAFs inhibit ER expression in the MCF-7 cell line and decrease clinical response to tamoxifen with worse patient outcome (Brechbuhl et al. 2017; Morgan et al. 2018). Fibroblasts have also been shown to promote therapy resistance in breast cancer cells through expression of JAG1 and exosomal transfer leading to activation of NOTCH3 and STAT1 signaling in cancer cells (Boelens et al. 2014). However, their connection to endocrine resistance is not so clear. CAFs and TAMs can collaborate via cell–cell interaction to promote endocrine resistance, and it is possible that Notch signaling may contribute in the cross-talk between these two cell types. If verified, targeting Notch signaling in CAFs and TAMs could be a promising therapeutic strategy to improve clinical outcome for endocrine resistant breast cancer patients.

Radiation resistance

Radiotherapy is another effective nonsurgical modality for breast cancer treatment (Charaghvandi et al. 2017) in which high-energy radiation destroys cancer cells through DNA damage, either directly or through generation of free radicals (Balaji et al. 2016). In comparison with chemotherapy, radiotherapy is more efficacious for local tumor control, with fewer side effects (Ozpiskin et al. 2019). Moreover, the pivotal role of adjuvant radiotherapy in leading to reduced recurrence and long-term mortality has been well established (Early Breast Cancer Trialists' Collaborative Group et al. 2011a). Nevertheless, some patients still may not benefit from this treatment owing to individual variation in radio-sensitivity and may experience recurrences that ultimately challenge their prognosis and quality of life. This could be due to the presence of CSCs in tumors (Choi et al. 2020). A few recent studies have associated aberrant Notch signaling with radio resistance and CSCs. For example, a modest induction of JAG1 expression on the surface of nonadherent CSC-enriched cells was observed after fractionated radiation (Eyler and Rich 2008). In another study, increased levels of activated NOTCH1 were present in the culture media of CSC-enriched cells after radiation (Phillips et al. 2006). Although this study showed a correlation between the levels of JAG and activated NOTCH1 and radiation treatment, more in depth research is required to determine whether the Notch pathway contributes to CSC-mediated radio resistance in breast cancer. In addition, characterization of the expression patterns of Notch receptors and ligands in response to radiation would be crucial for determining whether the use of specific Notch inhibitor in conjunction with radiation would represent a beneficial treatment option for breast cancer patients.

Another causal factor for radio resistance could be shedding of cancer cells from primary tumors in circulation in the form of circulating tumor cells (CTCs) (Yadav and Shankar 2019). Enumeration and characterization of CTCs hold great promise as predictive biomarkers for guiding optimal treatment. Interestingly, some recent reports demonstrated the association of increased CTCs and Notch signaling in HER2^{+} breast cancer (Jordan et al. 2016). Positive CTC status has been validated as prognostic of recurrence-free survival, breast cancer-specific survival in metastatic breast cancer (Franken et al. 2012; Janni et al. 2016). However, if Notch signaling-mediated CTCs are connected to radio resistance is not yet clear. With current lineage tracing technique and novel mouse reporter models to track Notch-activated cancer cells, it will be very interesting to determine whether the CTCs contain Notch receptor- or ligand-positive cells. It would be interesting to determine whether the Notch signaling-mediated CTCs has CSC function in future studies.

In addition to cancer cells, radiotherapy affects two major components of the TME-immune cells and tumor blood vessels, which later participate in the activation of different immune suppression pathways and induction of radio resistance. Very few studies address the role of Notch signaling in the TME in radio resistance. However, Notch signaling is frequently activated by hypoxia during tumor progression through activation of the hypoxia-inducible factor-1α (HIF-1α) transcription factor. Such hypoxia-driven Notch signaling may support the dormancy of CSCs, preserving their potential for proliferation and differentiation, thus protecting them from radiotherapy. In addition, HIF-1α tumor cell expression is tightly regulated within the TME and its activation promotes the

recruitment of immunosuppressive populations of TAMs with an M2 phenotype, MDSCs, and Treg cells into the TME of radio-resistant breast cancers (Carvalho and Villar 2018; Darragh et al. 2019; Jarosz-Biej et al. 2019). As such, additional insights into the signaling network between Notch signaling, HIF-1α, and the TME in breast cancer could provide new hints for overcoming radio resistance. Although Notch dysregulation is common in breast cancer, Notch signaling in radiation resistance is an evolving field. Thus, understanding of the context-dependent interactions between Notch and other therapeutically relevant pathways may provide insight in combining Notch therapeutics with radiotherapy for synergistic improvements.

Conclusions

In this review, we have summarized crucial roles for individual Notch receptors and ligands in the normal mammary gland development and progression of different breast cancer subtypes and highlighted significant preclinical and clinical studies revealing how aberrant Notch signaling modulates CSCs to induce drug and radio resistance. Indeed, Notch signaling has gained increasing attention as a potential therapeutic target to overcome current treatment failure. Notably, we also highlighted the function of Notch ligands in breast cancer development and drug and radiation resistance, which are highly understudied and need future thorough investigation. For example, determining which Notch ligands are specifically involved in certain subtypes of breast cancer might be useful for identifying patients who are most likely able to respond to different therapies, paving the way for reduction of the therapeutic complications associated with nonselective Notch inhibitors. Furthermore, we have highlighted how Notch signaling driven alterations in the TME impact the efficacy of current therapies in breast cancer patients. Detailed mechanistic analysis of the emergence of Notch-mediated resistance to chemotherapy, endocrine therapy, and radiotherapy are not well explored. However, studies outlined in this review suggest that combination therapies targeting both Notch signaling pathway and TME might promote new potential therapeutic intervention in the treatment of aggressive chemoresistant, endocrine-resistant, and radio-resistant breast cancer patients.

Acknowledgments

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