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Hypoxia, notch signalling, and prostate cancer

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Abstract

The notch signalling pathway is involved in differentiation, proliferation, angiogenesis, vascular remodelling, and apoptosis. Deregulated expression of notch receptors, ligands, and targets is observed in many solid tumours, including prostate cancer. Hypoxia is a common feature of prostate tumours, leading to increased gene instability, reduced treatment response, and increased tumour aggressiveness. The notch signalling pathway is known to regulate vascular cell fate and is responsive to hypoxia-inducible factors. Evidence to date suggests similar, therapeutically exploitable, behaviour of notch-activated and hypoxic prostate cancer cells.

Introduction

The notch pathway is an evolutionarily conserved signalling system that regulates the proliferation, differentiation, cell-fate determination, and self-renewal of stem and progenitor cells in both embryonic and adult organs.[1,2] It is increasingly recognized as a signalling pathway with oncogenic and tumour suppressor properties.[3,4] Notch deregulation has been reported in a wide range of tumours and is emerging as a novel therapeutic target.[5] The notch pathway is critical for normal cell proliferation and differentiation in the prostate. As a result, deregulation of this pathway has been proposed to facilitate prostatic tumorigenesis[6] and possibly influence the outcomes associated with anticancer hormonal treatments.[7]

Hypoxia is a common feature of prostate tumours and has been associated with disease progression and treatment resistance.[8] Many oxygen-responsive genes are regulated by the hypoxia-inducible factor 1 (HIF-1) complex. HIF-1α overexpression, as evidenced by increased immunostaining, has been reported in a variety of human cancers (including prostate cancer) and their metastases.[9,10] Recent evidence suggests that HIF-1 is recruited to

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notch-responsive promoters under hypoxic conditions to activate transcriptional targets.[11] In this Review, we evaluate the potential involvement of hypoxia in the deregulated expression of notch receptors, ligands, and targets in prostate cancer cells and consider the likely involvement of hypoxia and notch signalling in disease progression, treatment resistance, and the identification of novel therapeutic targets for prostate cancer.

Notch signalling in prostate

The human notch pathway is composed of ligands (jagged-1, jagged-2, and δ-like proteins 1, 3, and 4) and receptors (notch 1–4). Ligand–receptor interactions induce cleavage of the notch receptors, releasing the notch intracellular domain (NICD). The NICD translocates to the nucleus and binds a transcriptional repressor called recombining binding protein suppressor of hairless (RBPJ) converting it into a gene activator that induces the expression of downstream target genes, such as several helix–loop–helix transcription factors named transcription factor hes-1 and hairy/enhancer-of-split related with YRPW motif protein 1 (also known as hey-1; Figure 1).

Notch receptors

Expression of the notch pathway family members—which exhibit tumour-specific and metastatic-specific patterns—has been measured in prostate cancer cells *in vitro*, in the transgenic adenocarcinoma of the mouse prostate (TRAMP) mouse model, and in patient tumour specimens. Of the four notch receptors, the expression of notch 1 has been studied the most. In one study, notch 1 transcript levels were significantly higher in prostate cancer LNCaP cells than in normal prostate PNT2 cells.[12] *In situ* hybridization of tissue specimens from TRAMP mice showed that, although *notch 1* was undetectable or expressed at very low levels in normal mature prostate tissue, hybridization signals were strong in malignant cells.[13] In another study, the percentage of notch-1-positive cells was significantly greater in primary prostate tumours than in adjacent benign tissue, and was slightly greater (approaching statistical significance) in bone metastatic cancer compared with primary prostate cancer.[14] Despite elevated notch 1 expression, notch 1 signal transduction could be diminished in prostate cancer. Indeed, immunostaining of the cleaved receptor and its downstream effector hey-1 were significantly reduced in adenocarcinoma foci when compared with benign tissues in a series of prostate sections from 16 patients with prostate carcinoma.[15] Although the mechanistic basis for loss of cleaved notch 1 remains to be elucidated, notch 1 cleavage has been shown to correlate with loss of phosphatase and tensin homolog gene (PTEN) expression[15] and with calcium/calmodulin-dependent kinase II overexpression.[16] Nonetheless, *HES1* gene expression levels were higher in the metastatic PC3 cell line and in the strongly metastatic PC3-derived PC3M cell line than in the LNCaP metastatic cell line.[12]

Notch 2, notch 3, and notch 4 have been less extensively studied than notch 1. In one study, notch 2 mRNA levels were described as elevated in three prostate cancer cell lines (LNCaP, PC3, and PC3M) relative to normal prostate cells (PNT2), whereas expression of notch 3 and notch 4 was not detected in any of the cell lines.[12] Strong expression of the notch 2 receptor has also been reported in gliomas,[17] breast cancers,[18] and colorectal cancers.[19] In

breast cancers, high notch 1 levels have been associated with poor prognosis, whereas increased notch 2 levels have correlated with greater probability of survival.[20] Increased expression of the notch 3 and notch 4 receptors has been less commonly reported in cancers, but levels of these proteins seem to be elevated in glioblastomas,[21] salivary adenoid cystic carcinomas,[22] breast cancers,[23,24] cervical cancers,[25] and ovarian cancers.[26,27] The mechanistic significance of the expression of each notch receptor in tumours remains to be elucidated.

Notch ligands

Expression of the notch ligands jagged-1, jagged-2, and δ-like protein 1 (also known as DLL1) has been reported in LnCaP, PC3, and PC3M metastatic prostate cancer cells.[12] *DLL1* gene expression levels were higher in androgen-sensitive LNCaP cells than in androgen-independent PC3 and PC3M cells, suggesting that DLL1 might be associated with the androgen response.[12] The expression of jagged-1 was increased in all three cell lines relative to normal prostate cells, but jagged-2 expression did not vary significantly among the cell lines.[12] The notch ligand protein jagged-1 was significantly overexpressed in metastatic prostate cancer compared with localized prostate cancer or benign prostatic tissues (according to immunohistochemical analysis of human tumour samples from 154 men). Furthermore, high jagged-1 expression in a subset of clinically localized tumours was significantly associated with disease recurrence.[28] In clinical specimens, the notch signalling cascade was identified as one of the cellular pathways for which gene enrichment might distinguish high-grade (Gleason grade 8 [4 + 4]) from low-grade (Gleason grade 6 [3 + 3]) pathologically localized prostate cancer,[29] and expression of this pathway might be more pronounced in patients with a high BMI.[30]

Evidence to date suggests dominant roles for notch 1 and jagged-1 in the development of prostate cancer. Similarly, elevated expression of either protein has been associated with poor prognosis in patients with breast cancer.[31–33] The underlying mechanisms behind this genetic dominance remain to be elucidated, but could result from mutations in the notch 1 NICD[34] or F-box/WD repeat-containing protein 7 (FBXW7).[35] Differential expression of *FBXW7* isoforms has been shown to correlate with advanced pathological stage and clinical recurrence of prostate cancer.[36] This genetic dominance might, alternatively, be the result of increased activation of the Wnt/β-catenin signalling pathway, which has also been observed in prostate cancer.[37,38] Both Wnt/β-catenin and notch 1 signalling pathways are thought to have an important role in cell proliferation and survival. B-catenin was shown to regulate the level and transcriptional activity of notch 1 in mouse embryonic fibroblasts and human embryonic kidney 293 cells.[39] Preferential expression of notch 1 and β-catenin were associated with a stem-cell-like phenotype in prostate cancer cells.[40] Finally, numb, an important cell fate determinant protein, has been highlighted as a negative regulator of notch signalling in cancer.[41,42] Loss of numb expression has been shown to increase notch activity in lung cancer cells[43] and, in one study, was described in >50% of primary breast tumours.[44] The expression pattern of numb in prostate cancer, although yet to be reported, might provide some insight into the regulation of notch in prostate cancer development.

Notch signalling and hypoxia

A number of studies have reported crosstalk between notch and hypoxia signalling pathways.[45] Notch activity has been associated with expression of vascular endothelial growth factor (VEGF)[46] and HIF-1α[47]—two well-recognized markers of tumour hypoxia. HIF-1 is recruited to notch-responsive promoters under hypoxic conditions to activate transcriptional targets and potentially regulate cell differentiation.[11] It can interact with the NICD to augment the notch downstream response. This crosstalk between notch and hypoxia signalling seems to be mediated by the activation of factor-inhibiting HIF-1 (FIH-1).[48] The accumulation of HIF-1α and HIF-2α in breast cancer cells with hypoxia is thought to potentiate stabilization of the NICD of the notch 1 receptor[46,49,50] and induce notch signalling via the NICD of the notch 3 receptor.[50] Low oxygen levels (1%) also increases the expression of *HEY1* and *HES1* notch target genes, as well as *NOTCH3, JAG1*, and *JAG2* genes in breast cancer cell lines, indicating that hypoxia could regulate notch signalling in breast cancer.[50] In these cells, notch signalling mediates epithelial to mesenchymal transition (EMT), cell migration, cell invasion, and cell tube formation under hypoxic conditions. Activation of *JAG2* by hypoxia might enhance the metastatic potential of breast cancer cells.[51] Reduced oxygen concentrations were also associated with increased activation of notch 1 in lung tumours,[52,53] melanoma,[54] and malignant mesothelioma.[55]

Although most of the research to date has concentrated on other cancer types, a relationship between notch signalling and hypoxia in prostate cancer is possible. In one study, prostate cancer cell lines exposed to low oxygen concentrations assumed a neural-like phenotype, accompanied by upregulation of three neuroendocrine markers and a significant decrease in levels of notch 1, notch 2, DLL1, DLL4, hes-1, and hey-2 mRNA and protein expression. However, activation of neuroendocrine differentiation, which has been associated with poor prognosis and limited response to androgen deprivation therapy (ADT), was only seen in the androgen-sensitive LnCaP cell line.[56]

A number of parallels exist between the cellular behaviour of notch-activated and hypoxic cells, suggesting that the notch pathway might be responsive to hypoxia in prostate cancer cells. Downregulation of jagged-1 in prostate cancer cells has been associated with cell growth inhibition and S phase cell cycle arrest. As a result, jagged-1 has been proposed as a potential therapeutic target for the treatment of prostate cancer.[57] Hypoxia is known to reduce proliferation rates and trigger S phase arrest in p53-mutant cells,[58] suggesting a potential role of jagged-1 in the hypoxic response. p53-mediated upregulation of notch 1 expression in human cancer cell lines, including PC3 and LNCaP, has been shown to contribute towards cell fate determination after genotoxic stress.[59] Similarly, transfection of prostate cancer cell lines LNCaP, DU145, and PC3 with a constitutively active form of notch 1 has been shown to result in reduced proliferation rates.[13] PTEN has also been reported to inhibit the phosphoinositide 3-kinase (PI3K) pathway, participating in a reduction in HIF-1α cytoplasmic levels. Loss of PTEN has been associated with an elevated HIF-1α activation response to hypoxia[60] and proposed as a critical event in the development of resistance to notch inhibition in T-cell lymphoma.[61]

Cancer stem cells

Over the past decade, a substantial amount of evidence has been published that highlights the importance of interplay between hypoxia and the notch pathways in tumour progression and treatment resistance resulting from the maintenance of the stem cell population.[62–64] In breast cancer cells, the interaction of p66Shc with notch 3 following hypoxic exposure leads to increased cell survival and stem cell self-renewal via expression of the hypoxia-survival gene carbonic anhydrase IX and the notch ligand jagged-1.[23] Inhibition of notch 1 signalling in adenocarcinoma of the lung has been shown to reduce hypoxic cell survival.[53] In one study, HIF-2α maintained a stem cell phenotype in glioblastomas.[65] Consequently, the antitumour potential of novel anti-HIF strategies seems to be closely related to their ability to inhibit notch signalling and target cancer stem cell populations.[66–69]

Although this interplay has not yet been established in prostate cancer, existing evidence strongly supports this link. A number of studies have highlighted a link between notch signalling and the prostate stem cell population. Gene expression analysis indicates a higher level of notch-signalling-pathway activity in prostate stem cells than in parental cell lines.[6,70] Treatment of DU145 prostate cancer cells with the hepatocyte growth factor leads to the induction of a stem-cell-like phenotype associated with activation of notch signalling.[71] Increases in notch 1 and hey-1 expression are thought to prevent the differentiation of prostate epithelial cells.[72] Immunochemistry for EMT markers in formalin-fixed, paraffin-embedded tissue from surgically resected prostate cancer specimens has outlined a key role for notch 1 expression in EMT and the metastatic process of prostate cancer.[14] In human metastatic prostate cancer cells, loss of miR-8/200 has been associated with inhibition of jagged-1 and proliferative activity.[73] Docetaxel resistance in prostate cancer cells has been attributed to a notch-overexpressing subpopulation of cells with stem-like properties.[74]

Similarly, a few studies have highlighted a link between hypoxia signalling and the prostate stem cell population. Profiling experiments have revealed significant overlap between hypoxic cells in primary prostate tumours and human embryonic stem cell gene signatures. Immunostaining of these patient specimens revealed that 69% of the primary prostate tumour cells that were positive for the stem cell marker NANOG were also HIF-1α-positive.[75] Exposure to hypoxic conditions has also been shown to enrich the CD44/CD41-positive cell population in PC3 cells[76] and induce pluripotency and EMT in metastatic prostate cancer cells.[77] Thus, reports of hypoxia-induced notch signalling in prostate cancer stem cells are expected to emerge soon.

Androgen deprivation conditions

A fundamental challenge in prostate cancer research involves understanding the transition of cancer cells from androgen dependence to androgen independence. Hypoxia is thought to participate in androgen resistance under conditions of androgen deprivation via the induction of androgen hypersensitivity[78] and the amplification of androgen receptor (AR) activity.[79] In the presence of antiandrogens, receptor inhibition is likely to involve recruitment of co-repressor proteins, which interact with an antagonist-occupied receptor, but inhibit receptor-

dependent transcription. Hey-1 has been shown to compete with AF-1 to repress transcription from AR-dependent promoters.[80]

Although, in one study, amplification of the hey-containing region of chromosome 8 was reported in the majority of individuals with androgen-independent prostate cancer,[81] hey protein expression was reduced in prostate cancer foci when compared with adjacent benign tissue.[15] Further investigation revealed that hey-1 localization is altered in cancer and hey-1 is excluded from the nucleus of prostate cancer cells.[82] Researchers have proposed that sequestration of hey-1 in the cytoplasm of prostate cancer cells prevents repression of AR-dependent genes and has a role in the aberrant hormonal responses observed in prostate cancer, ultimately participating in androgen resistance.[82]

ADT causes atrophy of the prostatic epithelium as a result of apoptosis and reduced cell proliferation. A decrease in mitotic index, low Ki67, and down-regulated VEGF are all associated with a favourable effect of antiandrogen treatment.[83–85] Recent evidence suggests a relationship between the notch-responsive, androgen-responsive, and hypoxia-responsive pathways. HIF-1α is recruited to notch-responsive promoters under hypoxic conditions to activate transcriptional targets[11] and crosstalk between AR and HIF-1α has been identified in prostate cancer cells.[86] PSA expression is induced by both hypoxia and dihydrotestosterone via a hypoxia-responsive region in the human PSA promoter, suggesting that HIF-1α competes with hey-1 under hypoxic conditions to conserve survival-promoting gene expression (Figure 2). Further research is needed to confirm the role of the notch pathway in conditions of hypoxia and in determining androgen-dependent cell fate.

Treatment resistance

The notch pathway might participate in the development of resistance to chemotherapy and radiotherapy in hypoxic cells. Given that AR activity has been associated with cytotoxicity,[87] it seems reasonable to assume that the AR might participate in the chemoresponse of hypoxic cells. Recent studies have reported that response to taxanes, the only chemotherapeutic agent proven to be efficacious in prolonging overall survival in patients with prostate cancer, is maximized under conditions of AR activity.[88] On the other hand, HIF-1α overexpression in hypoxic cells has been shown to reduce the effectiveness of microtubule-disrupting agents in a drug-dose-dependent manner in human ovarian cell lines.[89] Similarly, we have reported sensitivity of hypoxic prostate cancer cells to docetaxel and a novel microtubule disrupting agent pyrrolo-1,5-benzoxazepine 15 (PBOX-15).[90,91] Understanding the relationship between these three pathways could, therefore, uncover key targets for optimizing treatment response.

The notch pathway interacts with the PI3K and NFkB pathways, both of which are switched on by hypoxia and ionizing radiation (and possibly also by chemotherapeutic agents). These stress-responsive signalling pathways influence the cellular concentration and interaction affinity of HIF-1α, suggesting a cooperative effort of hypoxia and radiation on therapeutic resistance. Activation of RAS, RAF-1, mitogen-activating protein kinase (MAPK), p38, and PI3K pathways has been associated with radioresistance[92] and could account for hypoxia-induced survival advantages via direct inhibition of apoptosis or enhanced HIF-1α

activity.[93,94] Breast cancer, chronic lymphoid leukaemia, and myeloma cells with aberrant notch 1 signalling become chemoresistant upon inhibition of the p53 pathway by the mTOR-dependent PI3K/AKT/PKB pathway.[95,96] Silencing of notch 1 has been shown to promote docetaxel-induced cell growth inhibition, apoptosis, and cell cycle arrest in PC3 cells,[97] whereas expression of the notch 2 homologue protein has been associated with radiosensitivity in patients with locally advanced adenocarcinoma of the rectum.[98]

No studies have identified a role for notch in the radiation response of prostate cancer cells. Nonetheless, proliferative activity and neuroendocrine differentiation have both been associated with radiation therapy failure in patients with prostate cancer,[99] suggesting that the notch pathway could potentially provide a prognostic signature. High DLL4 expression has been linked with favourable outcome to radiotherapy in patients with locally advanced squamous cell head and neck cancers.[100] Inhibition of notch has been shown to enhance the sensitivity of cancer stem cells to radiation *in vitro* in a number of cancers, including nasopharyngeal carcinoma,[101] colorectal carcinoma,[102,103] pancreatic cancer,[104] and glioma.[105] Given its ability to control survival of cancer stem cells, notch targeting could help to overcome resistance to radiation therapy, as the number of cancer stem cells is a determinant of tumour control.[106]

Tumour vasculature

The notch signalling pathway is essential to the regulation of blood vessel structure[107] and defects in this pathway cause inherited vascular and cardiovascular diseases.[108] Tumour blood vessels are thin-walled, with abnormal branching and blind endings.[109] Their endothelial lining is incomplete, showing fenestrations and loss of intercellular junctions.[110] The basement membrane is also often incomplete or absent, and associated with a paucity of smooth muscle cells and pericytes.[111,112] These features suggest that cancer is a vascular disease, associated with deregulation of the notch pathway. The role of the notch pathway in the regulation of tumour angiogenesis has been reviewed previously.[47]

The blood vessel network is central to tumour biology and an important structure to consider during the development of anticancer treatments. Evidence has connected tumour aggressiveness and poor patient survival with density of microvessels, which are structurally and functionally defective in many human malignancies, including prostate cancer.[113,114] Microvessel density has also been proposed as a molecular marker for identifying high-grade prostatic intraepithelial neoplasia (HGPIN) lesions that are more likely to progress[115] and improving the prognostic stratification of patients with moderately differentiated prostatic adenocarcinoma after radical prostatectomy.[116,117] Although not yet demonstrated, notch signalling is also likely to be involved in the angiogenesis of prostate tumours.

Perfusion is relatively ineffective (owing to arteriovenous shunts) and ≤30% of the total blood flow in tumours can bypass the exchange system of capillaries.[118,119] Solid (mechanical) stress generated by proliferating tumour cells also compresses vessels in tumours.[120] As a result, the tumour microcirculation suffers from impaired, multidirectional, and intermittent blood flow, impaired interstitial fluid drainage, increased interstitial fluid pressure, and increased vascular permeability.[121] In adult rat vascular smooth muscle cells,

mechanical stress has been shown to result in reduced proliferation rate and enhanced apoptosis induction via reduced expression of notch 3, jagged-1, and notch target gene products.[122,123] This mechanism could be responsible for several vascular diseases, including arthrosclerosis. Reversal of this effect occurred when cells were transduced with a notch 3 expressing vector.[122,123] Circulation defects within tumour blood vessels might trigger a response similar to that of mechanical injury in normal vessels.

Because tumour blood vessels provide unique and specific markers, such as VEGF, many targeted cancer therapies have been developed to deprive cancer cells of nutrients and prevent tumour expansion by vascular destruction. Destruction and remodelling of the tumour vasculature have been observed by interfering with VEGF signalling.[124] Despite the fact that vascular destruction is effective at inducing tumour necrosis, the effect of antiangiogenic therapies seems to be transient. Loss of endothelial cells is not necessarily accompanied by simultaneous loss of pericytes and surrounding basement membrane, which together can result in regrowth of tumour vessels.[125] Upon completion of antiangiogenic therapy, the tumour vasculature rapidly regains its pretreatment state.[126]

VEGF stimulates the notch ligand DLL4 to act as a negative-feedback regulator that delays vascular sprouting and branching.[127] Anti-DLL4s have been shown to inhibit tumour growth *in vivo* by triggering excessive, but nonfunctional, angiogenesis—suggesting potential roles as novel anticancer agents.[128] In a murine model, DLL4 blockade resulted in the formation of a dense and disorganized capillary network that was unable to supply limb muscles with adequate perfusion (femoral artery ligation).[129] However, the blockade of notch pathway via treatment with a low or intermediate dose of a soluble DLL4 fusion protein (DLL4-Fc) prompted the growth and maturation of new vessels in a hind limb ischaemia mouse model, enabling enhanced perfusion and increased vascular proliferation.[130] By contrast, PC3 cells transplanted with a retroviral vector to induce DLL4 overexpression displayed decreased vessel density and vessel number (yet exhibited larger vessels with larger lumina) and decreased numbers of apoptotic cells, resulting in better perfusion and decreased hypoxia *in vivo*.[131] These contradictory data could indicate that DLL4 has a role in the development of tumour vasculature, but its effect depends on the amount of protein present in the cell.

The jagged-2 ligand is upregulated in hypoxic breast cancer cells, and its expression significantly correlates with genes involved in the angiogenic process.[51] Depletion of jagged-2 from the epithelial T47D breast cancer cell line has been shown to result in a reduction of MS1 cell tube formation.[50] Other research suggests that hypoxic conditions lead to the induction of the notch ligand DLL4 and the notch target genes hey-1 and hey-2 in endothelial progenitor cells. Hey factors are also capable of repressing HIF-1α-induced gene expression in endothelial progenitor cells, suggesting a negative feedback loop to prevent excessive hypoxic gene induction.[132] Induction of the notch ligand jagged-1 by growth factors (via MAPK) in head and neck squamous cell carcinoma cells triggers notch activation in neighboring endothelial cells and promotes capillary-like sprout formation.[133]

A combination of intermittent hypoxia and ionizing radiation results in HIF-1α overexpression, enhanced endothelial cell migration and tube formation capacity, and increased radioresistance.[134] The expression of DLL4 in endothelial cells is synergistically

upregulated by VEGF and basic fibroblast growth factor, as well as by HIF-1α under hypoxic conditions.[135] Downregulation of hey-1 in response to DLL4-targeted RNA interference leads to inhibition of endothelial cell proliferation, migration, and network formation. Thus, hey overexpression resulting from hypoxia-induced DLL4 upregulation might contribute to radioresistance.

Modulation of notch signalling

A number of studies have investigated the potential of the notch pathway as a novel therapeutic target.[136] The notch 1 receptor and jagged-1 ligand both have key roles in the progression and metastasis of prostate cancer and, therefore, represent potential therapeutic targets.[13] Downregulated expression of the genes encoding notch 1 or jagged-1 has been shown to result in decreased prostate cancer cell invasion.[57,137,138] Downregulation of notch 1 and jagged-1 with small interfering RNA (siRNA) led to inhibition of cell growth, migration, invasion, and induction of apoptosis in PC3 prostate cancer cells.[139] In one study, inhibition of notch 1 and jagged-1 also led to activation of the AKT pathway, mTOR, and genes downstream of *NFκB*.[138] In PC3 and C42B cell lines, downregulation of notch 1 with siRNA significantly decreased expression of *FOXM1*, which is overexpressed in prostate cancer cells and has been associated with carcinogenesis.[139] This effect seems to be caused by the inactivation of AKT, one of the notch 1 downstream target genes.[139] Although most studies have focused on the downregulation of notch 1, one study established that the retroviral transduction of the active form of notch 1 into DU145 cells led to reduced cell migration and reduced repopulation of wounded monolayers when compared with nontransduced controls.[15] Finally, treatment with the isoflavone genistein resulted in significant downregulation of notch 1, AKT phosphorylation, and foxM1 expression and inhibition of tumour growth in the PC3 and C42B cell lines.[139]

The RBPJ transcriptional repressor is another identified target.[140] RBPJ expression was successfully knocked down by lentiviral-based transfer of RBPJ-specific small hairpin RNA in the PC3-CMVluc prostate-derived cell line. This knockdown was associated with decreased cell proliferation, changes in the expression of notch pathway genes, and loss of RBPJ DNA binding activity.[46] Modulation by miRNA has also been studied. Transient transfection of vectors expressing miR-200 and miR-141 decreased the concentration of jagged-1 and inhibited cell proliferation in PC3 cells.[73] On the other hand, overexpression of miR-34a decreased the expression of notch 1, AR, and PSA in LNCaP and C42B prostate cancer cell lines.[141] Γ-secretase inhibitors are the only notch inhibitors currently being evaluated in clinical trials, with clinical benefit reported in patients with advanced solid tumours[142,143] and breast cancer.[144] The efficacy of these compounds remains to be assessed in men with prostate cancer.

Conclusions

The notch signalling pathway has been associated with tumorigenesis in a number of cancer types. Current research suggests its involvement in prostate cancer. At the same time, hypoxia is becoming widely accepted as a feature of solid tumours, but evidence of its role in disease progression remains limited, despite the fact that the hypoxic response triggers

critical molecular pathways. The notch pathway has been proposed as a therapeutic target for the treatment of prostate cancer, lung cancer, and other hypoxic tumour types. A number of studies have experimented with modulation of the notch signalling pathway in order to develop novel treatments for prostate cancer, yet few have studied the role of notch in the response of cells to hypoxia. Close examination of the interplay between notch and hypoxia signalling pathways has the potential to provide further insight into the regulation and treatment response of the cancer stem cell population while expanding our understanding of angiogenesis in prostate tumours. Further evidence for this potential crosstalk is required in prostate cancer. Future studies should contribute to the identification of a molecular signature for disease progression and treatment response in prostate cancer and, ultimately, help to identify novel therapeutic targets.

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Key points

- Evidence to date suggests dominant roles for notch 1 and the notch 1 ligand jagged-1 in the development of prostate cancer
- A number of parallels exist between the cellular behaviour of notch-activated and hypoxic prostate cancer cells
- The antitumour potential of novel strategies that target hypoxia-inducible factor 1 (HIF-1) seems closely related to their ability to inhibit notch signalling and target the cancer stem cell population
- Notch and hypoxia signalling pathways might compete to control androgen-dependent molecular responses

Review criteria

The PubMed database was searched using combinations of the search terms “notch”, “hypoxia”, “prostate cancer”, “stem cell”, “androgens”, and “radiation”. Peer-reviewed English-language papers were considered for inclusion in the manuscript. The reference lists of identified publications were searched for additional articles.

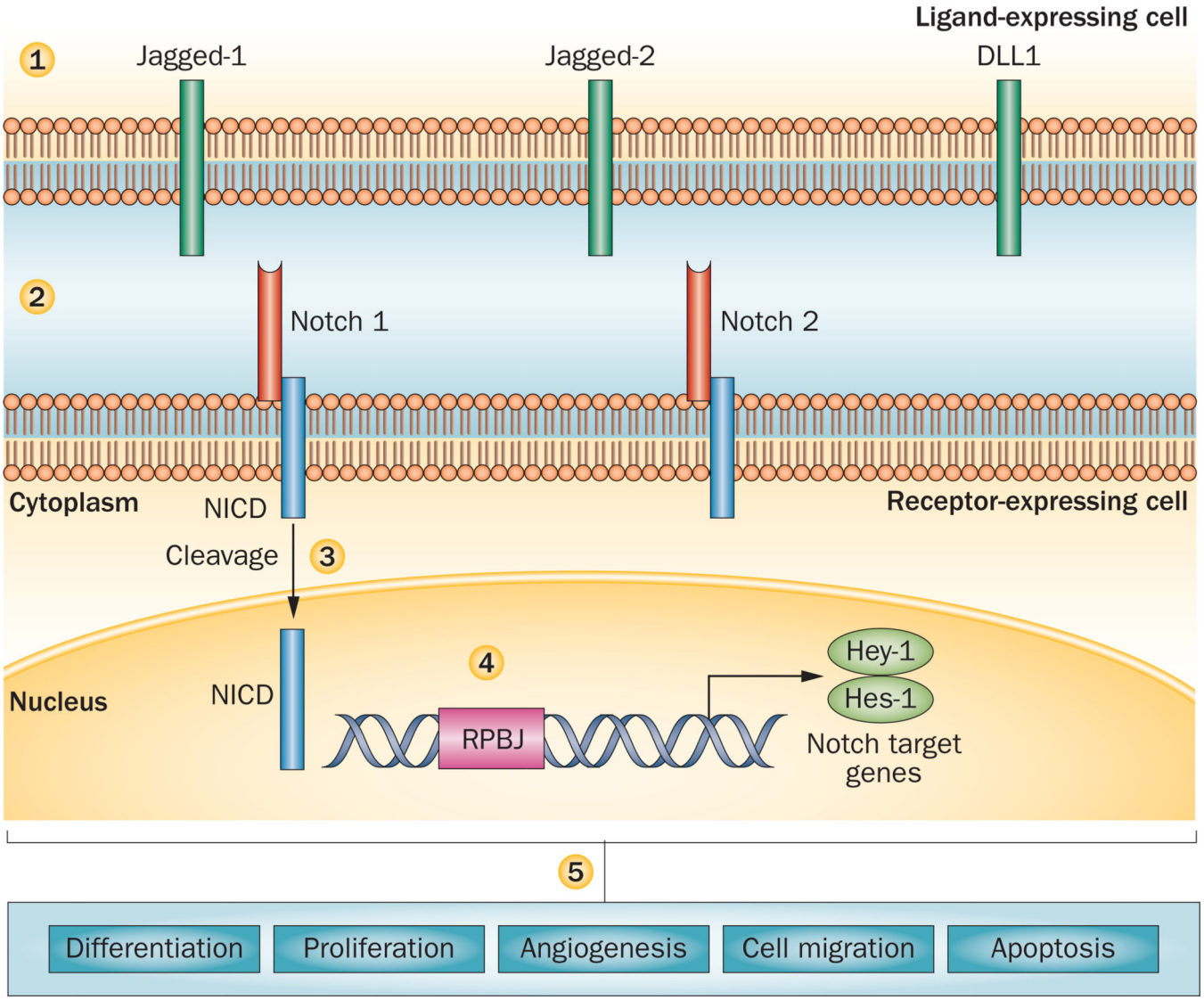

Figure 1.

The notch signalling pathway in prostate cancer. The notch ligands jagged-1, jagged-2, and DLL1 are overexpressed in prostate cancer cells (1). The notch receptors notch 1 and notch 2 are overexpressed in prostate cancer cells (2). Ligand-receptor interaction leads to cleavage of the notch intracellular domain (NICD), which translocates to the nucleus (3). Binding of the NICD to the transcriptional repressor RBPJ in the nucleus leads to the expression of notch target genes *HES1* and *HEY1* (4) and the regulation of differentiation, proliferation, angiogenesis, cell migration, and apoptosis (5). Abbreviations: NICD, notch intracellular domain; RBPJ, recombining binding protein suppressor of hairless.

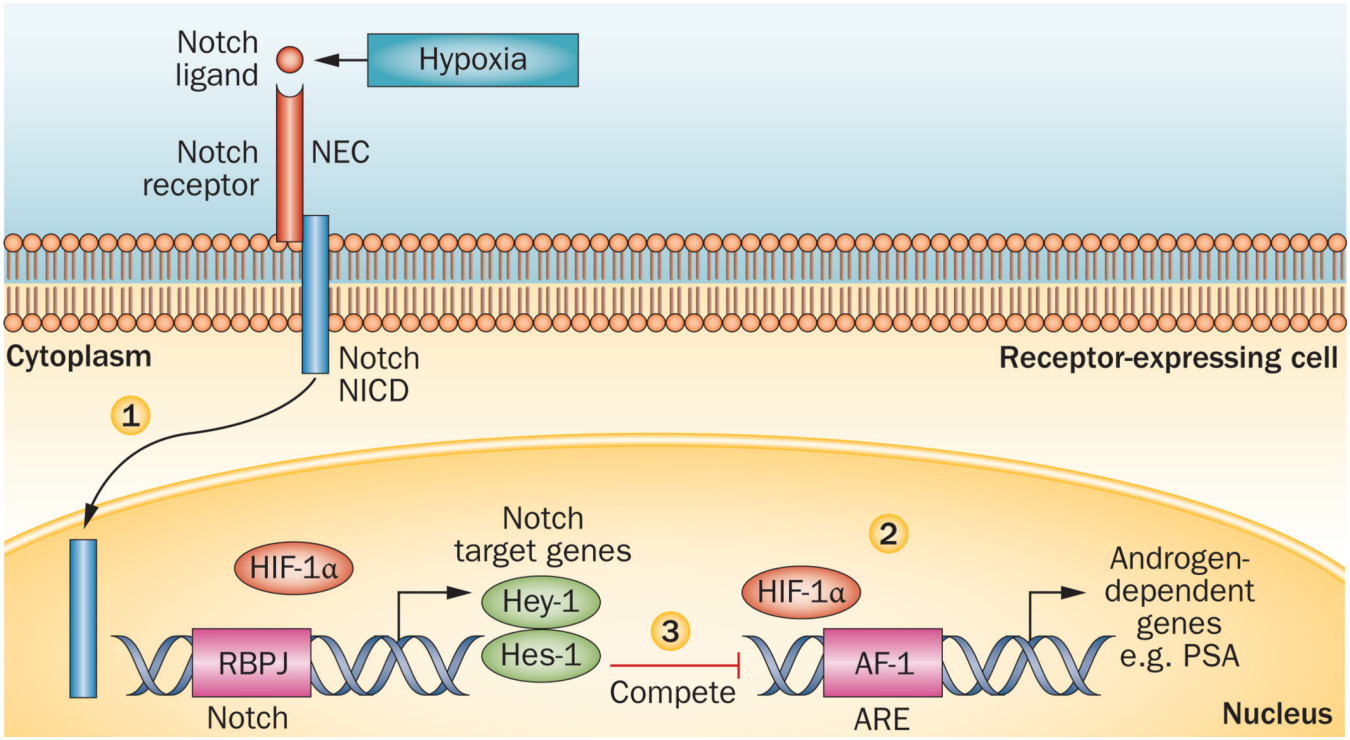

Figure 2.

Hypoxia can activate notch, and androgen-dependent gene expression. Hypoxia increases the expression of notch ligands and triggers the notch signalling pathway (1). HIF-1α is recruited to notch-responsive promoters and induces expression of the notch target genes *HES1* and *HEY1*. PSA expression can be induced by hypoxia owing to the presence of a hypoxia-responsive region in the human PSA promoter (2). Hey-1 competes with AF-1 to repress transcription of androgen-dependent gene expression (3). Hey-1 and HIF-1α might, therefore, compete to maintain androgen-dependent gene expression. Abbreviations: ARE, androgen responsive element; NED, notch extracellular domain; NICD, notch intracellular domain; RBPJ, recombining binding protein suppressor of hairless.