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NUMB inhibition of NOTCH signalling as a therapeutic target in prostate cancer

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Abstract

Prostate cancer is among the most prevalent life-threatening cancers diagnosed in the male population today. Various methods have been exploited in an attempt to treat this disease but these treatments, alongside preventative tactics, have been insufficient to control mortality rates and have usually resulted in detrimental adverse events. An opportunity to devise more-specific and potentially more-effective approaches for the eradication of prostate tumours can be found by targeting specific biological pathways. NUMB (protein numb homologue), a key regulator of cell fate, represents an attractive, actionable target in prostate cancer. NUMB participates in the observed deregulation of NOTCH (neurogenic locus notch homologue protein) signalling in prostate tumours, and the NUMB–NOTCH interaction regulates cell fate. NUMB has potential both as a target for control of prostate tumorigenesis and as a biomarker for identification of patients with prostate cancer who are likely to benefit from NOTCH inhibition.

Introduction

Many population forecasts predict a significant increase in the number of older people in the next 20 years.¹ As men increase in age, their risk of developing prostate cancer rises exponentially—about six cases in 10 are diagnosed in men aged 65—so prostate cancer incidence is set to rise dramatically. In parallel, worldwide obesity has nearly doubled since 1980. In 2008, more than 1.4 billion adults 20 years of age were overweight, and 200 million men and nearly 300 million women were obese.² Evidence suggests obesity is associated with an elevated incidence of aggressive prostate cancer, and increased risks

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of biochemical failure following radical prostatectomy and external-beam radiotherapy, cardiovascular complications and 'metabolic syndrome' following androgen-deprivation therapy and increased prostate-cancer-specific mortality.³ The design of strategies to improve the detection, diagnosis and treatment of prostate cancer, and survivorship of prostate cancer patients, is, therefore, essential.

Tumour growth is associated with tumour stem cell proliferation and tumour vascularization, so the eradication of cancer stem cells and complete vascular destruction are key to tumour control.^{4,5} Adaptation of the prostate cancer stem cell population to radiation therapy and chemotherapy is thought to be associated with loss of asymmetric cell divisions and an acceleration of differentiation, leading to progressive dominance of cells with a neuroendocrine phenotype.^{6,7} NUMB (protein numb homologue) is the human homologue of the protein numb that was initially discovered in *Drosophila melanogaster* as an adaptor protein responsible for recruiting proteins into different signalling pathways.⁸ NUMB is an evolutionarily conserved protein well-known for its multifaceted role in neurogenesis^{9,10} and cellular homeostasis within the peripheral and central nervous systems.^{8,11} The antagonistic influence of NUMB on the NOTCH pathway and the associated regulation of cell fate has drawn attention to the potential role of NUMB in tumorigenesis in a number of solid tumours, including those arising in the prostate.

The NOTCH (neurogenic locus notch homologue protein) pathway is an evolutionarily conserved signalling system that regulates cell proliferation, differentiation, cell-fate determination and self-renewal of stem cells and progenitor cells in both embryonic and adult organs.^{12,13} NOTCH inhibition is under increasing investigation as a novel anticancer strategy, and so the examination of the interaction between NUMB and NOTCH is warranted.

In 50% of human mammary carcinomas, the control of NOTCH signalling by NUMB is abrogated by ubiquitination and proteasomal degradation of NUMB.¹⁴ Although evidence for the role of NUMB in several types of cancer is accumulating,^{15,16} NUMB has not been extensively studied in relation to prostate cancer. In this Review we evaluate how NUMB is likely to participate in the observed deregulation of NOTCH signalling in prostate tumours, and we highlight the potential clinical implications of the NUMB–NOTCH interaction in prostate cancer.

The multiple regulatory functions of NUMB

The consistency of the biological functions of NUMB proteins within rats, chickens, birds, flies, humans and mice has highlighted their essential role in maintenance of cellular homeostasis within both the peripheral and central nervous systems.^{8,11} NUMB has diverse isoforms derived from alternative splicing of mRNA.^{17–19} Six human NUMB isoforms have been identified, with molecular weights between 50 and 75 kDa.^{18,20} In all these isoforms, the N-terminus carries a phosphotyrosine-binding (PTB) domain while the C-terminus contains Eps15 homology regions: aspartate-proline-phenylalanine tripeptide (DPF) and asparagine-proline-phenylalanine tripeptide (NPF) (Figure 1a). Studies in *Drosophila* have elucidated functional roles for these domains. The NPF domain can

bind to the endocytic machinery components Eps15 and Eps15R.²¹ At the centre of the NUMB protein, a proline rich (PRR) domain exists in certain isoforms, containing Src homology binding sites involved in intracellular signal transduction.²² The PTB domain has a role in tyrosine-kinase-mediated signalling pathways and is also crucial for membrane localization.^{8,19,23} An endocytic function for NUMB proteins has been proposed on the basis of studies identifying a PTB domain that specifically binds to acidic phospholipids and that is rich in basic residues.^{8,23,24} The membrane localization of NUMB is mediated by G-protein-coupled receptor (GPCR)-activated protein hydrolysis and protein kinase C (PKC)-dependent phosphorylation interactions within the 218–366 amino acid regions of NUMB proteins.^{19,23}

The C-terminus of NUMB directly interacts with the endocytic machinery. Adaptor protein 2 (AP-2) is an integral component of clathrin-coated pits²⁵ and functions in endocytic trafficking of transmembrane receptors for internalization and relocalization.²¹ During asymmetric cell division in neurogenesis, NUMB colocalizes with AP-2 through binding to α -adaptin. Mutation of α -adaptin results in NUMB loss-of-function effects such as loss of neurons and glia and gain of hairs and sockets at external sensory organs.^{26–30} NUMB can also interact with adaptor protein 1 (AP-1), which is involved in protein sorting and recycling.³¹

NUMB has a prominent role in the control of cell fate within the Hedgehog and p53 signalling pathways (Figure 1b, 1c).²³ In breast cancer, inactivity of the tumour suppressor protein p53 through loss of NUMB results in activation of NOTCH and reduction of tumour suppression.²⁶ NUMB inhibits E3 ubiquitin–protein ligase Mdm2, blocking degradation of p53 transcription factors.^{23,26,27} Direct methylation of the PTB binding domain of NUMB by lysine methyltransferase Set8 proteins results in p53 ubiquitination and degradation.²⁸ By maintaining high levels of p53 within the cell, an aggressive tumour phenotype can be suppressed, whereas a p53-dependent phenotype is strengthened.²⁷ With a p53-dependent phenotype, genes associated with apoptotic responses to cellular stresses can be expressed and can impede tumorigenesis.^{26,29} Although p53 has been primarily studied in breast cancer, disruption of normal p53 pathways has been reported in most, if not all, cancers,²⁶ including prostate cancer.³⁰

Inhibition by NUMB of the Hedgehog signalling pathway could also contribute to regulation of prostate tumorigenesis. Hedgehog signalling is the primary regulator of embryonic and developmental tissue patterning and cell proliferation.³² In prostate cancer, activation of the pathway is required for regeneration of the prostate epithelium, and continuous activation induces tumorigenic properties in prostate progenitor cells.³³ Several Hedgehog pathway inhibitory drugs are under clinical development, with one (vismodegib) recently approved by the FDA for the treatment of basal cell carcinoma.^{34–36} Activation of Hedgehog signalling is triggered by protein–protein interactions between Hedgehog ligands (SHH, IHH and DHH) and Patched (PTC) transmembrane receptors that are regulated by Hedgehog ligand gradient formation.^{32,37} This binding results in signalling by the GPCR Smoothened protein that activates downstream GLI family transcription factors (GLI1–3).³⁷ NUMB promotes ubiquitination and degradation of GLI1 through recruitment of Itch, the E3 ubiquitin–protein ligase Itchy homologue (Figure 1c).^{37,39}

The importance of GLI proteins in prostate cancer has been reported:⁴⁰ high expression of hedgehog ligands and GLI2 correlate with metastasis potential and therapeutic resistance. Inhibition of the Smoothened protein reduced cell viability and spheroid formation, and induced apoptosis, in prostate cancer stem cells in NOD/SCID IL2R γ null mice.³⁸ GLI proteins also interact with the androgen receptor and might contribute to androgen independence.^{40,41} Normal (PNT-2) and tumorigenic (DU145 and PC3) androgen-independent cells displayed higher GLI activity than androgen-dependent LNCaP prostate cancer cells.⁴² GLI1 was shown to act as a corepressor of the androgen receptor and to block transactivation mediated by the androgen receptor.⁴³ In LNCaP cells, androgen R1881 strongly suppressed the expression of Hedgehog ligands and GLI2 expression.⁴⁴ LNCaP cells overexpressing GLI1 were viable in the presence of the androgen receptor inhibitor bicalutamide and the transcriptome of these cells was significantly closer to DU145 and PC3 cells than to untransfected controls.⁴² Further examination is warranted of the relationship between NUMB and GLI expression levels within the context of androgen independence.

NUMB proteins are regulated by alternative splicing, and transcriptional and post-transcriptional modifications. Musashi1 (Msi1) is a neural RNA-binding protein that represses NUMB translation,⁴⁵ preserving the immature neural stem cell state,⁴⁶ and increasing NOTCH signalling.⁴⁶ Low-level expression of Msi1 has been reported in prostate cancer cells.⁴⁷ Several RING finger proteins are involved in ubiquitin-dependent degradation of NUMB, including LNX,⁴⁸ Siah-1⁴⁹ and Mdm2.⁴⁹ Specific microRNAs such as MiR-146a are involved in transcriptional repression of NUMB, which inhibits NOTCH signalling in muscle cells by delaying myogenic differentiation.⁵⁰ In prostate cancer, MiR-146a is associated with increased cancer risk,⁵¹ and loss of MiR-146a function is associated with suppression of tumour growth in castration-resistant disease.^{52,53} In mammals, phosphorylation of NUMB by protein kinase C (PKC) or regulation of membrane binding by GPCR-mediated lipid hydrolysis, lead to relocation of NUMB from the cortical membrane to the cytosol,¹⁹ rendering it unable to complete its function. This loss of function prevents NUMB-mediated NOTCH inhibition and could explain the increase in oncogenic expression of NOTCH in the prostate.⁵⁴

NOTCH is a key pathway in prostate cancer

The NOTCH pathway is increasingly recognized as a major deregulated pathway in many solid malignancies including renal and prostate cancers.^{55,56} The pivotal function of NOTCH in tumorigenesis stems from the control of cell fate decisions, stem cell maintenance, cellular proliferation and apoptosis.¹³ Additionally, NOTCH is involved in cellular differentiation in multicellular organisms, angiogenesis, epithelial–mesenchymal transitions and cell adhesion,⁵⁵ and is responsive to hypoxia.⁵⁷

The human NOTCH pathway is composed of ligands (Jagged-1, Jagged-2 and Delta-like proteins 1, 3 and 4) and receptors (Notch 1–4).⁵⁸ Activation, regulation and degradation of the Notch signal require endosomal trafficking of both ligands and receptors.^{59,60} Activation of the NOTCH pathway begins with binding of type 1 ligands from the DSL families (Delta, Jagged) to Notch transmembrane receptors,^{61–64} triggering a series of proteolytic cleavages within the plasma membrane and intracellular compartment, and initiating signal

transduction. ADAM family proteins TACE (ADAM 17) and Kuzbanian (ADAM 10) mediate regulation of Notch extracellular domain shedding.^{65,66} The final cleavage occurs by a γ -secretase complex releasing the Notch intracellular domain (NICD).^{62,63,65,67–70} The NICD functions as a transcription factor within the nucleus, interacting with recombining binding protein suppressor of hairless (RBPJ) transcription factor and regulating target genes including those of several helix–loop–helix transcription factors such as Hes-1 and Hey-1.^{62,63,71,72} During NICD–RBPJ interaction, the transcriptional activator protein Mastermind is also recruited to initiate transcription (Figure 2). The NOTCH pathway contributes to prostate tumorigenesis,^{73,74} has been proposed to influence the outcome of anticancer hormonal^{75,76} and cytotoxic (docetaxel)⁷⁷ treatments and might be more active in patients with prostate cancer who have a high BMI.⁷⁸ The NOTCH pathway is essential to regulation of blood vessel structure,^{79,80} which is altered within tumours.^{81,82}

Inhibition of NOTCH signalling

Possible strategies for NOTCH inhibition include receptor activation inhibition, modification of receptor–ligand interactions, inhibition by antibodies, disruption of γ -secretase cleavage of Notch, alteration of NICD post-translational modifications, inhibition of protein–protein interactions within the nucleus and disruption of assembly of the coactivator NICD–RBPJ complex (Figure 2).

Receptor activation inhibition

Ligand-induced ADAM-type metalloprotease cleavage of Notch transmembrane receptors at the extracellular negative regulatory region (NRR) is essential to initiation of the regulated intramembrane proteolytic (RIP) cascade that is required for release of NICD by γ -secretase cleavage.⁸³ If the ligand-induced Notch receptor conformational change is blocked, the NOTCH pathway remains inactivated. Inhibition of receptor cleavage by ADAM proteases⁶⁵ or γ -secretase⁸⁴ also abolishes NOTCH signalling.

Modification of receptor–ligand interaction

Blocking ligand ubiquitination and transendocytosis, which serve to activate ligands,⁸⁵ and decreasing the availability of *O*-Fucose, which is required for normal NOTCH signalling,⁸⁶ renders Notch transmembrane receptors unable to interact with ligands and to initiate signal transduction. Creating a soluble NOTCH decoy peptide composed of epidermal growth factor (EGF)-like repeats enables the ligand-binding region of Notch transmembrane receptors to be mimicked, preventing NOTCH pathway signalling by competitive inhibition.⁸⁷ These approaches have not yet been examined in the setting of prostate cancer. Decoys can potentially abrogate NOTCH signalling through disruption of receptor–ligand interactions, with effects that depend on pharmacokinetics, biodistribution and decoy abundance. Decoys are soluble structures created to imitate the biological structure of extracellular protein receptors and their ligands. Decoys of DSL ligands⁸⁸ and Jagged⁸⁹ have produced promising results in establishing NOTCH inhibition, but the levels of decoys required to consistently impede receptor–ligand interactions have not yet been determined.

Antibody inhibition of NOTCH

Two classes of blocking monoclonal antibodies (mAbs) have been developed to inhibit NOTCH. The first class inhibits Notch receptors by maintaining Notch in an inactive conformation on binding to the NRR domain.^{90,91} The second class impedes ligand binding through competitive inhibition at the Notch EGF-repeat region.⁹⁰ Other mAbs have also been shown to inhibit NOTCH signalling by targeting ligands, such as DLL4.⁹² These blocking antibodies have inhibited NOTCH signalling in endothelial cells.⁹²

γ -Secretase inhibitors

Activation of NOTCH signalling is highly dependent upon γ -secretase initiating the final cleavage to release NICD,⁹³ and so γ -secretase inhibitors are being investigated as potential anticancer agents. These compounds have shown promising results in various carcinomas,⁵⁶ despite their off-target effects.

Post-transcriptional modification of NICD

Post-transcriptional modifications of NICD could be targets for therapeutic regulation of NOTCH activity. Although NICD is a short-lived protein with a half-life of approximately 4 hours,^{94,95} it can be modified by phosphorylation, ubiquitination, hydroxylation, glycosylation and acetylation.

Phosphorylation—Phosphorylation of NICD was first discovered in *Drosophila*,⁶⁷ and phosphorylated NICD isoforms have also been reported in mammalian cells.^{95–98} Phosphorylation can either activate or deactivate NICD, depending on the number of cleavages the Notch transmembrane proteins experience, and the specific kinase protein that is involved. Several phosphorylated forms of NICD can exist within the nucleus. Glycogen synthase kinase-3 β (GSK β or Shaggy) is a serine/threonine kinase that increases the half-life of NICD but inhibits induction of nuclear genes, such as Hes-1.⁹⁹ However, when granulocyte colony stimulating factor (G-CSF) phosphorylates NICD at the Ser^{2,078} residue, NICD is inactivated.¹⁰⁰ Hyperphosphorylation has been identified within the nucleus and is stringently associated with NICD interaction with RBPJ proteins,⁹⁸ NICD nuclear accumulation^{96,97,101} and NICD transformations.¹⁰¹

Ubiquitination—Despite the activation of NOTCH signalling by monoubiquitination,¹⁰² several cytoplasmic proteins promote NOTCH downregulation by inducing endocytosis followed by lysosomal degradation.¹⁰³ Itch has an evolutionarily conserved role as a negative regulator of NOTCH.¹⁰⁴ However, Deltex, a homologue of Itch, is a positive regulator of NOTCH signalling and binds to NICD. Therefore, the suppressor of Deltex, E3 ubiquitin–protein ligase Su(dx), is a negative regulator of NOTCH signalling.^{66,105–108} Within Su(dx) molecules, WW domains select target proteins for ubiquitination, and WW modules can also be found in the developmentally down-regulated NEDD-4 (E3 ubiquitin–protein ligase NEDD4) family proteins that are expressed in neural precursor cells.¹⁰⁵ The loss of NEDD-4 function activates *NOTCH* and *Deltex* mutant phenotypes while NEDD-4 hyper-activation promotes degradation of Notch and Deltex.¹⁰⁹ Other ubiquitin–protein ligases, such as c-Cbl (casitas B-lineage lymphoma), target receptors for endosomal and lysosomal sorting by facilitating receptor translocation from the cell surface to the

degradation machinery.^{110,111} In comparison, Mam-1 (Mastermind-like protein 1) recruits CDK8 kinase for phosphorylation of NICD that is followed by E3 ubiquitin–protein ligase hAgo-dependent degradation of Notch.^{112,113} Furthermore, β -arrestin/Kurtz¹¹⁴ can also promote Notch inactivation by inducing proteasomal degradation, but has yet to be studied in the setting of prostate cancer.

Hydroxylation—Under hypoxic conditions, cells will adapt by initiating a cellular response involving the stabilization of HIF-1 α (hypoxia-inducible factor 1 α), activating transcription, increasing angiogenesis, increasing erythropoiesis and utilizing glycolytic metabolism.^{115,116} During this response, regulation of NOTCH signalling occurs when FIH-1 (factor inhibiting HIF-1) negatively regulates NICD by hydroxylating the Asn^{1,945} and Asn^{2,012} residues that are associated with Notch-mediated transcriptional activation, resulting in severe impairment of NICD function.^{116,117} NICD function can be restored by Mam-1.¹¹⁶ Preclinical studies involving mice and targeting FIH-1 have not yet resulted in significant overexpression of NOTCH phenotypes, suggesting a complex relationship exists between FIH-1 and NOTCH.¹¹⁸

Glycosylation—Glycosylation is essential for normal functioning of NOTCH transmembrane glycoproteins.¹¹⁹ *O*-fucosylation by O-FucT-1 (GDP-fucose protein *O*-fucosyltransferase 1) at NOTCH EGF domains facilitates ligand–receptor binding,¹¹⁹ and elongation of *O*-linked Fucose residues at Notch EGF-like sequences by both mFng (β -1,3-N-acetylglucosaminyltransferase manic fringe) and lFng (β -1,3-N-acetylglucosaminyltransferase lunatic fringe) impedes receptor–ligand binding.¹²⁰ Specifically, mFng and lFng prevent Notch activation by Jagged ligands but leave Delta–Notch interactions unaffected.^{120,121} Fringe proteins can positively and negatively regulate the ability of ligands to activate the Notch receptor,¹²² so Fringe is able to inhibit activation of Notch by Serrate and Jagged ligands but stimulates Notch activation by Delta ligands.¹²¹ The diverse roles of Fringe in Notch activation and inactivation need to be further examined in the setting of prostate tumorigenesis.

Acetylation—Despite the ability of Mam-1 to recruit CDK8 for NICD phosphorylation, resulting in proteasomal degradation, the presence of Mam-1 in the nucleus is also highly correlated with decreased ubiquitination of Notch and increased Mam-1-dependent acetylation of Notch by p300 HAT (histone acetyltransferase p300).¹²³ Acetylation by p300 HAT stabilizes NICD, whereas phosphorylation by CDK8 induces NICD degradation. Protein acetylation is reversible, and is regulated by histone deacetylases (HDACs), NAD-dependent protein deacetylases and histone acetyltransferases.¹²⁴ Preclinical studies have reported that HDAC inhibitors that stabilize protein hyperacetylation have resulted in a significant decrease in both *in vivo* and *in vitro* growth of T-cell leukemia in *NOTCH3* transgenic mice.¹²⁴ By comparison, hSIRT1 in association with NICD, by functioning as an NICD deacetylase, promotes NICD destabilization and alters NICD protein turnover in endothelial cells lacking active SRT1 protein.¹²⁵ Inhibition of NICD1 has been seen with both p300 and Tip60 HATs,^{125,126} but NICD3 inhibition is apparently p300 HAT-dependent.¹²⁴ Prostate cancer therapy by acetylation-based regulation of NICD activity will depend on the relative involvement of specific NICD isoforms in prostate tumorigenesis.

Nuclear protein–protein interactions—Following nuclear translocation, NICD can interact with the Notch transcriptional activation complex (NTC), which is composed of DNA-bound transcription factor RBPJ and Mastermind-like family proteins, to begin targeting specific genes.¹²⁷ Disruption of this NTC complex by blocking peptides can negatively regulate NOTCH signalling.¹²⁸

Natural compounds—Several natural compounds have been investigated for the ability to inhibit NOTCH signalling, but their effectiveness is not yet confirmed. Genistein, a product of soybeans, can inhibit NOTCH signalling by inducing cellular apoptosis and significantly reducing cell viability in prostate cancer cells.¹²⁹ Sulforaphane, a compound identified in broccoli, increases chemotherapeutic drug effects in both prostate cancer cells and pancreatic cancer stem cells.¹³⁰ Other substances, such as resveratrol, a phytoalexin compound found in grapes and red wine,¹³¹ and curcumin, a food flavouring compound found in *Curcuma longa*,¹³² have all caused downregulation of NOTCH signalling pathways but have yet to be tested in prostate cancer.

The NUMB–NOTCH interaction

An inverse relationship between NUMB and NOTCH has been described: cells inheriting NUMB are usually deficient in NOTCH activity while cells inheriting NOTCH lack active NUMB.^{133–135} Although the precise mechanisms for Notch transmembrane receptor inhibition by NUMB remain unknown, several models have been proposed (Figure 2).

The endocytosis model

NOTCH signalling requires endosomal trafficking for activation, regulation and degradation of the signal.^{59,60} NUMB interacts with the AP-2 adaptor complex and the endocytic proteins α -adaptin and Eps15, in clathrin-coated pits and in endosomes.¹³³ This ability of NUMB to localize in endocytic organelles, cotraffic with internalizing receptors and physically interact with endocytic machinery has raised the possibility of NOTCH inhibition by NUMB through endocytosis.

The postinternalization model

The NUMB–NOTCH interaction was proposed as a key regulator of asymmetric cell division in *Drosophila* peripheral neurogenesis, through regulation of Sanpodo.²¹ Sanpodo, a membrane-associated protein that interacts with both NUMB and Notch, is responsible for Notch transmembrane protein trafficking and endocytosis.^{31,136} A model was proposed suggesting that NUMB could inhibit AP-1-mediated recycling of Notch–Sanpodo complexes in p11b cells postinternalization.^{21,136} In *Drosophila* neural stem cells (NSCs), Numb/Notch signalling plays a key role in the balance between self-renewal and differentiation. While this model has been proposed in *Drosophila*, the transition between symmetric and asymmetric cell division is critical to the regulation of the cancer stem cell population.¹³⁷ In colorectal cancer, NUMB was reported as a key factor in this switch.¹³⁸ Examination of this NUMB–NOTCH interaction could have implications for (prostate) cancer stem cell biology.

The downstream inhibition model

Following NICD internalization, NUMB could inhibit NOTCH signal transduction by preventing NICD from initiating gene transcription. In the NOTCH signalling pathway, NICD interacts with RBPJ to form a complex in the nucleus, involving Mastermind transcriptional activation proteins, histone deacetylase transformations into histone acetyl transferases and chromatin modifications.^{58,63,66,139} Ubiquitin ligases also contribute to function of the complex.^{140,141} The RBPJ–NICD complex binds to DNA within the nucleus, activating gene transcription.¹⁴² With cotransfection of NOTCH and RBPJ, RBPJ localizes to the cytoplasm, and exposure to Delta ligands leads to RBPJ relocation to the nucleus.^{143,144} Preliminary data collected by Frise *et al.*¹⁴³ show that, with coexpression of NUMB and Notch, binding of Delta to Notch results in suppression of RBPJ translocation into the nucleus. NUMB can inhibit NOTCH signalling by binding directly to the NICD domain, preventing access of NICD to the nucleus.^{143–145} NUMB is also able to directly inhibit NOTCH by recruiting Itch for polyubiquitination and degradation of Notch proteins.¹⁴⁶

Clinical implications

The clinical potential of inhibition of the NOTCH pathway is under increasing investigation. However, the intricate relationship between NOTCH and NUMB has been poorly reported. Determination of NUMB expression in patients with prostate cancer could be central to the identification of patients most likely to benefit from NOTCH inhibition. For instance, loss of NUMB has been associated with resistance to cisplatin in malignant pleural mesothelioma,¹⁴⁷ and to docetaxel in breast cancer cells.¹⁴⁸ Treatment with the γ -secretase inhibitor PF-03084014 resensitized cells to docetaxel.¹⁴⁸ The two NUMB isoforms hNUMB5 and hNUMB6 are encoded by mRNAs that lack exon 10 and are expressed in cells known for polarity and migratory behavior, such as human amniotic fluid cells, glioblastoma and metastatic tumour cells. These isoforms seem to be less antagonistic to NOTCH signalling than other NUMB isoforms and could have a more prominent role in carcinogenesis.²⁰ In patients diagnosed with non-small cell lung cancer, tumour-associated increases in *NUMB* exon 9 inclusion correlate with reduced levels of NUMB protein expression and activation of the NOTCH signalling pathway.¹⁴⁹ Loss of NUMB is associated with tumour aggressiveness and BRCA1 status in patients with breast cancer,¹⁵⁰ and poor prognosis in patients with salivary gland carcinomas.¹⁵¹

Conclusions

Identifying a molecular target with a significant role in cancer cell proliferation and survival is essential for the development of a more specific, more effective and potentially less toxic approach to treating cancer. As both NOTCH and NUMB have evolutionarily conserved roles in cell-fate determination and tumour angiogenesis, their biological pathway is currently being examined as a potential target for anticancer therapeutics. NUMB is able to control various oncogenic signalling pathways, including the p53, Hedgehog and NOTCH pathways. Although the precise mechanism of NOTCH inhibition by NUMB is not yet known, the complexity of the isoforms and functions of NUMB enable it to

encompass a variety of functions within different signalling pathways. It might be possible to selectively target the involvement of NUMB in prostate cancer, and to identify patients most likely to benefit from NOTCH inhibition. However, NUMB is poorly characterized in the setting of prostate cancer despite being firmly established as a tumour suppressor in various other carcinomas.¹⁵² The role of NUMB in prostate cancer, and specifically its role in the regulation of NOTCH signalling, should be examined as an issue of the utmost importance.

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Key points

- NUMB is a complex protein with multiple cellular functions
- NUMB negatively regulates the NOTCH signalling pathway
- The NUMB–NOTCH interaction regulates cell fate in prostate tumours
- Targeting NUMB has potential to control prostate tumorigenesis
- NUMB profiling could assist the identification of patients with prostate cancer who are likely to benefit from NOTCH-inhibition strategies

Review criteria

The PubMed database was searched using combinations of the search terms “NOTCH”, “NUMB”, “prostate neoplasm” and “inhibition”. Peer-reviewed English-language papers were considered for inclusion in the manuscript. The reference lists of identified publications were searched for additional articles.

Figure 1.

The multiple functions of NUMB. **a** | Structure of the NUMB protein. All six isoforms of the human NUMB protein have a similar structure. At the N-terminus, a phosphotyrosine-binding (PTB) domain facilitates tyrosine kinase signalling, endocytosis and membrane localization. Eps15 homology regions DPF and NPF are located at the C-terminus. The NPF domain can bind to the endocytic machinery components Eps15 and Eps15R. At the centre of the NUMB protein, proline rich (PRR) domains exist in certain isoforms, containing Src homology binding sites involved in intracellular signal transduction. **b** |

Control of p53 activity by NUMB. p53 activity is induced by many stress-related signals (1). A negative regulatory feedback loop controls cellular levels of p53: p53-dependent transcription of *MDM2* promotes p53 degradation (2). MDM2 also inhibits p53 activity by blocking p53 transcriptional activity and favouring p53 nuclear export (3). By inhibiting MDM2 activity, NUMB amplifies p53 activity (4). Transcription of target genes promotes p53 tumour-suppressor properties (5). NUMB-MDM2 interaction might also alter p53-independent MDM2 activities (6). **c** | Regulation of the Hedgehog/GLI pathway by NUMB. Sonic hedgehog (SHH) inhibits the repression of Smoothened (SMO) by Patched (PTC) (1). Activation of SMO transmits the signal intracellularly and triggers the nuclear action of the GLI proteins. GLI proteins regulate target genes that participate in tumour proliferation and survival (2). NUMB interacts with Itch to promote ubiquitination and degradation of GLI (3). In prostate cancer cells, GLI proteins bind to the androgen receptor and affect androgen signalling. Abbreviations: BAD, Bcl-2-associated agonist of cell death; GADD45, growth arrest and DNA-damage-inducible; Itch, E3 ubiquitin–protein ligase Itchy homologue; MDM2, E3 ubiquitin–protein ligase Mdm2; NOXA, PMA-induced protein 1; PGM, phosphoglycerate mutase; PTEN, phosphatase and tensin homolog; PUMA, Bcl-2-binding component 3; SCO2, synthesis of cytochrome c oxidase 2; TSC1, tuberous sclerosis 1. Permission for part b obtained from Nature Publishing Group © Chène, P. *Nat. Rev. Cancer* **3**, 102–109 (2003) and Dotto, G. P. *Nat. Rev. Cancer* **9**, 587–595 (2009). Permission for part c obtained from Nature Publishing Group © Ruiz i Altaba, A. *et al. Nat. Rev. Cancer* **2**, 361–372 (2002).

Figure 2.

Inhibition of the NOTCH pathway by NUMB. NUMB interacts with AP-2 and Eps15 to disrupt endocytosis of Notch ligands and receptors (1). The binding of ligands (Delta, Jagged [JAG]) to Notch transmembrane receptors 1–4 triggers a series of proteolytic cleavages by ADAM family proteins (ADAM 10, ADAM 17) and a γ -secretase complex that lead to the release of the Notch intracellular domain (NICD) (2). The NICD is translocated into the nucleus, interacts with the recombining binding protein suppressor of hairless (RBPJ) transcription factor and the transcriptional activator protein Mastermind

to regulate target genes such as Hes-1 and Hey-1. NUMB might disturb this transcriptional machinery and inhibit Notch-dependent gene expression (3). Phosphorylation of NICD by CDK8 leads to hAgo-dependent proteosomal degradation of NICD (4). NUMB prevents translocation of NCID into the nucleus (5). Strategies under development for the therapeutic inhibition of the NOTCH pathway are indicated in boxes.