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REVIEW ARTICLE

NOTCH3 signalling as a therapeutic nexus: Bridging cerebral small vessel disease and breast cancer pathophysiology

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Abstract

The neurogenic locus notch homolog protein 3 (NOTCH3), is central in both vasculogenesis and oncogenesis and, therefore, has been considered an important factor in the development of cerebral small vessel disease (CSVD) and breast cancer (BC). Pathogenic mutations of NOTCH3 induce vascular smooth muscle cell degeneration, microvascular dysfunction and neurovascular damage in cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), which is a genetic cause of CSVD. Meanwhile, NOTCH3 aberrant signalling in BC promotes tumour progression, metastasis and chemoresistance, especially in aggressive subtypes, such as triple-negative BC. A growing body of evidence points to a common molecular pathway whereby NOTCH3 dysregulation mediates vascular and tumour pathologies, thus providing an important link between these conditions. This narrative review synthesises current insights into the dual role of NOTCH3, focusing on translational relevance as a therapeutic target. Targeting NOTCH3 may mitigate vascular damage in CSVD and simultaneously inhibit tumour progression and metastasis in BC. The review further discusses NOTCH3 as a biomarker for early diagnosis and risk stratification, besides novel therapeutic strategies involving γ-secretase inhibitors and monoclonal antibodies. Future directions include studies into the ligand-independent functions of NOTCH3, its role within the tumour microenvironment, and the development of therapies with dual-action potential. This review discusses, for the 1st time, common mechanisms between CSVD and BC, thereby opening new avenues for therapies that could effectively target both conditions. By translating these laboratory findings into clinical applications, this approach aims to improve outcomes for patients affected by these devastating disorders.

Keywords: Cerebral small vessel disease; Breast cancer; Neurogenic locus notch homolog protein 3; Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy; Tumor

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1. Introduction

Breast cancer (BC) and stroke are two different medical disorders, each with unique pathophysiological causes. Recent discoveries suggest a possible connection between these two unconnected entities,[1,2] identifying the roles of neurogenic locus notch homolog protein 3 (NOTCH3) signalling as important participants in both illnesses.

One member of the NOTCH family that has become important in both oncogenesis and vasculogenesis is NOTCH3. Variations in the *NOTCH3* gene have been linked to several cardio- and cerebral-vascular diseases, such as cerebral small vessel disease (CSVD), which is a precursor to stroke and is characterised by cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL).[3] The pathological hallmark of CSVD is the accumulation of NOTCH3 extracellular domain within small penetrating arteries, which is noteworthy.[4] Despite CSVD is often silent, many affected individuals remain asymptomatic. Their manifestation is frequently unintentionally detected during normal magnetic resonance imaging (MRI) brain scans, showing distinctive cerebral white matter lesions.[5]

Moreover, pre-clinical studies have revealed that NOTCH3 plays a dual role in BC, contributing to both tumor development and metastatic progression.[6,7] Alarmingly, increased cancer invasiveness, partly mediated by the development of lymphovascular emboli, is associated with elevated NOTCH3 expression in inflammatory BC.[8] These results are further supported by observations that hypercoagulation and vascular impairment related to cancer are associated with a higher risk of stroke and that stroke-related death is more common in BC patients than in non-cancer patients.[9]

Given the ageing global population and the increasing incidence of both CSVD and BC, the clinical overlap between these conditions is of growing interest. CSVD contributes significantly to ischemic stroke, a leading cause of death in cancer patients, and the underlying shared molecular mechanisms involving NOTCH3 signalling may suggest an urgent need for integrative research. Uncovering these connections holds the potential to transform clinical approaches by identifying shared therapeutic targets and improving outcomes in patients at the intersection of these pathologies. The intersection of these research avenues demands a reassessment of the clinical environment, where the symptomatology of BC and CSVD may counterintuitively resemble one another, ultimately predisposing individuals to ischemic stroke.

Thus, the purpose of this narrative review is to compile the body of knowledge clarifying the dynamic interactions among NOTCH3 signaling, CSVD and BC. Across exploring the common mechanical foundations of these illnesses, we hope to uncover new understandings of their relationship. These disclosures could lead to the creation of novel preventive and therapeutic approaches, which would be an example of 'killing two birds with one stone' in the fight against these powerful enemies.

2. BC

BC is an array of distinguished malignancies that occur within the mammary glands.[9] Indications of BC can encompass a breast lump, alterations in breast contour, skin dimpling, nipple discharge, inverted nipple (turns inward), and/or the presence of a red or scaly skin patch.[10,11] In addition, individuals with the disease or tumours that have metastasised to distant sites may experience symptoms such as bone pain, difficulty in breathing, enlarged lymph nodes, and/or jaundice.[12] Factors that increase the risk of developing BC encompass a sedentary lifestyle, obesity, alcohol consumption, early onset of menstruation, ageing, delayed or absent childbearing, hormone replacement therapy during menopause, exposure to ionising radiation, a prior BC diagnosis and a family history of the disease.[13]

2.1. BC epidemiology and present trends

BC is the second most common cause of cancer-related mortality in women globally.[14] The World Health Organization reported that in 2020, BC was diagnosed in 2.3 million women worldwide, resulting in 685,000 fatalities. By the end of 2020, there were 7.8 million women who had received a BC diagnosis within the past 5 years, solidifying its status as the most widespread cancer globally. Moreover, BC affects women of all ages in every country after they reach puberty, with higher incidence rates in later life. Alarmingly, around 0.5 – 1% of BC cases are observed in males.[15]

Moreover, BC has experienced a significant rise in both the number of cases and fatalities in the past 30 years. Between 1990 and 2016, BC cases more than doubled in 60 out of 102 countries, including Afghanistan, Argentina, Brazil and the Philippines. Simultaneously, BC-related deaths also doubled in 43 out of 102 countries, including Libya, Paraguay, Saudi Arabia and Yemen.[16] It has been suggested that according to the projections for the year 2030, there will be approximately 2.7 million new cases of BC diagnosed globally each year, with around 0.87 million annual deaths.[17] Moreover, the incidence of BC in low- and middle-income countries is anticipated to rise even more, primarily because of the increasing adoption of Western lifestyles, which include the previously mentioned risk factors. In addition, improved cancer registration and diagnostic capabilities also contribute to the observed increase in reported cases.[18]

2.2. Genetics of BC: Present updates

While BC research has predominantly focused on understanding the molecular mechanisms behind tumourigenesis, metastatic spread to distant organs following the surgical removal of the primary tumour remains a significant factor contributing to unfavourable outcomes.[19] Hence, it is crucial to gain a deeper comprehension of the molecular mechanism that drives cancer cell seeding and the development of metastases. This knowledge is beneficial for the development of novel therapies specifically targeting breast tumour metastasis-initiating cells, with the goal of halting the advancement of the tumour.

Despite only about 5 – 10% of BC cases having a genetic basis, multiple genetic mutations have been identified as strongly linked to an elevated risk of developing BC. Two prominent genes with a significant penetrance, *BRCA1* (located on chromosome 17) and *BRCA2* (situated on chromosome 13), are primarily associated with an elevated risk of BC development.[20] The mutations in these genes are typically inherited through an autosomal dominant pattern, but sporadic mutations are also frequently documented. Additional BC genes with significant penetrance, such as *TP53*, *CDH1*, *PTEN* and *STK11,* have also been reported.[21-24] In addition to the heightened risk of BC, individuals with these mutations are also at an increased susceptibility to ovarian cancer.

In addition, genetic predisposition plays a critical role in a subset of BC cases, particularly those with early onset or strong family history. While 5 – 10% of BCs are hereditary, several high- and moderate-penetrance genes significantly elevate the associated risk.[20,25] *BRCA1* and *BRCA2* mutations remain the most studied, with high lifetime risks for BC and ovarian cancer. Other key genes, such as *TP53*, *PALB2*, *CHEK2* and *ATM* influence tumour suppression and DNA repair pathways. These mutations typically follow autosomal dominant inheritance patterns. Table 1 summarises the most clinically relevant genes, their functions and associated cancer risks.

In addition to genes related to BC, NOTCH receptors have been reported to be likely to exert distinct regulatory effects on BC cells.[36] Therefore, it is crucial to define the specific functional roles of individual NOTCH receptors in propelling tumour advancement. Later in this narrative review, we provide evidence that the expression of NOTCH3 is associated with the pathogenesis of cancer cell dissemination and the progression of BC metastases. Moreover, there is an increasing body of evidence suggesting that BC can lead to the onset and progression of ischemic stroke, and BC is currently being referred to as a risk factor for stroke and its subtypes. In the next section, we will discuss the precursor for ischemic stroke, CSVD and its relation to BC.

3. CSVD

CSVD is responsible for approximately 25% of ischemic strokes, most intracerebral haemorrhages in individuals over the age of 65, as well as the primary cause of vascular dementia. A recent study also linked CSVD with vascular Parkinsonism.[37] In addition, it is linked to issues with mobility, gait, neurobehavioural functions and mood disorders.[38] The pathophysiological foundation of CSVD encompasses alterations in the structure and function of the microvasculature within the deep subcortical regions. These alterations primarily affect arteries, including tributaries of the middle cerebral artery, and arterioles, resulting in phenomena, such as fibrinolysis, lipohyalinosis, necrosis and microthrombosis.[39,40]

CSVD is a condition that becomes increasingly prevalent with age and is frequently encountered as an incidental discovery during neuroimaging. This condition is frequently underestimated by healthcare professionals because of its covert (silent) nature, as it often presents without symptoms. Clinically, it commonly presents with subtle but progressive symptoms such as forgetfulness, depression, slowed thinking, balance issues, and urinary urgency.[38] Present neuroimaging indicators (or manifestation) of CSVD based on the Standards for Reporting Vascular Changes on Neuroimaging 2 encompass recent small subcortical infarcts, white matter hyperintensities (WMHs) of presumed vascular origin, lacunar infarcts (of presumed vascular origin), enlarged perivascular spaces, cerebral microbleeds, cortical superficial siderosis, brain atrophy and cortical cerebral microinfarcts.[41] Frequent cardio-cerebrovascular risk factors for sporadic CSVD, including ageing, type 2 diabetes, hypertension, smoking and dyslipidaemia, elevate the risk of pathological alterations in arteries and arterioles, potentially resulting in vessel blockage, which in turn leads to the development of arteriosclerosis and arteriolosclerosis.[40]

Several aetiopathogenic classifications have been proposed for CSVD. Nevertheless, the most widely acknowledged categories of CSVD include amyloidal CSVD (e.g., sporadic and hereditary cerebral amyloid angiopathy) and non-amyloid CSVD, which encompasses age-related and small vessel disease related to vascular risk factors (such as arteriolosclerosis and ageing).[42] Meanwhile, the less prevalent categories of CSVD encompass inherited or genetic (monogenic) forms, which exhibit distinct characteristics separate from cerebral amyloid angiopathy. Examples include Fabry's disease and CADASIL, as well

Table 1. Prominent genes linked to an elevated risk of breast cancer incidence

Gene	Chromosome site	Function and degree of penetration	Risk for breast cancer (%)
ATM[26]	11q22.3	• DNA repair and cell cycle regulation • Moderate penetration	20 – 60
BRCA1[27]	17q21.31	• DNA repair and cell cycle regulation • High penetration	45 – 87
BRCA2[28]	13q13.1	• DNA repair and cell cycle regulation • High penetration	50 – 85
BRIP1[29]	17q23.2	• Participation in *BRCA1* function • Moderate penetration	Limited Data
CDH1[30]	16q22.1	• Cell adhesion regulation • Regulate epithelial cell proliferation and mobility • High penetration	63 – 83
CHEK2[31]	22q12.1	• Cell cycle regulation • Moderate penetration	20 – 25
PALB2[32]	16p12.2	• DNA repair • Moderate penetration	33 – 58
PTEN[33]	10q23.31	• Cell cycle regulation • High penetration	50 – 85
STK11[34]	19p13.3	• Cell cycle regulation • Upkeep of energy balance • High penetration	32 – 54
TP53[35]	17p13.1	• DNA repair, cell cycle regulation, triggering apoptosis, promoting senescence and sustaining cellular metabolism are all crucial cellular processes • High penetration	20 – 85

Note: Risk percentages reflect ranges from recent meta-analyses (2020 – 2024).
Abbreviations: ATM: Ataxia telangiectasia mutated; BRCA1: Breast cancer gene 1; BRCA2: Breast cancer gene 2; BRIP1: BRCA1 interacting protein c-terminal helicase 1; CDH1: Cadherin 1; CHEK2: Checkpoint kinase 2; PALB2: Partner and localizer of BRCA2; PTEN: Phosphatase and tensin homolog; STK11: Serine/Threonine kinase 11; TP53: Tumour protein 53.

as inflammatory and immune-mediated CSVD, venous collagenopathies and other forms of CSVD, including non-amyloid microvessel degeneration in Alzheimer's disease and post-radiation angiopathy.[43] Various pathomechanisms and molecular cascades have been proposed for the onset and progression of CSVD, and many of which are interrelated across most, if not all, CSVD categories.[44-50]

4. Putative clinicopathological correlates of BC and CSVD

CSVD is a precursor for ischemic stroke, a complex pathological event leading to sudden neurological damage, with cancer being just one of the numerous associated risk factors. Simultaneously, it seems that the occurrence and frequency of both conditions are rising within the elderly population. Similarly, in cancer patients, cerebrovascular disease emerges as the second most prevalent neurological condition after metastases.[51] However, this connection is frequently overlooked by the clinician. CSVD or even ischemic stroke may manifest at any stage during malignancy and, in some cases, serve as the initial sign of a silent malignancy in up to 3% of patients.[52] In addition, post-mortem examinations of cancer patients show the presence of ischemic stroke in 15% of cases, with half of these cases being silent or asymptomatic.[53] Considering that CSVD could potentially serve as the initial indication of neoplasia, in this case, BC. This necessitates a precise determination of the underlying cause to tailor treatment appropriately and enhance clinical outcomes.[52]

BC and malignancies in general, share numerous risk factors with CSVD. These risk factors are more prevalent among the elderly population, which also tends to have a higher burden of vascular risk factors. Studies have indicated that the prevalence of these vascular risk factors, including diabetes mellitus, obesity, hypertension, hyperlipidemia, smoking, alcoholism, and atrial fibrillation, is comparable between ischemic stroke patients with cancer and stroke patients without cancer.[2,54] Considering both higher pathogenicity and prevalence of vascular risk factors, it comes as no surprise that these factors continue to be the most common cause of ischemic stroke, even among individuals with cancer, specifically BC.[54] Alarmingly, studies have shown

that the conventional ischemic stroke mechanisms, such as cardioembolic and lacunar strokes, are roughly the same among patients with and without cancer.[54] Furthermore, previous studies have indicated that atherosclerosis is the leading cause of ischemic stroke in patients with neoplasia.[1]

The exact mechanisms of CSVD and ischemic stroke in the context of BC remain vague. Given that vascular risk factors are widespread among CSVD and/or ischemic stroke patients, whether both CSVD and BC emerge independently and simultaneously, or if BC directly impacts the pathophysiology of CSVD and/or ischemic stroke, remains uncertain. Interestingly, hypercoagulability has been considered the primary mechanism behind ischemic stroke in individuals with cancer.[55] Tumour cells secrete pro-coagulant molecules, including tissue factors and cancer pro-coagulants, such as cysteine protease, which enhance the coagulation process. Furthermore, they release various cytokines, such as tumour necrosis factor-alpha, and interleukins (ILs), including IL-1 and IL-6.[56] These molecules function as pro-coagulants through three mechanisms: First, by inducing cells to express tissue factors; second, by impeding the activation of Protein C; and finally, by causing shedding of vascular endothelial cells, thereby contributing to increased blood viscosity and clot formation.[54] This constitutes a paraneoplastic phenomenon that remains poorly understood but is associated with reduced survival in affected individuals.[57]

Numerous studies have attempted to employ laboratory markers to assess coagulation abnormalities. D-dimer serves as an indicator of an activated coagulation system, including in patients with CSVD. Cancer patients who experience strokes tend to have elevated D-dimer levels in comparison to stroke patients without cancer.[2,54,57] D-dimer is also an independent predictor for CSVD and strokes caused by non-conventional mechanisms, which are notably linked to cancer in several studies.[58] Moreover, previous studies have reported a greater occurrence of micro-embolisms detected by transcranial Doppler inpatients who experienced strokes due to cancer, especially in cases involving unconventional stroke mechanisms. This correlation was notably significant and aligned with elevated D-dimer levels.[59] Nevertheless, it's important to note that D-dimer is a non-specific marker, as it can become elevated in various situations, even in cancer patients who do not have a stroke.[57] Hence, new and alternative approaches are needed to further strengthen the association between CSVD and/or stroke and cancer, specifically BC in this context.

4.1. Roles of NOTCH3 in BC and CSVD

The *Notch* gene received its initial identification during research on irregular notched-wing *Drosophila melanogaster* in 1914.[60] NOTCH signalling contributes to a range of biological processes in different species, including organ development, tissue maintenance, and tissue regeneration. Consequently, disrupted NOTCH signalling can lead to pathological outcomes. In addition, NOTCH protein and its homologs, including NOTCH1, NOTCH2, NOTCH3, NOTCH4, LIN-12, and glucagon-like peptide 1, have been detected in genomes across all biological kingdoms, signifying the ongoing diversification of the NOTCH family. Their length spans from approximately 110 amino acids in bacteria to about 4,500 amino acids in animals.[61] Members of the NOTCH family have evolutionarily preserved type-1 transmembrane glycoproteins, serving the dual role of acting as transmembrane receptors for ligands and functioning as transcription factors.[62] To date, there are four paralogous NOTCH transmembrane receptors encoded by four distinct genes, namely, NOTCH1 to 4 were found in mammals.

Structurally, the human NOTCH family receptor comprises an extracellular domain (NOTCH extracellular domain, NECD), a transmembrane domain, and an intracellular domain (NOTCH intracellular domain, NICD) (Figure 1).[63] The NECD comprises between 29 and 36 epidermal growth factor-like repeats (EGF-like domains), a number that varies depending on the receptor type, along with a negative regulatory region (NRR). The NRR consists of three cysteine-rich LIN-12/NOTCH repeats (LNRs) and a heterodimerisation domain. Each EGF-like repeat contains six cysteines, resulting in the formation of three disulphide bonds that contribute to the protein's three-dimensional structure.[3] Moreover, a recent study detailed that the NICD is comprised of an RBPJκ-associated molecule (RAM) domain, a domain with seven ankyrin repeats (ANK), nuclear localization sequences (NLS), a transcriptional activation domain (TAD), and a C-terminal Pro-Glu-Ser-Thr (PEST) domain (Figure 1).[64] While NOTCH receptors are largely preserved, they exhibit structural variability, particularly in the number of EGF-like repeats, the presence of the TAD domain, and the length of the segment between the ANK repeats and the C-terminal region.[65]

5. NOTCH3 signalling

The *NOTCH3* gene encodes a receptor of approximately 2321 amino acids, which dictates the fate of vascular smooth muscle cells (VSMCs) within the brain's arterial network.[66] Activation of the NOTCH (in this case NOTCH3) signalling pathway occurs through cellular interactions with a NOTCH ligand. In mammals, there are five NOTCH ligands have been reported, such as delta-like ligands (DLL) 1, 3, and 4 and jagged (JAG) 1, and 2. Before reaching the cell membrane, the intact NOTCH3

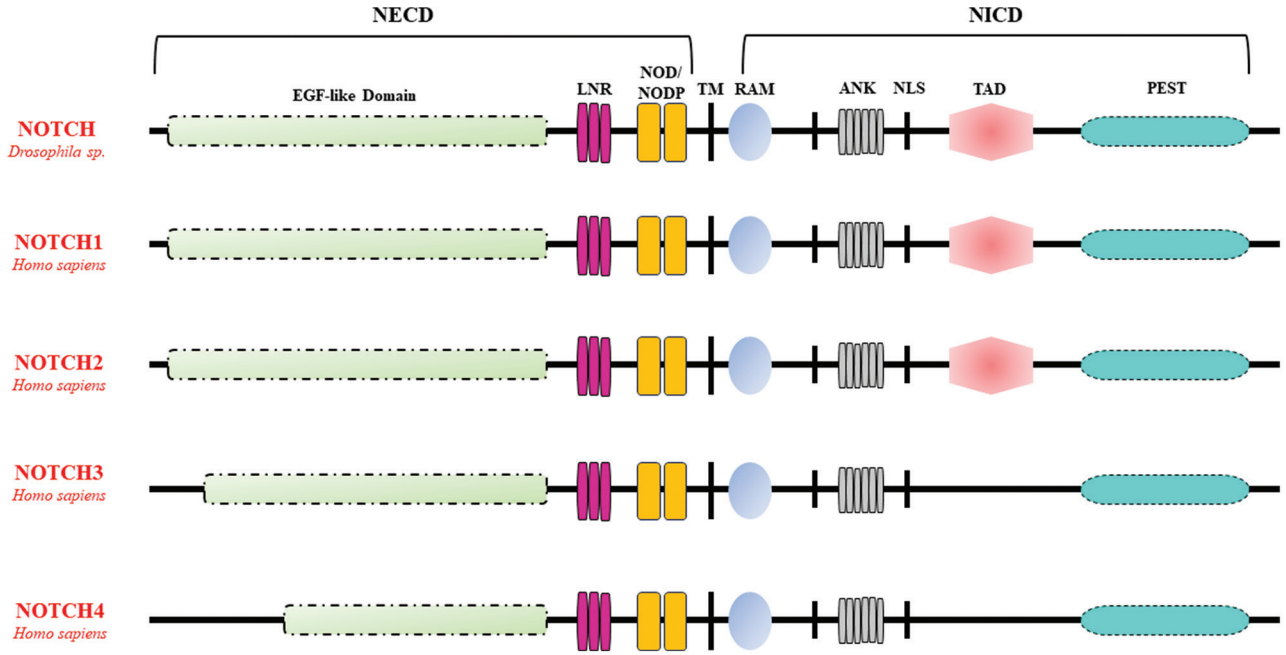

Figure 1. An overview of the structural characteristics of NOTCH family receptors, between *Drosophila melanogaster* and Homo sapiens NOTCH family receptors are depicted with key domains labelled, encompassing the NECD that includes EGF, cysteine-rich LNR repeats and NOTCH domain present in multiple NOTCH proteins (NOD/NODP). NECD was separated from the NICD by the TM. NICD includes RAM domain, ANK, NLS, TAD, and PEST domain. Image created by the authors. Abbreviations: ANK: Ankyrin repeat domain; EGF: Epidermal growth factor; LNR: LIN-12/NOTCH repeat (LNR); NECD: NOTCH extracellular domain; NICD: NOTCH intracellular domain; NLS: Nuclear localization sequences; PEST: Pro-Glu-Ser-Thr; RAM: RBPJκ-associated molecule; TAD: Transcriptional activation domain; TM: Transmembrane domain.

receptor undergoes an initial cleavage, often referred to as S1 cleavage, within the Golgi apparatus. When a NOTCH ligand (JAG or DLL) on the cell membrane of an adjacent signal-sending cell engages with a NOTCH3 receptor on the cell membrane of the signal-receiving cell, it triggers the activation of the NOTCH3 receptor, leading to a subsequent proteolytic cleavage. The processes known as S2 and S3 cleavage are initiated by a disintegrin and metalloprotease domain-containing protein 10 and the γ-secretase complex, respectively, as reported in previous studies.[62,67] Following these cleavages, the NICD is released and translocated into the nucleus, where it forms a complex by binding to the DNA-binding transcription factor CBF-1 (RBPJ)/suppressor of hairless/Lag1 (CSL). This complex then recruits the transcriptional co-activator known as mastermind-like (MAML) protein to stimulate the transcription of downstream target genes. Finally, the NOTCH3 receptor or NICD undergoes degradation in the proteasome or lysosome.[62,67,68]

The existing evidence suggests that the activation of NOTCH3 occurs through the stereotypical ligands containing DSL domains, following the mechanism described above.[69] However, recent findings suggest that NOTCH3 exhibits a significant baseline of ligand-independent signalling, which may be relevant to both its physiological functions and pathological conditions.[70] Although the mechanism remains elusive, it has been reported that the native NRR fold of EGF is comparatively less stable in NOTCH3 than in other studied NOTCH proteins. Whether this ligand-independent process is contingent upon NOTCH3 endocytosis, like the activation mechanisms observed in *Drosophila sp*., remains to be determined. Furthermore, it cannot be ruled out that non-canonical ligands may play a role. For instance, Y-box binding protein 1, a protein related to cold shock domains, has been reported to activate NOTCH3 by binding to EGF-modules 20 – 23, potentially influencing immune and inflammatory responses.[71]

5.1. Roles of NOTCH3 in vasculogenesis

As discussed, NOTCH3 plays a significant part in vascular development, specifically within the lineage responsible for determining the fate of VSMCs. A pre-clinical animal study on post-natal mice lacking NOTCH3 revealed defects in the maturation of smooth muscle cells, arterial differentiation, and morphology when compared to wild-type mice. This resulted in thinner and improperly structured smooth muscle cell layers, which typically

surround arterial vessels.[72] Moreover, *in vitro* studies suggest that the expression of JAG-1 in endothelial cells triggers the activation of NOTCH3 in VSMCs, and this process forms an auto-regulatory loop that sustains the expression of NOTCH3 in VSMCs.[69] In this process, a pivotal downstream effector is the platelet-derived growth factor (PDGF) signalling pathway, and NOTCH3 activation leads to the upregulation of PDGF.[73]

Additional research has demonstrated that NOTCH3 plays earlier roles within the VSMC lineage, although these functions may be concealed by the partial redundancy with other NOTCH homologues. In one animal study on zebrafish, researchers have found that Notch2 and Notch3 cooperate to govern the embryonic production of both mesoderm-derived and neural crest-derived mural cells, which serve as precursors for VSMCs.[74] This was supported by a later study that revealed a comparable redundancy in mice models, as double mutants lacking both NOTCH2 and NOTCH3 are embryonically lethal, and this is associated with a profound reduction in VSMCs and the presence of vascular abnormalities.[75] Nonetheless, additional research employing primary cell cultures has unveiled distinctive roles for NOTCH2 and NOTCH3 within the VSMCs. For example, NOTCH2 exerts an inhibitory effect on VSMC proliferation, whereas NOTCH3 facilitates proliferation and serves as a protective factor against VSMC apoptosis.[76] The functional overlap and interactions between various NOTCH proteins are therefore intricate and greatly context-dependent.

5.2. NOTCH3 and cancer

While the primary role of NOTCH appears to be the regulation of vasculogenesis, where its dysregulation has been associated with various vascular disorders, it can also exhibit tumour-suppressing or oncogenic functions. Numerous studies have demonstrated the common occurrence of aberrantly elevated NOTCH3 expression in human cancer tissues. Table 2 summarises the tumour-suppressive and oncogenic roles of human NOTCH3.

The overexpression of NOTCH3 in cancer tissues is associated with various clinicopathological characteristics, including larger tumour size, advanced tumour-node-metastasis stage, higher pathological grade, tumour metastasis, and an unfavourable prognosis. This is reflected in diminished disease-free survival, progression-free survival, relapse-free survival, and overall survival among cancer patients.[100,101] The predominant cause for NOTCH3 overexpression in cancer is genetic alterations within the *NOTCH3* gene. According to the Cancer Genome Atlas, the *NOTCH3* gene was modified in 5% of cancer samples, primarily through mutations and gene amplification.[102] Moreover, one study reported that mutations in the NRR and PEST domains of the *NOTCH3* gene could lead to NOTCH3 activation, characterised as gain-of-function or activating mutations. These mutations have been observed in human T-ALL.[103]

An important function of NOTCH3 is the preservation of cancer stem cell (CSC) stemness. CSCs, a subset of self-renewing cells possessing strong tumourigenic potential, are known to be stimulated by NOTCH3 signalling in various cancer types, thereby contributing to cancer progression through intricate mechanisms. Another significant aspect of NOTCH3 signalling is its ability to promote resistance to multiple types of chemotherapeutic drugs, such as platinum agents, doxorubicin, epidermal growth factor receptor (EGFR)–tyrosine kinase inhibitors, taxanes, and gemcitabine. It's important to highlight that the support of CSC activity by NOTCH3 is also implicated in the mechanisms of tumour chemo-resistance, angiogenesis, and metastasis.[36,104,105] These underscore the pivotal role of NOTCH3 signalling in cancer.

6. Roles of NOTCH3 in BC

In the context of BC, NOTCH3 typically functions as an oncogene, with a few exceptions. Notably, NOTCH3 has been shown to induce the development of mammary tumours in transgenic mice.[6] Among BC cell lines, NOTCH3 signalling remains persistently active, and when compared to other NOTCH receptors, its activity alone is adequate to drive tumour growth both *in vitro* and *in vivo*.[7] Recently, there has been an acknowledgement of the role of juxtacrine NOTCH signalling between tumour cells and distinct cell types within the tumour microenvironment (TME).[106]

The communication between cancer cells and the TME, which includes both juxtacrine and paracrine signalling, holds significance in the development of novel targeted therapies for BC progression and addressing drug resistance.[107] Certainly, the activation of NOTCH3 in tumour cells stimulates the release of various soluble factors that can exert paracrine effects on cells within the TME.[108] These effects extend to immune and stromal cells, including cancer-associated fibroblasts (CAFs) and endothelial cells. However, it's important to highlight that CAFs can also enhance NOTCH3 signalling and contribute to resistance by secreting multiple pro-inflammatory cytokines and chemokines.[109,110] Indeed, Studebaker *et al.*[111] observed that BC CAFs activate NOTCH3 through the secretion of IL-6.

Apart from that, a previous study by Yamaguchi *et al.*[112] highlighted the significance of NOTCH3 signalling when compared to other NOTCH family members. They revealed that reducing NOTCH3

Table 2. Tumor-suppressive and oncogenic roles of human NOTCH3

Type of cancer	Clinicopathological features
Breast cancer	• In TNBC, NOTCH3 amplifications are more frequently observed than mutations.[77] • In TNBC, mutations have been observed within the PEST domain, which plays a role in negatively regulating gene expression.[78] • NOTCH3 is linked to increased invasiveness and a higher rate of metastasis in breast cancer.[79] • NOTCH3 is connected to the development of chemoresistance.[80] • NOTCH3 can enhance chemo-sensitivity in doxorubicin-resistant breast cancer.[80]
Colorectal cancer	• NOTCH3 is frequently found to be expressed at markedly elevated levels compared to its expression in normal tissue.[81] • NOTCH3 is linked to the invasiveness and metastasis of the tumour.[82] • NOTCH3 is connected to chemo-resistance and leads to poorer clinical outcomes.[83]
Haemangioma	• The expression of NOTCH3 is increased in stem cells of infantile hemangioma.[84]
Hepatocellular carcinoma	• NOTCH3 is linked to the invasiveness and metastasis of the tumour.[85] • NOTCH3 expression is markedly elevated (especially in larger tumours) compared to its expression in normal tissue.[86]
Nasopharyngeal carcinoma	• Knocking down the expression of NOTCH3 in nasopharyngeal carcinoma enhanced the sensitivity to cisplatin chemotherapy.[87]
Non-small cell lung cancer	• 40% of non-small cell lung cancers had NOTCH3 overexpression.[88] • NOTCH3 suppressed apoptosis and reduced cell proliferation.[88] • NOTCH3 is linked to the invasiveness and metastasis of the tumour.[89] • NOTCH3 is connected to radiotherapy resistance, and chemo-resistance and leads to poorer clinical outcomes.[90]
Ovarian cancer	• The expression NOTCH3 is notably elevated in ovarian cancer when compared to normal ovaries or benign ovarian tumours.[91] • The expression of NOTCH3 is linked to a higher tumour grade, lymph node, and distant metastasis, as well as a more advanced clinical stage.[91] • NOTCH3 overexpression is linked to tumour recurrence and a higher mortality rate.[92] • Stimulating the NOTCH3 intracellular domain resulted in the accumulation of p27kip1, causing cancer cells to arrest in the G0/G1 phase of the cell cycle.[93]
Prostatic adenocarcinoma	• The expression of NOTCH3 is positively associated with the Gleason score, and overexpression of NOTCH3 is observed in prostate cancers with a high risk for metastasis.[94,95] • NOTCH3 is connected to chemo-resistance.[95]
Squamous cell cancer	• The expression of NOTCH3 is notably elevated in comparison with normal cells.[96] • NOTCH3 is linked to the aggressiveness of oesophageal squamous cell carcinoma and resistance to 5-fluorouracil chemotherapy.[97]
T-cell acute lymphoblastic leukaemia (T-ALL)	• The activation of NOTCH3 signalling is connected to cancer cell survival and proliferation.[98] • Knockdown of NOTCH3 resulted in reduced MKP-1 levels, ultimately causing decreased tumourigenicity and an increase in apoptosis in T-ALL.[99]

Abbreviations: TNBC: Triple-negative breast cancer; MKP-1: Mitogen-activated protein kinase phosphatase 1; T-ALL: T-cell acute lymphoblastic leukaemia.

expression significantly inhibited growth and stimulated apoptosis in receptor tyrosine-protein kinase (ErbB2)-negative tumour cell lines. Interestingly, this effect was not seen in ErbB2-positive tumour cells. Conversely, silencing NOTCH1 using small interfering (siRNA) did not hinder the proliferation of either ErbB2-positive or ErbB2-negative cell lines. Nonetheless, one study demonstrated that CAFs can produce substantial amounts of the chemokine (C-C motif) ligand 2 (CCL-2).[109] This chemokine, in turn, can regulate the CSC phenotype and influence NOTCH1 expression in BC cells. Similarly, in a xenograft model where fibroblasts and BC cells were co-transplanted into NOD/SCID/IL-2Rg-null mice, the elimination of CCL-2 significantly reduced tumourigenesis and NOTCH1 expression. This suggests that CAFs can engage in crosstalk with cancer cells through a CCL-2/NOTCH1 axis.[113]

In addition, NOTCH3 activation has been observed in a human xenograft model of inflammatory BC through various methods, including real-time quantitative reverse transcription polymerase chain reaction, western blotting, and immunohistochemistry.[114] In triple-negative BC (TNBC), NOTCH3 aberrations, specifically the amplifications of NOTCH3, are more frequently observed compared to mutations. When mutations do occur, they tend to affect the PEST domain, which is generally associated with the negative regulation of gene expression.[77] Moreover, among the TNBC samples, 34% of the tumours tested positive for the NOTCH3 intracellular domain (NICD3), whereas only 4% were positive for the NOTCH1

intracellular domain (NICD1).[7] Nonetheless, the exact role of NOTCH3 in BC remains unclear. A study conducted by Chen *et al.*[93] proposed that the activation of the NICD3 resulted in the accumulation of p27kip1, leading to the arrest of cancer cell cycles in the G0/G1 phase.

Another extensively recognised immune component of BC is the presence of tumour-associated macrophages (TAMs). One study indicated that the JAG-mediated NOTCH signalling in BC has been linked to the differentiation of macrophages into TAMs within the TME in luminal BC patient samples.[115] When tumour cells and TAMs are co-cultured, it leads to the differentiation of macrophages into a predominant M2-type phenotype. While TAMs can exhibit both inflammatory and immunosuppressive behaviours, in BC patient samples they are primarily linked to the immunosuppressive M2 subtype. This association is largely due to the elevated secretion of macrophage colony-stimulating factors by tumour cells, which directs macrophages toward the M2 phenotype.[116] On the other hand, in the basal subtype of BC, NOTCH3 signalling through JAG-1 in tumour cells triggers the release of crucial macrophage-activating cytokines, including IL-β and CCL-2. These cytokines facilitate the recruitment of M2-type macrophages, which subsequently secrete transforming growth factor-β (TGF-β), thus activating TGF-β signalling within the tumour cells.[108] The interplay between tumour cells and TAMs mediated by NOTCH3 signalling could also be of significance in the development of drug resistance, although additional investigation is needed.[108]

The involvement of NOTCH3 in the epidermal-to-mesenchymal transition (EMT) in BC remains a topic of debate. Some researchers have indicated that NOTCH3 encourages tumour aggressiveness by instigating EMT, while others have shown evidence that NOTCH3 inhibits EMT, for instance, by upregulating the Hippo/Yap pathway.[7] It has been reported that EMT inhibition can also occur through the reduction of Fos-related antigen 1 (Fra1), an activator of the EMT process. This observation was made in human BC cells that developed resistance to Adriamycin, where Fra1 was expressed at elevated levels whilst NOTCH3 was downregulated.[80] Conversely, within metastatic BC cells, a reduction in NOTCH3 levels resulted in reduced levels of EMT-associated proteins, such as vimentin, fibronectin and Snail.

Previous study on animal models of allergic airway inflammation have shown that regulatory T-cells (Tregs), another prominent immune component associated with immune suppression, are elevated by both JAG-1 and JAG-2 through the involvement of mesenchymal stem cells.[117] Both JAG-1 and JAG-2 exhibit elevated expression levels in TNBC.[118] Given that Tregs play a role in evading immune surveillance and are associated with increased tumour invasiveness and unfavourable prognoses, the disruption of Tregs' function mediated by NOTCH3 signalling could significantly impact on BC therapy. Nonetheless, no study has directly examined NOTCH3 signalling within Tregs in the context of BC. Therefore, it is imperative to gain a better understanding of how different NOTCH3 ligands and receptors regulate the recruitment of Tregs. Intriguingly, NOTCH3 signalling can also hinder the function of Tregs, potentially diminishing their capacity for immunosuppression.[119]

6.1. NOTCH3 and BC metastasis and chemo-sensitivity

In terms of BC metastasis, NOTCH3 expression is notably higher in invasive cancer when compared to ductal carcinoma *in situ*.[78] In addition, the truncated isoform of the Fms-related receptor tyrosine kinase 1 has been linked to enhanced BC invasiveness and was found to be upregulated by NOTCH3.[120] Lymphovascular emboli in human inflammatory BC have shown elevated levels of NOTCH3 expression. When normal breast epithelial cells were transfected with NICD3, spheroid formation was observed, a phenomenon that was not seen after NICD1 transfection.[8] An exclusive elevation in NOTCH3 expression was also observed in human BC cell lines following exposure to TGF-β1 released by bone marrow osteoblasts. The formation of cancer cell colonies was impeded upon treatment with an anti-TGF-β1 antibody and chemical inhibition of NOTCH3. Furthermore, the expression of NOTCH3 was linked to the presence of osteolytic bone lesions.[121]

Another mechanism by which NOTCH3 can enhance the aggressiveness of BC is through its role in cellular metabolism. NOTCH3 activity is crucial for the survival of metastatic BC cells resistant to hormone therapy. Furthermore, it has been demonstrated to boost mitochondrial activity and facilitate the transition out of metabolic dormancy. In tamoxifen-resistant cells, mitochondrial DNA copy number, mitochondrial antigen expression, and oxidative phosphorylation were all reduced. The decline in mitochondrial activity was reversed through IL-6 treatment, which was orchestrated by NOTCH3.[79] The critical function of NOTCH3 in reversing the mitochondrial damage induced by tamoxifen was substantiated when IL-6 treatment proved ineffective in enhancing mitochondrial activity in cells with suppressed NOTCH3.[79] Multiple studies have supported that in metastatic BC, the presence of functional NOTCH3 is indispensable for IL-6 to sustain elevated levels of carbonic anhydrase, an enzyme linked to survival in hypoxic conditions and increased invasiveness in cancer.[122]

NOTCH3 can enhance chemo-sensitivity in doxorubicin-resistant BC by negatively regulating Fra1, a critical factor in the EMT of BC cell lines.[80,123] In doxorubicin-resistant BC cells, Fra1 was notably over-expressed while NOTCH3 was under-expressed. This resistance was attributed to the EMT induced by Fra1. It was possible to induce Adriamycin chemo-resistance in initially chemo-sensitive cells by suppressing NOTCH3 signalling.[80] Apart from that, the stromal cells can also further enhance chemo-resistance by stimulating the expression of NOTCH3 in BC cells, an effect that can be counteracted with NOTCH3 siRNA or a γ-secretase inhibitor (GSI).[110] Another mechanism by which cancer cells resist treatment is through a state of tumour dormancy. Activation of the NOTCH3 pathway induces cell cycle arrest at the G0/G1 phase and promotes the expression of DNp63a, Mix1 and Hes1 proteins, which encourage cellular quiescence.[124]

Given the constrained effectiveness of immunotherapy directed solely at tumour cells in BC, it becomes crucial to comprehend the regulatory function of NOTCH3 signalling in the interactions between cancer cells and immune cells within the TME across various subtypes of BC. This understanding can facilitate the identification of more effective drug targets involving NOTCH3 signalling and immune cells.

7. NOTCH3 and the brain

A previous study reported that adult mice with NOTCH3 deficiency exhibit ongoing progressive symptoms in the vasculature of the brain and retina, stemming from VSMC degeneration and loss through apoptosis.[125] This leads to a breakdown in vessel integrity, causing haemorrhaging and impairing the functionality of the blood–brain barrier (BBB). Furthermore, the expression of NOTCH3 in VSMCs contributes to the regulation of vascular tone and flow-mediated dilation in both cerebral and tail resistance arteries in mice.[125] Moreover, the functions of NOTCH3 may extend beyond vascular roles, whereby recent research has reported its involvement in neuronal stem cells and neuronal differentiation.[126]

The subependymal zone serves as a crucial stem cell niche within the adult mammalian brain, housing both active and dormant stem cell populations. There is a distinct functional difference between NOTCH1 and NOTCH3 in the regulation of these populations. In mouse model studies, NOTCH1 is expressed in active stem cells, stimulating their proliferation. On the other hand, NOTCH3 is primarily expressed in quiescent stem cells, and its role is essential for maintaining these cells by inhibiting their proliferation.[127] Apart from that, NOTCH3 participates in the regulation of satellite cells, which are stem cells responsible for the repair of skeletal muscle. A recent study demonstrated that mice lacking NOTCH3 exhibited significantly greater muscle growth than their wild-type counterparts following repeated injuries, which was attributed to the increased proliferation of activated satellite cells.[128] In contrast, NOTCH1 has been associated with promoting satellite cell activation and proliferation.[129]

7.1. *NOTCH3* gene aberration and risk for ischemic stroke

The exact pathophysiological mechanisms responsible for ischemic stroke and its various subtypes remain incompletely understood. A combination of genetic and environmental factors probably contributes to the onset and progression of the condition.[130] The *NOTCH3* gene is in the 19p13.12 region, spanning from 15,159,633 base pairs to 15,200,981 base pairs. It comprises 33 exons and 32 introns and encodes a protein consisting of 2,321 amino acids, which plays a critical role in neuronal development.[131] The *NOTCH3* gene variants, rs1044009 (g.45022C > T, c.6668C > T, p. Ala2223Val), and rs3815188 (g.13568C > T, c.303C > T, p. Thr101=), correspond to a missense mutation and a synonymous variant, respectively. Both polymorphisms are situated within the intracellular DUF3454 domain of the protein, and they have a direct impact on the signal transduction activity of NOTCH3. However, *NOTCH3* rs1043994 (g.13949A > G, c.606A > G, p. Ala202=) is a benign point mutation or synonymous variant situated in the conserved protein family of the calcium-binding endothelial growth factor (EGF)-like domain within the NOTCH3 protein.[132]

Certain mutations in the *NOTCH3* gene have been documented as a direct cause of CADASIL, an autosomal dominant disorder affecting cerebral small penetrating vessels. It presents typical clinical symptoms such as migraine with aura, recurrent ischemic strokes in early to middle adulthood, apathy, neuropsychological symptoms and cognitive impairment that advances to dementia.[132] The details on *NOTCH3* gene aberration and its roles in ischemic strokes and CSVD will be discussed in the later section.

7.2. NOTCH3 and inherited cause of CSVD

However, as far back as the 1970s, literature documented families who, despite lacking typical vascular risk factors, exhibited a heightened susceptibility to CSVD. Studies reported cases of families in which multiple-infarct dementia afflicted individuals in a manner consistent with autosomal-dominant inheritance.[133,134] The prevalence of deep white-matter lesions in these individuals indicated that they were likely influenced by a genetic factor causing

severe CSVD. Indeed, examinations of tissues samples from members of these families revealed distinct brain pathology, characterised by degenerative alterations in the walls of cerebral arteries.[135] Therefore, it became evident that while most cases of CSVD are sporadic or involve multiple genes, a certain portion is probably attributed to monogenic genetic variations.

Subsequently, an acronym was coined for the most prevalent monogenic autosomal-dominant form of CSVD, which is CADASIL. An analysis of numerous extensive and well-documented CADASIL families established a genetic linkage to a single disease locus on chromosome 19q12.[136] The defining pathological feature of CADASIL is the existence of granular osmophilic material (GOM) within the basement membrane of VSMCs. Common clinical manifestations in CADASIL patients encompass migraine with aura, transient ischemic attacks or ischemic strokes, intracranial haemorrhage, cognitive deficits, and psychiatric disturbances. Patients may exhibit one or more of these symptoms. Neuroimaging, typically MRI, frequently reveals WMHs, lacune infarcts and cerebral microbleeds.[137] Anomalies in periventricular regions appear as initial findings on fluid-attenuated inversion recovery and T2-weighted imaging. Over time, these anomalies spread symmetrically to affect other regions, including distinctive areas, such as the anterior temporal pole and the external capsule.[138]

The *NOTCH3* gene encodes a single-pass transmembrane NOTCH3 receptor. It has been pinpointed as the causative gene for CADASIL.[139] Exons 2 – 24 of the *NOTCH3* gene encode 34 epidermal growth factor-like repeats (EGFRs) within the extracellular domain of the NOTCH3 protein.[140] Each EGFR contains six cysteine residues that form three disulphide bonds, contributing to the receptor's structural stability. However, mutations that alter in the number of cysteine residues, changing from an even to an odd number, result in a structural change in the extracellular domain of the NOTCH3 receptor. This structural change leads to misfolding and the aggregation of the extracellular domain. Consequently, this misfolding and aggregation can give rise to the formation of GOM deposits, which are a distinctive characteristic of CADASIL. Furthermore, this process can also lead to the deterioration of VSMCs.[141]

CADASIL is a rare condition, and its estimated occurrence in the general population ranges from 1.98 to 4.6 cases/100,000.[142] Nonetheless, recent research indicates that *NOTCH3* gene mutations, which alter cysteine, are considerably more common than previously documented.[142] As an illustration, utilising exome databases that are publicly accessible, recent investigations have approximated the prevalence of *NOTCH3* gene mutations that modify cysteine to be between 2.2 and 3.4 cases/1000 individuals. This prevalence is nearly 100 times higher than the earlier estimates available for CADASIL.[143] The evident disparity in the estimates of disease and mutation occurrence lacks a comprehensive understanding but probably stems from variations in the extent of penetrance or the severity linked to these genetic mutations. This explanation aligns with evidence suggesting that not all individuals carrying *NOTCH3* gene mutations, whether altering cysteine or sparing cysteine, or those with legitimate NOTCH3 loss-of-function mutations, exhibit symptoms.[143] Hence, some individuals can be asymptomatic, which reflects the typical nature of CSVD.

7.3. Impact of aberrant NOTCH3 signalling in CADASIL and NOTCH3-related CSVD

The pathophysiological mechanisms underlying CADASIL involve NOTCH3 receptor activation by its ligands, leading to proteolytic cleavages and release of the NICD. The NICD then translocate to the nucleus, where it influences gene transcription and cellular processes. However, in CADASIL, the aberrant receptor structure disrupts this signalling cascade, promoting VSMC apoptosis and impairing vascular function.[144]

Moreover, recent findings highlight a significant baseline of ligand-independent NOTCH3 signalling, which could be relevant in both normal and pathological contexts.[145] This signalling variability underscores the complexity of NOTCH3-related diseases and suggests potential targets for therapeutic intervention. Understanding the precise molecular mechanisms and cellular effects of NOTCH3 mutations is essential for developing targeted treatments for CADASIL and NOTCH3-related CSVD.

In conclusion, aberrant NOTCH3 signalling plays a critical role in the pathogenesis of CADASIL and NOTCH3-related CSVD. Advances in genetic and molecular research continue to unravel the complexities of this signalling pathway, offering hope for novel therapeutic strategies aimed at mitigating the clinical burden of these debilitating conditions.

8. NOTCH3 as a potential link between the BC and CSVD

The interplay between BC and CSVD through aberrant NOTCH3 signalling presents a compelling narrative that underscores the intricate pathophysiological mechanisms linking these two seemingly distinct conditions. This manuscript has delineated the critical role of NOTCH3 in both oncogenesis and vasculogenesis, particularly highlighting its involvement in CADASIL and BC

metastasis. Figure 2 summarises the roles of NOTCH3 as a potential molecular link between BC and CSVD (i.e., CADASIL).

Recent experimental studies have further elucidated the dual role of NOTCH3 signalling in both cerebrovascular and oncogenic contexts. In CSVD, particularly in CADASIL models, aberrant NOTCH3 activity has been shown to induce VSMC degeneration, arterial wall remodelling deficits, and compromised BBB integrity through increased apoptotic signalling pathways.[72,125] These pathophysiological processes contribute to the hallmark features of CSVD, including WMHs and microvascular ischemia.

Moreover, mutations in the *NOTCH3* gene lead to the misfolding and aggregation of the receptor, resulting

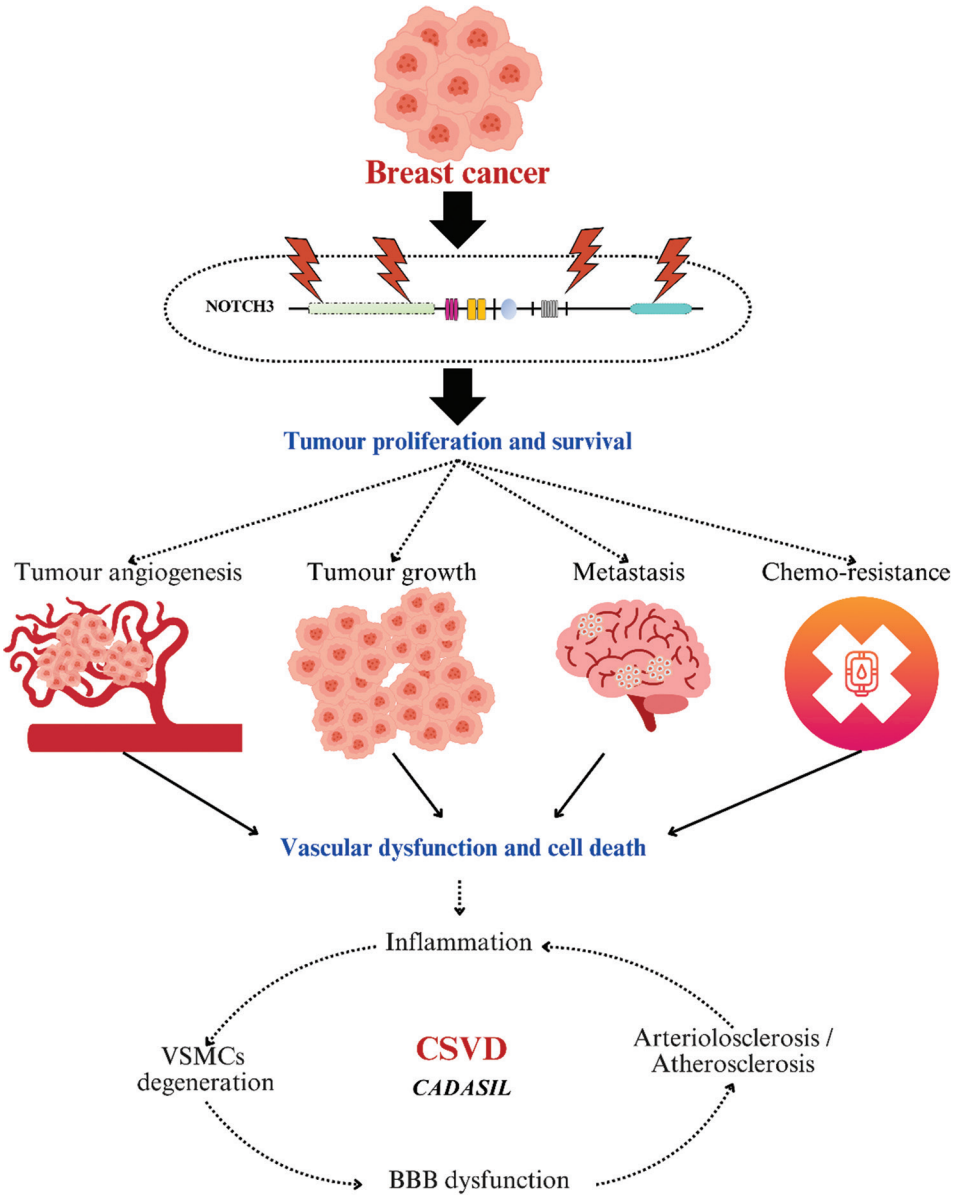

Figure 2. Schematic illustration of the central role of NOTCH3 in linking BC and CSVD. In BC, NOTCH3 activation promotes tumor growth, metastasis, and chemo-resistance, as depicted by tumor cells, the microenvironment, and blood vessels. In CSVD, *NOTCH3* gene mutations lead to VSMC degeneration, endothelial dysfunction, and ischemic stroke. A potential targeted therapy against NOTCH3 aberration may serve as a dual strategy to potentially mitigate tumor progression in BC and prevent vascular damage in CSVD. Image created by the authors. Abbreviations: BC: Breast cancer; CSVD: Cerebral small vessel disease; VSMC: Vascular smooth muscle cell; BBB: Blood–brain barrier; CADASIL: Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy.

in the degeneration of VSMCs and the formation of GOM, a hallmark of CADASIL. These pathophysiological changes disrupt vascular integrity, contributing to the clinical manifestations of CADASIL, including migraines, recurrent strokes, cognitive decline and psychiatric disturbances.

In BC, NOTCH3 signalling has been implicated in tumour growth, metastasis and chemo-resistance. Notably, studies have demonstrated that IL-6-induced NOTCH3 activation promotes mitochondrial recovery in tamoxifen-resistant BC cells, enabling the transition out of metabolic dormancy and enhancing survival under hypoxic stress.[79] Elevated NOTCH3 expression has been associated with increased invasiveness and higher metastatic potential, particularly in TNBC.[77] Furthermore, NOTCH3 plays a critical role in maintaining CSC properties, facilitating EMT, and remodelling the TME through its interaction with TAMs and cytokines such as IL-6 and TGF-β.[108,115] The convergence of these pathomechanisms suggests a shared pathway that may underline both CSVD and BC. This dual role of NOTCH3 in vascular and oncogenic processes opens new avenues for therapeutic interventions targeting this signalling pathway. By inhibiting aberrant NOTCH3 activity, it may be possible to mitigate the vascular damage seen in CADASIL and suppress tumour progression and metastasis in BC.

Future research should prioritise elucidating the molecular mechanisms of ligand-independent NOTCH3 signalling, which remains poorly understood but is increasingly recognised as relevant in both normal physiology and disease states. Studies investigating the structural instability of NOTCH3's NRR and its role in spontaneous receptor activation could uncover novel regulatory checkpoints. Furthermore, the role of NOTCH3 within the TME, particularly its crosstalk with CAFs, TAMs and Tregs, warrants further exploration to identify immunomodulatory functions that contribute to tumour aggressiveness and resistance.

Therapeutically, targeting NOTCH3 offers a promising dual-action approach. Strategies under development include GSIs, which block NOTCH receptor cleavage and downstream signalling,[146] monoclonal antibodies specific to NOTCH3 or its ligands, such as DLL4 and JAG1, and selective NOTCH3 inhibitors. These agents may be used alone or in combination with conventional therapies to address vascular degeneration in CSVD and metastatic progression in BC. Pre-clinical models and early-phase trials should focus on optimising dosage, minimising off-target effects, and assessing tissue-specific responses. Ultimately, advancing these research directions could lead to integrated therapeutic strategies that transcend traditional disease boundaries and significantly improve outcomes for patients affected by both CSVD and BC. Despite the promising role of NOTCH3 as a therapeutic target, several challenges must be addressed to enable clinical translation. One major concern is the off-target effects, particularly when using non-selective agents, such as GSIs, which affect multiple NOTCH receptors and may lead to gastrointestinal and immunological side effects.[147] Furthermore, tissue-specific variability in NOTCH3 expression complicates the prediction of therapeutic responses and raises the risk of unintended impacts on normal vascular or immune function. Ensuring selectivity and safety in targeting NOTCH3, especially in the context of long-term treatment, is critical and warrants the development of more refined, receptor-specific strategies and delivery systems. In summary, the exploration of NOTCH3 signalling as a common link between CADASIL and BC underscores the importance of integrated research approaches that consider the multifaceted roles of key molecular players in disease pathogenesis. This comprehensive understanding could lead to innovative strategies for prevention, diagnosis, and treatment, ultimately benefiting patients affected by these debilitating conditions.

9. Conclusion

The intricate role of NOTCH3 in both CSVD and BC underscores its significance as a shared molecular driver of vascular degeneration and tumour progression. This review highlights how aberrant NOTCH3 signalling contributes to VSMC dysfunction and white matter damage in CSVD, while simultaneously promoting proliferation, chemo-resistance, and immune evasion in BC. These insights position NOTCH3 as a promising dual-purpose therapeutic target with potential benefits across neurovascular and oncologic domains. However, this synthesis also reveals several limitations and gaps in present knowledge. Notably, direct mechanistic studies that experimentally link NOTCH3 signalling between CSVD and BC are limited. The biological relevance of ligand-independent NOTCH3 activation remains incompletely understood, as does the precise role of NOTCH3 in mediating immune-tumour interactions. Furthermore, while pre-clinical models suggest promising avenues for targeted therapies, the clinical translation of NOTCH3 inhibitors requires caution due to potential off-target effects and tissue-specific variability. In conclusion, the convergence of NOTCH3 signalling in CSVD and BC represents both a scientific opportunity and a clinical challenge. Future studies should prioritise mechanistic elucidation, therapeutic refinement and cross-disciplinary collaboration to fully unlock the translational potential of targeting this signalling axis.

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References

1. Kim K, Lee JH. Risk factors and biomarkers of ischemic stroke in cancer patients. *J Stroke*. 2014;16(2):91-96.

   doi: 10.5853/jos.2014.16.2.91

2. Quintas S, Rogado J, Gullón P, *et al*. Predictors of unknown cancer in patients with ischemic stroke. *J Neurooncol*. 2018;137(3):551-557.

   doi: 10.1007/s11060-017-2741-0

3. Muiño E, Gallego-Fabrega C, Cullell N, *et al*. Systematic review of cysteine-sparing NOTCH3 missense mutations in patients with clinical suspicion of CADASIL. *Int J Mol Sci*. 2017;18:1964.

   doi: 10.3390/ijms18091964

4. Dupré N, Drieu A, Joutel A. Pathophysiology of cerebral small vessel disease: A journey through recent discoveries. *J Clin Invest*. 2024;134(10):e172841.

   doi: 10.1172/JCI172841

5. Che Mohd Nassir CMN, Mohamad Ghazali M, Ahmad Safri A, *et al*. Elevated circulating microparticle subpopulations in incidental cerebral white matter hyperintensities: A multimodal study. *Brain Sci*. 2021;11:133.

   doi: 10.3390/brainsci11020133

6. Hu C, Dievart A, Lupien M, Calvo E, Tremblay G, Jolicoeur P. Overexpression of activated murine Notch1 and Notch3 in transgenic mice blocks mammary gland development and induces mammary tumors. *Am J Pathol*. 2006;168:973-990.

   doi: 10.2353/ajpath.2006.050416

7. Choy L, Hagenbeek TJ, Solon M, *et al*. Constitutive NOTCH3 signalling promotes the growth of basal breast cancers. *Cancer Res*. 2017;77(6):1439-1452.

   doi: 10.1158/0008-5472.CAN-16-1022

8. Xiao Y, Ye Y, Zou X, *et al*. The lymphovascular embolus of inflammatory breast cancer exhibits a Notch 3 addiction. *Oncogene*. 2011;30(3):287-300.

   doi: 10.1038/onc.2010.405

9. Feng Y, Spezia M, Huang S, *et al*. Breast cancer development and progression: Risk factors, cancer stem cells, signalling pathways, genomics, and molecular pathogenesis. *Genes Dis*. 2018;5(2):77-106.

   doi: 10.1016/j.gendis.2018.05.001

10. Henry NL, Shah PD, Haider I, Freer PE, Jagsi R, Sabel MS. Cancer of the breast. In: Niederhuber JE, Armitage JO, Doroshow JH, Kastan MB, Tepper JE, editors. *Abeloff's Clinical Oncology*. 6th ed. Philadelphia, PA: Elsevier; 2020.

11. Jagsi R, King TA, Lehman C, Morrow M, Harris JR, Burstein HJ. Malignant tumors of the breast. In: DeVita VT, Lawrence TS, Rosenberg SA, editors. *DeVita, Hellman, and Rosenberg's Cancer: Principles and Practice of Oncology*. 11th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2019.

12. Saunders C, Jassal S. *Breast Cancer*. 1st ed., Ch. 13. Oxford: Oxford University Press; 2009.

13. Fakhri N, Chad MA, Lahkim M, *et al*. Risk factors for breast cancer in women: An update review. *Med Oncol*. 2022;39(12):197.

   doi: 10.1007/s12032-022-01804-x

14. Torre LA, Siegel RL, Ward EM, Jemal A. Global cancer incidence and mortality rates and trends-an update. *Cancer Epidemiol Biomark Prev*. 2016;25(1):16-27.

   doi: 10.1158/1055-9965.EPI-15-0578

15. *World Health Organization (WHO). Breast Cancer*; 2023. Available from: https://www.who.int/news-room/fact-sheets/detail/breast-cancer [Last accessed on 2025 May 19.

16. Sharma R. Breast cancer incidence, mortality and mortality-to-incidence ratio (MIR) are associated with human development, 1990-2016: Evidence from Global Burden of Disease Study 2016. *Breast Cancer*. 2019;26:428-445.

doi: 10.1007/s12282-018-00941-4

17. Ferlay J, Laversanne M, Ervik M, *et al*. *Global Cancer Observatory: Cancer Tomorrow*. Lyon, France: International Agency for Research on Cancer; 2020. Available from: https://gco.iarc.fr/tomorrow [Last accessed on 2024 Dec 01].
18. Porter P. Westernizing women's risks? Breast cancer in lower-income countries. *N Engl J Med*. 2008;358:213-216.

    doi: 10.1056/NEJMp0708307
19. McKee MJ, Keith K, Deal AM, *et al*. A multidisciplinary breast cancer brain metastases clinic: The University of North Carolina experience. *Oncologist*. 2016;21(1):16-20.

    doi: 10.1634/theoncologist.2015-0328
20. Shiovitz S, Korde LA. Genetics of breast cancer: A topic in evolution. *Ann Oncol*. 2015;26:1291-1299.

    doi: 10.1093/annonc/mdv022
21. Shahbandi A, Nguyen HD, Jackson JG. TP53 mutations and outcomes in breast cancer: Reading beyond the headlines. *Trends Cancer*. 2020;6:98-110.

    doi: 10.1016/j.trecan.2020.01.007
22. Corso G, Veronesi P, Sacchini V, Galimberti V. Prognosis and outcome in CDH1-mutant lobular breast cancer. *Eur J Cancer Prev*. 2018;27(3):237-238.

    doi: 10.1097/CEJ.0000000000000405
23. Kechagioglou P, Papi RM, Provatopoulou X, *et al*. Tumor suppressor PTEN in breast cancer: Heterozygosity, mutations and protein expression. *Anticancer Res*. 2014;34(3):1387-1400.
24. Chen J, Lindblom A. Germline mutation screening of the STK11/LKB1 gene in familial breast cancer with LOH on 19p. *Clin Genet*. 2000;57(5):394-397.

    doi: 10.1034/j.1399-0004.2000.570511.x
25. Rahman N, Seal S, Thompson D, *et al*. PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. *Nat Genet*. 2007;39(2):165-167.

    doi: 10.1038/ng1959
26. Stucci LS, Internò V, Tucci M, *et al*. The ATM gene in breast cancer: Its relevance in clinical practice. *Genes (Basel)*. 2021;12(5):727.

    doi: 10.3390/genes12050727
27. Gorodetska I, Kozeretska I, Dubrovska A. BRCA genes: The role in genome stability, cancer stemness and therapy resistance. *J Cancer*. 2019;10(9):2109-2127.

    doi: 10.7150/jca.30410
28. Qu Y, Qin S, Yang Z, *et al*. Targeting the DNA repair pathway for breast cancer therapy: Beyond the molecular subtypes. *Biomed Pharmacother*. 2023;169:115877.

    doi: 10.1016/j.biopha.2023.115877
29. Khan U, Khan MS. Prognostic value estimation of BRIP1 in breast cancer by exploiting transcriptomics data through bioinformatics approaches. *Bioinform Biol Insights*. 2021;15:11779322211055892.

    doi: 10.1177/11779322211055892
30. Gamble LA, McClelland PH, Teke ME, *et al*. Defining features of hereditary lobular breast cancer due to CDH1 with magnetic resonance imaging and tumor characteristics. *NPJ Breast Cancer*. 2023;9(1):77.

    doi: 10.1038/s41523-023-00585-4
31. Rainville I, Hatcher S, Rosenthal E, *et al*. High risk of breast cancer in women with biallelic pathogenic variants in CHEK2. *Breast Cancer Res Treat*. 2020;180(2):503-509.

    doi: 10.1007/s10549-020-05543-3
32. Hu ZY, Liu L, Xie N, *et al*. Germline PALB2 mutations in cancers and its distinction from somatic PALB2 mutations in breast cancers. *Front Genet*. 2020;11:829.

    doi: 10.3389/fgene.2020.00829
33. Fusco N, Sajjadi E, Venetis K, *et al*. PTEN alterations and their role in cancer management: Are we making headway on precision medicine? *Genes (Basel)*. 2020;11(7):719.

    doi: 10.3390/genes11070719
34. Angeli D, Salvi S, Tedaldi G. Genetic predisposition to breast and ovarian cancers: How many and which genes to test? *Int J Mol Sci*. 2020;21(3):1128.

    doi: 10.3390/ijms21031128
35. Børresen-Dale AL. TP53 and breast cancer. *Hum Mutat*. 2003;21:292-300.

    doi: 10.1002/humu.10174
36. Leontovich AA, Jalalirad M, Salisbury JL, *et al*. NOTCH3 expression is linked to breast cancer seeding and distant metastasis. *Breast Cancer Res*. 2018;20(1):105.

    doi: 10.1186/s13058-018-1020-0
37. Che Mohd Nassir CMN, Damodaran T, Yusof SR, *et al*. Aberrant neurogliovascular unit dynamics in cerebral small vessel disease: A rheological clue to vascular parkinsonism. *Pharmaceutics*. 2021;13(8):1207.

    doi: 10.3390/pharmaceutics13081207
38. Wardlaw JM, Smith C, Dichgans M. Small vessel disease: Mechanisms and clinical implications. *Lancet Neurol*. 2019;18:684-696.

    doi: 10.1016/S1474-4422(19)30079-1
39. Smith EE. Clinical presentations and epidemiology of vascular dementia. *Clin Sci (Lond)*. 2017;131:1059-1068.

    doi: 10.1042/CS20160607
40. Prabhakaran S, Naidech AM. Ischemic brain injury after intracerebral hemorrhage: A critical review. *Stroke*. 2012;43:2258-2263.

doi: 10.1161/STROKEAHA.112.655910

41. Duering M, Biessels GJ, Brodtmann A, *et al*. Neuroimaging standards for research into small vessel disease-advances since 2013. *Lancet Neurol*. 2023;22(7):602-618.

doi: 10.1016/S1474-4422(23)00131-X

42. Pantoni L. Cerebral small vessel disease: From pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol*. 2010;9(7):689-701.

doi: 10.1016/S1474-4422(10)70104-6

43. Mustapha M, Nassir CMNCM, Aminuddin N, Safri AA, Ghazali MM. Cerebral small vessel disease (CSVD) - lessons from the animal models. *Front Physiol*. 2019;10:1317.

doi:10.3389/fphys.2019.01317

44. Chojdak-Łukasiewicz J, Dziadkowiak E, Zimny A, Paradowski B. Cerebral small vessel disease: A review. *Adv Clin Exp Med*. 2021;30(3):349-356.

doi: 10.17219/acem/131216

45. Zhuang FJ, Chen Y, He WB, Cai ZH. Prevalence of white matter hyperintensities increases with age. *Neural Regen Res*. 2018;13(12):2141-2146.

doi: 10.4103/1673-5374.241465

46. Li T, Huang Y, Cai W, *et al*. Age-related cerebral small vessel disease and inflammaging. *Cell Death Dis*. 2020;11(11):932.

doi: 10.1038/s41419-020-03137-x

47. Jann K, Shao X, Ma SJ, *et al*. Evaluation of cerebral blood flow measured by 3D PCASL as biomarker of vascular cognitive impairment and dementia (VCID) in a cohort of elderly Latinx subjects at risk of small vessel disease. *Front Neurosci*. 2021;15:627627.

doi: 10.3389/fnins.2021.627627

48. Baezner H, Blahak C, Poggesi A, *et al*. Association of gait and balance disorders with age-related white matter changes: The LADIS study. *Neurology*. 2008;70(12):935-942.

doi: 10.1212/01.wnl.0000305959.46197.e6

49. Naraoka M, Matsuda N, Shimamura N, Asano K, Ohkuma H. The role of arterioles and the microcirculation in the development of vasospasm after aneurysmal SAH. *Biomed Res Int*. 2014;2014:253746.

doi: 10.1155/2014/253746

50. Li Q, Yang Y, Reis C, *et al*. Cerebral small vessel disease. *Cell Transplant*. 2018;27(12):1711-1722.

doi: 10.1177/0963689718795148

51. Zhang YY, Chan DKY, Cordato D, Shen Q, Sheng AZ. Stroke risk factor, pattern and outcome in patients with cancer. *Acta Neurol Scand.* 2006;114(6):378-383.

doi: 10.1111/j.1600-0404.2006.00709.x

52. Uemura J, Kimura K, Sibazaki K, Inoue T, Iguchi Y, Yamashita S. Acute stroke patients have occult malignancy more often than expected. *Eur Neurol.* 2010;64(3):140-144.

doi: 10.1159/000316764

53. Kim SG, Hong JM, Kim HY, *et al*. Ischemic stroke in cancer patients with and without conventional mechanisms: A multicenter study in Korea. *Stroke*. 2010;41(4):798-801.

doi: 10.1161/STROKEAHA.109.571356

54. Dearborn JL, Urrutia VC, Zeiler SR. Stroke and cancer-a complicated relationship. *J Neurol Transl Neurosci*. 2014;2(1):1039.

55. Grazioli S, Paciaroni M, Agnelli G, *et al*. Cancer-associated ischemic stroke: A retrospective multicentre cohort study. *Thromb Res*. 2018;165:33-37.

doi: 10.1016/j.thromres.2018.03.011

56. Grisold W, Oberndorfer S, Struhal W. Stroke and cancer: A review. *Acta Neurol Scand*. 2009;119(1):1-16.

doi: 10.1111/j.1600-0404.2008.01059.x

57. Schwarzbach CJ, Schaefer A, Ebert A, *et al*. Stroke and cancer: The importance of cancer-associated hypercoagulation as a possible stroke etiology. *Stroke*. 2012;43(11):3029-3034.

doi: 10.1161/STROKEAHA.112.658625

58. Álvarez-Pérez FJ, Verde I, Usón-Martín M, Figuerola-Roig A, Ballabriga-Planas J, Espino-Ibañez A. Frequency and mechanism of ischemic stroke associated with malignancy: A retrospective series. *Eur Neurol*. 2012;68(4):209-213.

doi: 10.1159/000341343

59. Seok JM, Kim SG, Kim JW, *et al*. Coagulopathy and embolic signal in cancer patients with ischemic stroke. *Ann Neurol*. 2010;68(2):213-219.

doi: 10.1002/ana.22050

60. Dexter JS. The analysis of a case of continuous variation in *Drosophila* by a study of its linkage relations. *Am Nat*. 1914;48(576):712-758.

61. Durieux I, Ginevra C, Attaiech L, *et al*. Diverse conjugative elements silence natural transformation in *Legionella* species. *Proc Natl Acad Sci U S A*. 2019;116(37):18613-18618.

doi: 10.1073/pnas.1909374116

62. Kopan R, Ilagan MX. The canonical Notch signalling pathway: Unfolding the activation mechanism. *Cell*. 2009;137(2):216-233.

doi: 10.1016/j.cell.2009.03.045

63. Yavropoulou MP, Maladaki A, Yovos JG. The role of Notch and Hedgehog signalling pathways in pituitary development and pathogenesis of pituitary adenomas. *Hormones (Athens)*. 2015;14(1):5-18.

doi: 10.1007/BF03401377

64. Hori K, Sen A, Artavanis-Tsakonas S. Notch signalling at a

glance. *J Cell Sci.* 2013;126(Pt 10):2135-2140.

65. Gozlan O, Sprinzak D. Notch signaling in development and homeostasis. Development. 2023;150(4):dev201138.

doi: 10.1242/dev.201138

66. Vlachakis D, Tsaniras SC, Ioannidou K, Papageorgiou L, Baumann M, Kossida S. A series of Notch3 mutations in CADASIL; insights from 3D molecular modelling and evolutionary analyses. *J Mol Biochem.* 2014;3(3):134.

67. Bray SJ. Notch signalling in context. *Nat Rev Mol Cell Biol.* 2016;17(11):722-735.

doi: 10.1038/nrm.2016.94

68. Xiu MX, Liu YM. The role of oncogenic Notch2 signalling in cancer: A novel therapeutic target. *Am J Cancer Res.* 2019;9(5):837-854.

69. Xia Y, Bhattacharyya A, Roszell EE, Sandig M, Mequanint K. The role of endothelial cell-bound Jagged1 in Notch3-induced human coronary artery smooth muscle cell differentiation. *Biomaterials.* 2012;33(8):2462-2472.

doi: 10.1016/j.biomaterials.2011.12.001

70. Xu X, Choi SH, Hu T, *et al*. Insights into autoregulation of Notch3 from structural and functional studies of its negative regulatory region. *Structure.* 2015;23(7):1227-1235.

doi: 10.1016/j.str.2015.05.001

71. Gera S, Dighe RR. The soluble ligand Y box-1 activates Notch3 receptor by binding to epidermal growth factor like repeats 20-23. Arch Biochem Biophys. 2018;660:129-136.

doi: 10.1016/j.abb.2018.10.009

72. Domenga V, Fardoux P, Lacombe P, *et al.* Notch3 is required for arterial identity and maturation of vascular smooth muscle cells. Genes Dev. 2004;18(22):2730-2735.

doi: 10.1101/gad.308904

73. Jin S, Hansson EM, Tikka S, *et al*. Notch signalling regulates platelet-derived growth factor receptor-beta expression in vascular smooth muscle cells. Circ Res. 2008; 102(12):1483-1491.

doi: 10.1161/CIRCRESAHA.107.167965

74. Ando K, Wang W, Peng D, *et al*. Peri-arterial specification of vascular mural cells from naïve mesenchyme requires Notch signalling. Development. 2019;146(2):dev165589.

doi: 10.1242/dev.165589

75. Wang Q, Zhao N, Kennard S, Lilly B. Notch2 and Notch3 function together to regulate vascular smooth muscle development. PLoS One. 2012;7:e37365.

doi: 10.1371/journal.pone.0037365

76. Baeten JT, Lilly B. Differential regulation of NOTCH2 and NOTCH3 contribute to their unique functions in vascular smooth muscle cells. J Biol Chem. 2015;290:16226-16237.

doi: 10.1074/jbc.M115.655548

77. Kawazu M, Kojima S, Ueno T, *et al*. Integrative analysis of genomic alterations in triple-negative breast cancer in association with homologous recombination deficiency. PLoS Genet. 2017;13(6):e1006853.

doi: 10.1371/journal.pgen.1006853

78. Doebar SC, Sieuwerts AM, de Weerd V, *et al.* Gene expression differences between ductal carcinoma *in situ* with and without progression to invasive breast cancer. Am J Pathol. 2017;187(7):1648-1655.

doi: 10.1016/j.ajpath.2017.03.012

79. Sansone P, Ceccarelli C, Berishaj M, *et al.* Self-renewal of CD133(hi) cells by IL6/Notch3 signalling regulates endocrine resistance in metastatic breast cancer. Nat Commun. 2016;7:10442.

doi: 10.1038/ncomms10442

80. Gu X, Lu C, He D, *et al*. Notch3 negatively regulates chemoresistance in breast cancers. Tumour Biol. 2016;37(7):9511-9519.

doi: 10.1007/s13277-016-5412-4

81. Serafin V, Persano L, Moserle L, *et al*. Notch3 signalling promotes tumour growth in colorectal cancer. J Pathol. 2011;224(4):448-460.

doi: 10.1002/path.2895

82. Suliman MA, Zhang Z, Na H, *et al*. Niclosamide inhibits colon cancer progression through downregulation of the Notch pathway and upregulation of the tumour suppressor miR-200 family. Int J Mol Med. 2016;38(3):776-784.

doi: 10.3892/ijmm.2016.2689

83. Xie T, Li Y, Li SL, Luo HF. Astragaloside IV enhances cisplatin chemosensitivity in human colorectal cancer via regulating NOTCH3. Oncol Res. 2016;24(6):447-453.

doi: 10.3727/096504016X14685034103590

84. Wu JK, Adepoju O, De Silva D, *et al.* A switch in Notch gene expression parallels stem cell to endothelial transition in infantile haemangioma. Angiogenesis. 2010;13(1):15-23.

doi: 10.1007/s10456-009-9161-5

85. Hu L, Xue F, Shao M, Deng A, Wei G. Aberrant expression of Notch3 predicts poor survival for hepatocellular carcinomas. Biosci Trends. 2013;7(3):152-156.

86. Gramantieri L, Giovannini C, Lanzi A, *et al.* Aberrant Notch3 and Notch4 expression in human hepatocellular carcinoma. Liver Int. 2007;27(7):997-1006.

doi: 10.1111/j.1478-3231.2007.01544.x

87. Man CH, Wei-Man Lun S, *et al.* Inhibition of NOTCH3 signalling significantly enhances sensitivity to cisplatin in EBV-associated nasopharyngeal carcinoma. J Pathol. 2012;226(3):471-481.

doi: 10.1002/path.2997

88. Chen CY, Chen YY, Hsieh MS, *et al*. Expression of Notch gene and its impact on survival of patients with resectable non-small cell lung cancer. J Cancer. 2017;8(7):1292-1300.

doi: 10.7150/jca.17741

89. Shi C, Qian J, Ma M, Zhang Y, Han B. Notch3 protein, not its gene polymorphism, is associated with the chemotherapy response and prognosis of advanced NSCLC patients. Cell Physiol Biochem. 2014;34(3):743-752.

doi: 10.1159/000363039

90. Ikezawa Y, Sakakibara-Konishi J, Mizugaki H, *et al*. Inhibition of Notch and HIF enhances the antitumor effect of radiation in Notch expressing lung cancer. Int J Clin Oncol. 2017;22(1):59-69.

doi: 10.1007/s10147-016-1031-8

91. Liu Z, Yun R, Yu X, *et al*. Overexpression of Notch3 and pS6 is associated with poor prognosis in human ovarian epithelial cancer. *Mediators Inflamm.* 2016;2016:5953498.

doi: 10.1155/2016/5953498

92. Jung SG, Kwon YD, Song JA, *et al*. Prognostic significance of Notch 3 gene expression in ovarian serous carcinoma. *Cancer Sci.* 2010;101(9):1977-1983.

doi: 10.1111/j.1349-7006.2010.01641.x

93. Chen CF, Dou XW, Liang YK, *et al*. Notch3 overexpression causes arrest of cell cycle progression by inducing Cdh1 expression in human breast cancer cells. *Cell Cycle.* 2016;15(3):432-440.

doi: 10.1080/15384101.2015.1127474

94. Ross AE, Marchionni L, Vuica-Ross M, *et al*. Gene expression pathways of high-grade localized prostate cancer. *Prostate.* 2011;71(14):1568-1577.

doi: 10.1002/pros.21373

95. Danza G, Di Serio C, Ambrosio MR, *et al*. Notch3 is activated by chronic hypoxia and contributes to the progression of human prostate cancer. *Int J Cancer.* 2013;133(11):2577-2588.

doi: 10.1002/ijc.28293

96. Yeasmin S, Nakayama K, Rahman MT, *et al*. Expression of nuclear Notch3 in cervical squamous cell carcinomas and its association with adverse clinical outcomes. *Gynecol Oncol.* 2010;117(3):409-416.

doi: 10.1016/j.ygyno.2010.03.004

97. Liu J, Fan H, Ma Y, *et al*. Notch1 is a 5-fluorouracil resistant and poor survival marker in human esophagus squamous cell carcinomas. *PLoS One.* 2013;8(2):e56141.

doi: 10.1371/journal.pone.0056141

98. Lee SH, Jeong EG, Yoo NJ, Lee SH. Mutational analysis of NOTCH1, 2, 3 and 4 genes in common solid cancers and acute leukemias. *APMIS.* 2007;115(12):1357-1363.

doi: 10.1111/j.1600-0463.2007.00751.x

99. Masiero M, Minuzzo S, Pusceddu I, *et al*. Notch3-mediated regulation of MKP-1 levels promotes survival of T acute lymphoblastic leukemia cells. *Leukemia.* 2011;25(4):588-598.

doi: 10.1038/leu.2010.323.

100. Tang XF, Cao Y, Peng DB, *et al*. Overexpression of Notch3 is associated with metastasis and poor prognosis in osteosarcoma patients. *Cancer Manag Res.* 2019;11:547-559.

doi: 10.2147/CMAR.S185495

101. Zhang Y, Chen B, Wang Y, *et al*. NOTCH3 overexpression and posttranscriptional regulation by miR-150 were associated with EGFR-TKI resistance in lung adenocarcinoma. *Oncol Res.* 2019;27(7):751-761.

doi: 10.3727/096504018X15372657298381

102. Xiu M, Wang Y, Li B, *et al*. The role of Notch3 signaling in cancer stemness and chemoresistance: Molecular mechanisms and targeting strategies. *Front Mol Biosci.* 2021;8:694141.

doi: 10.3389/fmolb.2021.694141

103. Bernasconi-Elias P, Hu T, Jenkins D, *et al*. Characterization of activating mutations of NOTCH3 in T-cell acute lymphoblastic leukemia and anti-leukemic activity of NOTCH3 inhibitory antibodies. *Oncogene.* 2016; 35(47):6077-6086.

doi: 10.1038/onc.2016.133

104. Papadakos KS, Bartoschek M, Rodriguez C, *et al*. Cartilage oligomeric matrix protein initiates cancer stem cells through activation of Jagged1-Notch3 signaling. *Matrix Biol.* 2019;81:107-121.

doi: 10.1016/j.matbio.2018.11.007

105. Fang CH, Lin YT, Liang CM, Liang SM. A novel C-Kit/phospho-prohibitin axis enhances ovarian cancer stemness and chemoresistance via Notch3-PBX1 and β-catenin-ABCG2 signaling. *J Biomed Sci.* 2020;27(1):42.

doi: 10.1186/s12929-020-00638-x

106. Meurette O, Mehlen P. Notch signaling in the tumour microenvironment. *Cancer Cell.* 2018;34(4):536-548.

doi: 10.1016/j.ccell.2018.07.009.

107. Hanahan D, Coussens LM. Accessories to the crime: Functions of cells recruited to the tumour microenvironment. *Cancer Cell.* 2012;21(3):309-322.

doi: 10.1016/j.ccr.2012.02.022

108. Shen Q, Cohen B, Zheng W, *et al*. Notch shapes the innate immunophenotype in breast cancer. *Cancer Discov.* 2017;7(11):1320-1335.

doi: 10.1158/2159-8290.CD-17-0037

109. Tsuyada A, Chow A, Wu J, *et al*. CCL2 mediates cross-talk between cancer cells and stromal fibroblasts that regulates

breast cancer stem cells. *Cancer Res.* 2012;72(11):2768-2779.

doi: 10.1158/0008-5472.CAN-11-3567

110. Boelens MC, Wu TJ, Nabet BY, *et al*. Exosome transfer from stromal to breast cancer cells regulates therapy resistance pathways. *Cell.* 2014;159(3):499-513.

doi: 10.1016/j.cell.2014.09.051

111. Studebaker AW, Storci G, Werbeck JL, *et al*. Fibroblasts isolated from common sites of breast cancer metastasis enhance cancer cell growth rates and invasiveness in an interleukin-6-dependent manner. *Cancer Res.* 2008;68(21):9087-9095.

doi: 10.1158/0008-5472.CAN-08-0400

112. Yamaguchi N, Oyama T, Ito E, *et al*. NOTCH3 signalling pathway plays crucial roles in the proliferation of ErbB2-negative human breast cancer cells. *Cancer Res.* 2008;68(6):1881-1888.

doi: 10.1158/0008-5472.CAN-07-1597

113. Huang TX, Guan XY, Fu L. Therapeutic targeting of the crosstalk between cancer-associated fibroblasts and cancer stem cells. *Am J Cancer Res.* 2019;9(9):1889-1904.

114. Fernandez SV, Robertson FM, Pei J, *et al*. Inflammatory breast cancer (IBC): Clues for targeted therapies. *Breast Cancer Res Treat.* 2013;140(1):23-33.

doi: 10.1007/s10549-013-2600-4

115. Liu H, Wang J, Zhang M, *et al*. Jagged1 promotes aromatase inhibitor resistance by modulating tumour-associated macrophage differentiation in breast cancer patients. *Breast Cancer Res Treat.* 2017;166(1):95-107.

doi: 10.1007/s10549-017-4394-2

116. Sousa S, Brion R, Lintunen M, *et al*. Human breast cancer cells educate macrophages toward the M2 activation status. *Breast Cancer Res.* 2015;17:101.

doi: 10.1186/s13058-015-0621-0

117. Cahill EF, Tobin LM, Carty F, Mahon BP, English K. Jagged-1 is required for the expansion of CD4+ CD25+ FoxP3+ regulatory T cells and tolerogenic dendritic cells by murine mesenchymal stromal cells. *Stem Cell Res Ther.* 2015;6:19.

doi: 10.1186/s13287-015-0021-5

118. Lamy M, Ferreira A, Dias JS, Braga S, Silva G, Barbas A. Notch-out for breast cancer therapies. *New Biotechnol.* 2017;39:215-221.

doi: 10.1016/j.nbt.2017.08.004

119. Charbonnier LM, Wang S, Georgiev P, Sefik E, Chatila TA. Control of peripheral tolerance by regulatory T cell-intrinsic Notch signalling. *Nat Immunol.* 2015;16(11):1162-1173.

doi: 10.1038/ni.3288

120. Mezquita B, Mezquita J, Barrot C, *et al*. A truncated-Flt1 isoform of breast cancer cells is upregulated by Notch and downregulated by retinoic acid. *J Cell Biochem.* 2014;115(1):52-61.

doi: 10.1002/jcb.24632

121. Sabol HM, Amorim T, Ashby C, *et al*. Notch3 signalling between myeloma cells and osteocytes in the tumour niche promotes tumour growth and bone destruction. *Neoplasia.* 2022;28:100785.

doi: 10.1016/j.neo.2022.100785

122. Ying J, Tsujii M, Kondo J, *et al*. The effectiveness of an anti-human IL-6 receptor monoclonal antibody combined with chemotherapy to target colon cancer stem-like cells. *Int J Oncol.* 2015;46(4):1551-1559.

doi: 10.3892/ijo.2015.2851

123. Antony J, Tan TZ, Kelly Z, *et al*. The GAS6-AXL signalling network is a mesenchymal (Mes) molecular subtype-specific therapeutic target for ovarian cancer. *Sci Signal.* 2016;9(448):ra97.

doi: 10.1126/scisignal.aaf8175

124. Kent S, Hutchinson J, Balboni A, Decastro A, Cherukuri P, Direnzo J. ΔNp63α promotes cellular quiescence via induction and activation of Notch3. *Cell Cycle.* 2011;10(18):3111-3118.

doi: 10.4161/cc.10.18.17300

125. Belin de Chantemèle EJ, Retailleau K, Pinaud F, *et al*. Notch3 is a major regulator of vascular tone in cerebral and tail resistance arteries. *Arterioscler Thromb Vasc Biol.* 2008;28(12):2216-2224.

doi: 10.1161/ATVBAHA.108.171751

126. Kawai H, Kawaguchi D, Kuebrich BD, *et al*. Area-specific regulation of quiescent neural stem cells by Notch3 in the adult mouse subependymal zone. *J Neurosci.* 2017;37(49):11867-11880.

doi: 10.1523/JNEUROSCI.0001-17.2017

127. Loerakker S, Stassen OMJA, Ter Huurne FM, Boareto M, Bouten CVC, Sahlgren CM. Mechano sensitivity of Jagged-Notch signalling can induce a switch-type behavior in vascular homeostasis. *Proc Natl Acad Sci U S A.* 2018;115(16):E3682-E3691.

doi: 10.1073/pnas.1715277115

128. Kitamoto T, Hanaoka K. Notch3 null mutation in mice causes muscle hyperplasia by repetitive muscle regeneration. *Stem Cells.* 2010;28(12):2205-2216.

doi: 10.1002/stem.547

129. Conboy IM, Rando TA. The regulation of Notch signalling controls satellite cell activation and cell fate determination in postnatal myogenesis. *Dev Cell.* 2002;3(3):397-409.

doi: 10.1016/s1534-5807(02)00254-x

130. Wei LK, Sutherland H, Au A, *et al*. A potential epigenetic

marker mediating serum folate and vitamin B12 levels contributes to the risk of ischemic stroke. *Biomed Res Int.* 2015;2015:167976.

doi: 10.1155/2015/167976

131. Joutel A, Vahedi K, Corpechot C, *et al*. Strong clustering and stereotyped nature of Notch3 mutations in CADASIL patients. *Lancet.* 1997;350(9090):1511-1515.

doi: 10.1016/S0140-6736(97)08083-5

132. Soong BW, Liao YC, Tu PH, *et al*. A homozygous NOTCH3 mutation p.R544C and a heterozygous TREX1 variant p.C99MfsX3 in a family with hereditary small vessel disease of the brain. *J Chin Med Assoc.* 2013;76(6):319-324.

doi: 10.1016/j.jcma.2013.03.002

133. Bekris LM, Yu CE, Bird TD, Tsuang DW. Genetics of Alzheimer disease. *J Geriatr Psychiatry Neurol.* 2010;23(4):213-227.

doi: 10.1177/0891988710383571

134. Paulson HL, Igo I. Genetics of dementia. *Semin Neurol.* 2011;31(5):449-460.

doi: 10.1055/s-0031-1299784

135. Ruchoux MM, Maurage CA. CADASIL: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *J Neuropathol Exp Neurol.* 1997;56(9):947-964.

136. Tournier-Lasserve E, Joutel A, Melki J, *et al*. Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy maps to chromosome 19q12. *Nat Genet.* 1993;3(3):256-259.

doi: 10.1038/ng0393-256

137. Stojanov D, Vojinovic S, Aracki-Trenkic A, *et al*. Imaging characteristics of cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL). *Bosn J Basic Med Sci.* 2015;15(1):1-8.

doi: 10.17305/bjbms.2015.247

138. O'Sullivan M, Jarosz JM, Martin RJ, Deasy N, Powell JF, Markus HS. MRI hyperintensities of the temporal lobe and external capsule in patients with CADASIL. *Neurology.* 2001;56(5):628-634.

doi: 10.1212/wnl.56.5.628

139. Joutel A, Corpechot C, Ducros A, *et al*. Notch3 mutations in CADASIL, a hereditary adult-onset condition causing stroke and dementia. *Nature.* 1996;383(6602):707-710.

doi: 10.1038/383707a0

140. Tikka S, Baumann M, Siitonen M, *et al*. CADASIL and CARASIL. *Brain Pathol.* 2014;24(5):525-544.

doi: 10.1111/bpa.12181

141. Schoemaker D, Arboleda-Velasquez JF. Notch3 signalling and aggregation as targets for the treatment of CADASIL and other NOTCH3-associated small-vessel diseases. *Am J Pathol.* 2021;191(11):1856-1870.

doi: 10.1016/j.ajpath.2021.03.015

142. Moreton F, Razvi S, Davidson R, Muir K. Changing clinical patterns and increasing prevalence in CADASIL. *Acta Neurol Scand.* 2014;130(3):197-203.

doi: 10.1111/ane.12266

143. Rutten JW, Hack RJ, Duering M, *et al*. Broad phenotype of cysteine-altering NOTCH3 variants in UK Biobank: CADASIL to nonpenetrance. *Neurology.* 2020;95(18):e1835-e1843.

doi: 10.1212/WNL.0000000000010525

144. Coupland K, Lendahl U, Karlström H. Role of NOTCH3 mutations in the cerebral small vessel disease cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy. *Stroke.* 2018;49(11):2793-2800.

doi: 10.1161/STROKEAHA.118.021560

145. Hosseini-Alghaderi S, Baron M. Notch3 in development, health and disease. *Biomolecules.* 2020;10(3):485.

doi: 10.3390/biom10030485

146. Song C, Zhang J, Xu C, Gao M, Li N, Geng Q. The critical role of γ-secretase and its inhibitors in cancer and cancer therapeutics. *Int J Biol Sci.* 2023;19(16):5089-5103.

doi: 10.7150/ijbs.87334

147. Takebe N, Nguyen D, Yang SX. Targeting notch signaling pathway in cancer: Clinical development advances and challenges. *Pharmacol Ther.* 2014;141(2):140-149.

doi: 10.1016/j.pharmthera.2013.09.005