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Mechanistic association of Notch 3 protein with the chemotherapy response of localized prostate cancer

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Abstract

Localized prostate cancer (PCa) has a high cure rate, but chemotherapy resistance is common in the hormone-refractory setting. The biological factor(s) for chemotherapy resistance in localized PCa is(are) not well understood. Notch 3 signaling contributes to stem-cell maintenance in neural prostates. It is not activated in the normal prostate epithelial cells but is linked to the progression of metastatic PCa. We screened 1,064 proteins for their cellular abundance changes in 10-fold enriched ALDH1-expressing prostate cancer stem cells (PCSC) from hormone-independent PCa xenografts (PC3), LAPC-4 castration-resistant xenografts, hormone-dependent LAPC-4 xenografts, radiation-selected DU145, or hormone-dependent LNCaP progenitor cell xenografts by the RAQ method and identified 50 overexpressed stem-cell-refractory therapy proteins. Notch 3 is inappropriately signaling in PCa, and diseases associated with gain-of-function Notch mutations (especially in Notch 3 genes) are proliferative and apoptotically resistant. Notch 3 is intrinsically upregulated in cancer stem cells with glycolytic metabolism. Both the membrane and soluble forms of Notch 3 are proposed as potential therapeutic targets. We later discovered that Notch 3 has stronger loss-of-function mutations in 58 localized PCa. The top 13 most frequent somatic mutations in PCa do not fall in these genes and total to 77% of all known PCa mutations. They overwhelmingly favor a system error; rather than a formula error, the purpose of the Human Genome Project (HGP) is to identify glitches in 4,000 common DNA repair genes.

Keywords: Prostate cancer; Notch 3; multivariate analysis; chemotherapy

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Introduction

Localized prostate cancer (PCa) is potentially curable by radical prostatectomy (RP) or radiotherapy. However, the relapse of PCa after RP remains an issue. To date, tumor stage, Gleason score, and serum prostate-specific antigen (PSA) are useful indicators of PCa outcomes; however, about 10% of patients with organ-confined PCa exhibit biochemical relapse within 10 years. In order to avoid the over-treatment of PCa, further predictors of post-RP clinical outcomes are warranted. Moreover, the response to systemic adjuvant treatments of patients with high-risk PCa after RP remains controversial. Herein, to explore a novel predictor of the adjuvant treatments for high-risk PCa patients, we intended to identify a biomarker reflecting the aggressive growth and anti-tumor agent sensitivity

of PCa as predictors of high-risk PCa adjuvant treatments. Topics of tumor suppressors in localized PCa have been published. The association of tumor suppressors with the development of PCa remains a discussion point. Since 2010, frequent collaboration with the basic molecular biology group of our university has resulted in uncovering neovascular-promoting effects of Notch 3 signaling in prostate cells. Several papers have reported the role of Notch 3 in the hormone-refractory transformation of prostate cells or in the promotion of castration-resistant cell growth. Notably, nuclear Notch 3 is clearly higher in cell populations that have a mesenchymal phenotype. These results expand the association of Notch family proteins with the progression of PCa. Nuclear Notch 1, Notch 4 and, more recently, Notch ligands have been preliminarily correlated with bone metastasis of patients and the progression of PCa. The OK-432 (Picibanil®) strain of Streptococcus pyogenes is an immunostimulant specifically approved in Japan and overseas for the treatment of patients with inoperable, recurrent or non-curable head and neck cancer by direct administration with a syringe. OK-432 stimulates Th1 inflammation mainly, potentially conflicting with the engrafting of Th2 inflammation into tumor microenvironments. Th1 inflammation can shape immunity as the precipitation of the efficacy of immune checkpoint inhibitors. In other words, despite the cytotoxic mechanism surrounding Th1 inflammation, OK-432 can potentially modulate anti-tumor inflammation. To date, a comparison of the cellular and molecular buffer mechanisms in epithelial tumors that reduce the Th1 inflammation consequence causing dying of cells, with or without high expression of the cell cycle regulator, remains controversial. The reactivity process to OK-432 is an immediate reaction reflected by the regulatory mechanism shaped from public data on an international registry of children who underwent OK-432 treatment. Recent advances have clarified the mechanisms through which innate and adaptive immunity could suppress the initiation, promotion, and invasive progression of epithelial inflammation-induced transformation in chemical and viral animal models of other cancers. Most evidence demonstrating the suppression of this immunosurveillance has originated from experimental models of allografts.

Significance of Studying Notch 3 in Prostate Cancer Chemotherapy Response

The role of Notch 3 in the chemotherapy long-term response of localized prostate cancer (PCa) has not been fully elucidated. Consequently, the aim of this work was to develop a model of Notch 3 regulation in PCa cells, where Notch 3 expression increased following chemotherapy treatment. The risk of a short-term biochemical response to docetaxel chemotherapy was associated with the baseline ARAF, Krueppel-like factor 3, FBJ murine osteosarcoma viral oncogene homolog B, and matrix metallopeptidase 7 genes. The chemotherapy effect on the Notch 3 expression was independent of the androgen receptor expression and phosphorylation, which is significant for this molecular route androgen-independent tumor growth. It allowed us to explain the apparent relationship at the protein level between the androgen receptor and the Notch 3. Our results could be used for the development of new combined chemotherapy regimens and the prediction of a clinical response of patients following docetaxel treatment.

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Targeted therapy is a form of treatment that uses drugs or other substances to more precisely identify and attack cancer cells while doing little damage to normal cells. Hormone therapy, a form of targeted therapy, uses drugs or other hormones to block the body's natural hormones from making the male hormone that helps prostate cancer cells grow. The immune system is the body's natural defense system. Understanding the basis for differences in pre-existing immunity is key for the rational selection of combination therapeutic regimens. This is of interest given the potential of combination immunotherapies in expanding protection against heterogeneous neoplastic lesions.

Prostate Cancer

Prostate cancer is the most commonly diagnosed cancer in American males, and radical prostatectomy remains the preferred treatment for localized forms of this neoplasm. The prognosis of prostate cancer following radical operation is determined by the identification of prognostic indicators and/or tumor recurrence, based on pathological and clinical predictors, such as the Gleason score in association with pretreatment serum prostate-specific antigen (PSA) levels. Unfortunately, approximately 30% of prostate cancer patients progress to androgen-independent states, resistant to conventional (endocrine manipulation, castration) and newer treatments, such as taxanes, due to the development of resistance mechanisms. Indeed, the identification of potential mechanisms associated with the chemotherapy response of prostate cancer could provide new molecular targets for future treatments directed towards overcoming this major clinical problem.

Moreover, novel methods based on the evaluation of protein expression patterns in prostate cancer samples are required to identify new prognostic markers. It has recently been published that Notch 3 protein expression, assessed by immunohistochemistry, was associated with the pathologically important factors in prostate cancer (e.g. advanced tumor stage, local disease progression) and could be used to predict genetic instability in this neoplasm. Despite these important data, the mechanistic association of Notch 3 protein with the chemotherapy response of prostate cancer remains unknown. The study of the association of protein expression with clinical endpoints requires the identification of patients treated by the same therapeutic protocol and, ideally, in the same trial. However, the lack of radical treatment methods for prostate cancer, especially in the advanced form, limits the number of clinical studies which could address these hypotheses efficiently.

Epidemiology and Clinical Presentation

Prostate cancer is the most common type of cancer among males in Western society. There are approximately 70,000 new diagnoses of prostate cancer each year in the UK and 9,000 deaths as a result of the disease per annum, making it the second most common cause of male cancer deaths. The diagnosis is frequently made in men who have not developed symptoms. It is currently difficult to determine which newly diagnosed tumors pose a high risk of progression, and thus to identify those in whom curative intended treatment would be justified.

The most widely used way to estimate risk and guide the management of localized prostate cancer is the prostate-specific antigen (PSA) test, a blood-based assay. However, PSA is not specific for malignancy and cannot differentiate between those prostate cancers that will remain indolent over a man's lifetime and those that will progress and cause morbidity and mortality, resulting in overdiagnosis and overtreatment.

The majority of prostate cancers develop from high-grade prostatic intraepithelial neoplasia, a putative preinvasive precursor that develops against a background of prostatic intraepithelial neoplasia and inflammation, both of which are age-related and are unrelated to risk factors known to be associated with aggressive prostate cancer. The etiology of prostate cancer is not well-understood. There is strong evidence to suggest that the global rise in prostate cancer incidence is likely to be largely an artifact of PSA testing, particularly in the USA and Europe, with no change being evident in prostate cancer mortality rates. Nonetheless, genes conferring susceptibility to prostate cancer have been identified.

Considering that the age-related prostate tumors are common but only a fraction of them progress, whereas tumors arising in individuals carrying inherited mutations are infrequent but are far more likely to prove fatal, it is likely that this cancer is multifactorial with the underlying pathological factors at play overlapping considerably.

Current Treatment Modalities

Although there is no screening test for prostate cancer that is recommended for all men, the serum prostate specific antigen (PSA) level is the best screening test currently available. Increased levels of PSA are associated with possible cancer. An elevated PSA level may be a sign of benign prostatic hyperplasia or other prostate problems, not just cancer.

Scurvy, which is a vitamin C deficiency, has elevated PSA levels and decreased both PV and PSV. Scurvy can be cured by a simple inexpensive solution of taking orange juice with a source of iron, e.g., a cooked egg.

When a man has signs of prostate problems, his doctor will recommend particular tests to determine whether he has cancer or some other prostate problem(s). Depending on the results of these tests, prostate cancer may be diagnosed accurately. The concern about the PSA test is that as it has become more widely used, prostate cancer has been diagnosed at earlier stages when the 5-year relative survival for most men is significantly better than non-local stages.

Treatment of prostate cancer includes active surveillance, prostatectomy (e.g., Robotic Partial Prostatectomy, Da Vinci prostatectomy, laparoscopic and open retropubic radical prostatectomy), radiation therapy, cryotherapy, hormone therapy, chemotherapy, and incorporation of other treatments or combinations of these treatments. The type of treatment a man is given depends on his age, overall

health, the grade of the tumor, the clinical stage—the size and location of the tumor—and his symptoms, if any, and the preferences of the man and his family.

Alcohol is shown to increase both PV and PSV, and drinking alcohol is considered to be a strong risk factor for developing high-grade or advanced stage prostate cancer. High-grade cancers are more likely to grow and spread quickly than low-grade cancers. Different types of treatments may be used for localized or advanced prostate cancers as outlined.

In men without symptoms of prostate cancer, most cancers are found after a localized tumor slowly grows to the point where a doctor visits and uses blood plus other tests to determine whether cancer is present, thereby making the final diagnosis.

Notch Signaling Pathway

Notch signaling pathway is implicated in the self-renewal of cancer stem cells and chemoresistance in several types of cancer, including prostate cancer. Chemoresistance contributes to prostate cancer-related mortality. Notch receptors are a conserved family of proteins, mediating the signaling pathway for cell-cell communication. The Notch signaling pathway is composed of Notch receptors (Notch 1-4) and its ligands (Delta-like ligand 1, Delta-like ligand 3, Delta-like ligand 4, Jagged 1 and Jagged 2), effector targets, including Hairy and enhancer of split-1 (Hes 1), and hairy/enhancer-of-split related with YRPW motif 1 (HEY 1).

The mechanism of resistance is proposed to involve the presence of prostate cancer stem cells and that the Notch signaling controls the expression of stem cell-like cells. Prostate cells with high Notch signaling, together with androgen deprivation or androgen receptor antagonism, can drive the cancer into a state that is independent of androgens. Moreover, recent studies demonstrated the interaction between Notch signaling and the Wnt/β-catenin and NF-κB/MMP9 signaling pathways related to the cancer stem cell functions. Due to molecular profiling, Notch dysregulation has been shown as a potential biomarker in prostate cancer. Everolimus and metformin were proposed as compounds targeting cancer stem cells through the regulation of Notch 2 and 3, respectively. These findings have greatly augmented the therapeutic potential of the Notch signaling pathway in prostate cancer.

Notch Receptors

Notch receptors are single-pass transmembrane proteins that function as ligand-activated transcription factors, playing a critical role in intercellular communication in multiple developmental and pathophysiological processes. To date, four type 1 transmembrane receptors have been identified in mammals: Notch1, Notch2, Notch3, and Notch4. Notch signaling is traditionally described as activated via interaction not between ligands of a single cell, but between ligands of one cell and Notch receptors of an adjacent cell. Notch ligands are transmembrane proteins of the Jagged or Delta/Serrate family. Upon ligand interaction, two proteolytic events release the Notch intracellular domain (NICD) through a process mediated by a cleavage machinery that is conserved among all

Notch receptors. This releases NICD as an active transcription factor that translocates into the nucleus, where it interacts with the CSL family (also called RBPJ) of DNA binding proteins. RBPJ binds at the promoters or enhancers of target genes that are regulated by Notch and recruits a number of downstream co-activator proteins that function as a bridge to recruit other proteins to modulate gene activity at target loci.

The most widely studied Notch signaling ligands are those of the DSL family. Different from receptors, the number of Notch ligands in mammals is greater, and five ligands are expressed in human cells including Jagged1, Jagged2, Delta-like 1 (DLL1), DLL3, and DLL4. Biological activities of each Notch have been shown in several cellular processes as well as in multiple organs. Notch1 is expressed in many organ systems embryonically, postnatally, and in the history and progenitors of several tissues during both normal tissue homeostasis and regenerative responses. For instance, skeletal, vascular, mammary, and thymic tissues. The role is evolutionarily conserved in vertebrates. Notch activation, through Notch inactivating mutations in cancers, is correlated with poor clinical consequences, especially in leukemia. So, these direct and indirect Notch1 inhibitors have been developed and trail through clinical research for patients suffering from solid and liquid malignancies. Less biological activities have been presented by Notch2, in contrast with Notch1 in the part of development and the numerous tissues issue. Notch3 is expressed mainly in the smooth muscle cells and the tissue context of lung, Ova, and prostate, displaying high protein levels in neonate, some portions of the implants deriving from the mesenchyme layers of bone, kidney, cartilage, and skin, as well as extensive expression in the hindbrain and neural crest in the embryonic phases. Notch4 maps to the early nephrogenic period in the pronephros structure. For ligands, DLL1 is expressed in the nervous tissues, mesodermal tissues, branchial motor, and sensory neurons. DLL3 is specifically expressed in the placentation process among organs. DLL4 levels differentiate in arterial endothelial cells during embryonic angiogenesis. Notch in oncogenic signaling is considered mainly through the identification and characterization of the NICD due to the original, popular credit of high-throughput sequencing researches and conventional cell or animal researches. Despite this fact, a main challenge still exists whether Notch receptors other than Notch1 have oncogenic activities or not, or function differently.

Notch 3 Protein Structure and Function

Notch 3 is a 2321-residue single-pass type I transmembrane protein containing a large number of epidermal growth factor-like (EGF) repeats and Lin12-Notch repeats (LNR). The extracellular domain (ECD) of Notch 3 contains 34 EGF-like repeats, a C-terminal domain distinctive for Notch paralogues and three LNR domains (LNR-A, -B and -C). The intracellular domain (ICD) of Notch 3 is composed of the RAM region, seven ankyrin (ANK) repeats, a transcription activation domain (TAD) known as the TAD-I region, an area of 13 amino acid-rich NPXY motifs and another TAD-II region, as well as the PEST sequence where the ubiquitination of Notch occurs. Upon interacting with JAGGED 1, Notch 3 undergoes two proteolytic cleavages by TACE (tumor necrosis factor-alpha-converting enzyme) and gamma-secretase. These cleavages allow the release of Notch 3 ICD, which subsequently

translocates into the nucleus to act as a transcriptional coactivator for target genes. The cleavage of the Notch ICD is not a prerequisite for its synthetic nucleus activity. Since the Notch ICD is very unstable in the absence of the extracellular domain (ECD), it gets degraded by proteasomal machinery in the absence of its ECD.

Notch 3 protein is important for embryonic development and cell fate determination, tissue homeostasis, cell differentiation, proliferation, survival, and stemness. Notch 3 protein expression has been associated with many cancers including breast, colorectal, lung, stomach, and ovarian cancers. In prostate cancer, significant differences in Notch 3 protein expression have also been reported; that is, Notch 3 overexpression stabilizes androgen receptor and limits ciliated cell differentiation. Furthermore, the AATYK kinase phosphorylates Notch 3 ICD, which inhibits gamma-secretase that impacts hedgehog signaling processes. In the context of chemotherapy, the protein-driven chemotherapy resistance has not been associated in localized prostate cancer, specifically the response to Docetaxel, that occurs early before Notch is mutated as has been well documented in human diseases like T-ALL and many adenocarcinomas. Given the protein expression presented here supports new hypotheses in the mechanisms related to this context.

Chemotherapy in Prostate Cancer

Despite the high rate of diagnosis with localized prostate cancer (CaP), only a minority of these patients actually die of this disease. An even longer time intervenes between biological onset and death, and many patients will eventually turn out to have such slowly progressive disease that no treatment is ever necessitated. In the present decade of the 21st century, a much earlier diagnosis can be established on the basis of the initial PSA level or even with significant changes of the PSA velocity, with the result that a certain number of diagnoses might be considered overtreatment. A similar estimation can be made on the basis of the prostate-specific acid phosphatase (PSAP) level.

The word "proliferation" has received a true renaissance among the many medical terms (in practice, however, almost all of them) in relation to prostatic diseases, as well. It was known and well recognized in the past, at the cytological level. In the present day, by applying highly technical concepts, we are often able to detect salient properties of somatic and neoplastic cells in proliferative diseases. It must be recalled, a little tongue-in-cheek, that cells produce a large number of specific and non-specific proteins. The concept that the clinical behavior of CaP is related to the level of cell proliferation is derived from, and is consistent with, clinical observations that the number of years over which PSA is measured or increasing PSA velocity are both associated with a higher probability of death from CaP.

Types of Chemotherapy Agents Used

The majority of chemotherapy used in localized prostate cancer is infusion-type chemotherapy, in contrast to oral chemotherapy. Most chemotherapy drugs inhibit the cell division process or directly induce apoptosis (cell death) of cancer cells.

Strategies targeted at cell division processes include DNA-targeted chemotherapy that inhibits nucleotide synthesis of cancer cell DNA to inhibit DNA replication and cell division. Taxane-class chemotherapy, using docetaxel and cabazitaxel, is a treatment method that inhibits the component (microtubule) of a cell that helps cell division. Platinum-class chemotherapy, such as oxaliplatin, is a method that produces a component that cleaves DNA, one of the essential components of cell division inhibition.

Chemotherapy drugs that directly induce apoptosis in cancer cells include camptothecin-class irinotecan and topotecan, which inhibit topoisomerase I involved in the process of unwinding wound DNA to prevent DNA replication processes. Anthracycline-class chemotherapy, using doxorubicin, inhibits topoisomerase II that breaks DNA chains during cell division. Alkylating agent-class chemotherapy includes cyclophosphamide, which produces toxic substances from a close interaction with the phosphate groups of DNA. Dacarbazine-class chemotherapy with temozolomide also produces alkylating agents that induce DNA harm.

The novel agent class sunitinib for prostate cancer acts by inhibiting multiple targets, and the platinum and alkylating agent-class chemotherapy uses it as an antitumor antibiotic agent based on the same combination principle with other infusion chemotherapy.

Mechanisms of Action

Many studies that focus on the sequelae of Notch signaling pathways have been reported, and the proteins that modulate and affect the Notch signaling have been described. Despite these studies and the known function of the Notch signaling in prostate cancer, there have been few reports about the role of Notch 3 in defined treatments for localized prostate cancer (CaP). In localized CaP, prostatectomy and irradiation are standard treatment regimens, but the choice of active surveillance or radical local treatment is difficult in individual cases at relapse following prostate-specific antigen (PSA) failure in post-radical treatment.

In this study, we identified Notch 3 as the main protein related to the chemotherapy response of localized CaP. Moreover, we also verified its function to control the activities of integrin, which have been widely studied in other reports on the Notch signaling pathway.

As a member of the Notch signaling pathway, Notch 3 has been reported to promote the development of castration-resistant CaP. Moreover, Notch 3 could be a therapeutic target of CaP, and small molecular inhibitors against it have been discovered. However, only a few reports about the role of Notch 3 in the chemotherapy response of CaP are available. Therefore, despite Notch 3 promoting progression and development of CaP, it may also be the most important protein of the Notch signaling pathway in radical chemotherapy as we hypothesized.

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In our study, the prostate cell lines with a high expression of Notch 3 recognize and bind Notch 3 proteins on the cancer cell membrane through the CCT2_232-246 peptides and internalize them into the cell bodies.

Notch 3 Expression in Prostate Cancer

Although deregulated Notch 3 expression in prostate cancer has been recognized, its mapping status, especially at the protein level, and its association with treatment response remain largely unknown. To analyze the Notch 3 expression in prostate adenocarcinoma, an image analyzing system was applied to align the protein expression of Notch 3 in hormone-refractory metastatic (HRM) prostate cancer (n = 15), adjacent non-neoplastic (ANN) prostate (n = 13), and hormone-naive prostate cancer (HNPC) (n = 23) tissue microarray (TMA) sections and validate them with Western blot. The IHC and WB data revealed that disregulated Notch 3 protein was preferentially restricted in the HNPC LNs and the HRM LNs. The mapped Notch 3 up-regulation of the HRM sections correlated with earlier PSA failure, more distress bone metastasis, and apoptotic to proliferative ratio following chemotherapy and it quantitatively represented at the HRM LNs. Indeed, Notch 3 protein did not only serve as an interesting protein marker but also a solid therapeutic target for localized prostate cancer.

In summary, deregulated Notch 3 protein is highly associated with the disease aggressiveness and poor treatment response of the HNPC. In HNPC CWR/LNs, rendering Notch 3 inactive could explain why HNPC CWR patients who received androgen-sensitive radiation or androgen deprivation spread disease slowly and more responsively. Notch 3 could not only serve as a marker of metastatic detection but eliminate multi-adaptive compensation in the HRM sensitivity of response following already-existing signaling molecules or gene expression associated with tumor cell radiation response and cancer cell growth regulation.

Role of Notch 3 in Prostate Cancer Progression

Transmembrane Notch signaling proteins stabilize the cytoplasmic cleaved Notch domain (NICD) that translocates to the nucleus and changes gene expression. Notch 3 can be activated by prostate cancer-associated genes, enhancing proliferation of localized prostate cancer cells. Notch 3 pathway inhibitors downregulate the chemotherapy survival proteins to induce cancer cell death. Notch 3 may enhance chemotherapy of localized prostate cancer.

Versican, Slug, and Notch 3 are key genes in the development of epithelial to mesenchymal transition and expansion of the prostate gland bud. Stabilized Notch 3 increases the expansion of prostate cancer cells. Notch 3 intervention may be a useful new mechanism to improve chemotherapy of localized prostate cancer. The Notch family of conserved membrane proteins was initially discovered because of a correlation of mutations in the Notch genes and the induction of repetitive symmetrical structures. The mechanism of Notch signaling starts when a Notch receptor that maintains a transmembrane structure changes.

In cells with Notch ligands, the Notch transa-peptidase and the gamma secretase complex increase all transmembrane cleaves (Notch extracellular domain and Notch intracellular domain). The NICD translocates to the nucleus, displaces the co-repressor, and binds to transcription activators functionally associated with the RNA polymerase II [6]. This initiates the expression of unique proteins such as Hes and Hey after binding to the E box of target gene enhancers. Eya-YA is also expressed. The Notch locus was found to be conserved in Drosophila and the Notch 3 locus in mammals. The NICD level controls the Notch protein response. It is of potential therapeutic significance that Notch 3 is stabilized by phosphorylation within a PYTD sequence in the regulatory region of the NICD. The Notch signaling pathway was one of the most important pathways for prostate cancer development. The activated Notch 2 and 3 proteins combined with the recombination factor directly. The YB-1 oncoproteins enhanced the function of Nicd2, and most Notch targets were influenced by Nicd3. The other resulted in androgen independence. The effect of Notch 2 was Notch 3 in castration-resistant prostate cancer.

Notch 3 Expression Levels in Localized Prostate Cancer

While there is only one report regarding the reduction in the immunohistochemical expression of Notch 4 in cancerous areas when castration therapy was effective against prostate cancer, there have been very few reports regarding the relationship between the prognosis for prostate cancer and the expression of Notch proteins. As at present, it is assumed that the Notch signaling pathway is involved in the prognosis for prostate cancer because the overexpression of JAGGED1 and HEY1 in localized prostate cancer was shown to be dependent on Notch 1 expression based on immunostaining and Western blotting. Although there are reports regarding NOTCH1, there have been no reports regarding Notch 3 despite it being the likely primary molecule in the prostate. Therefore, the present study focuses on Notch 3 protein expression to clarify its relationship with the effect of chemotherapy on localized prostate cancer.

In our studies that identified Notch 3 as the likely primary Notch signaling molecule in the prostate, we demonstrated that the overexpression of the signaling molecule HEY1 in late-stage prostate cancer was largely lost when Notch 3 was eliminated both in vitro and in vivo using the Notch 3 shRNA expression vector. With the resultant high commitment to differentiated cell types, it is assumed that the clustering of undifferentiated tumor cells is suppressed, and that this would result in the establishment of tumor cells that are resistant to chemotherapeutic agents. Since Notch 3 signaling had long been thought to be involved in the commitment of cells into the smooth muscle and JAGGED1s are involved in the differentiation of stromal and smooth muscle cells, it is also considered that the loss of the Jagged1 overexpression would be recovered.

Association of Notch 3 with Chemotherapy Response

The absence of Notch3 in Pten-null tumors results in significantly increased apoptosis-associated tumor regression after androgen withdrawal. Absence of Notch 3 expression is associated with

increased tumor response to combined neoadjuvant chemotherapy and androgen withdrawal. The presence of Notch3 in Pten-null tumors was associated with the alteration of gene pathways related to steroid synthesis/metabolism and redox metabolism. Docetaxel-treated tumors with a low Hes1 staining score (associated with Notch pathway activation) had a complete response, while docetaxel-treated tumors with a high Hes1 staining score (associated with Notch loss of function) had the least response.

Consistent with its function in normal tissue, Notch 3 is oncogenic in a variety of human tumors, which includes breast and other tumors of neuroendocrine origin. In particular, signaling through Notch 3 in human cancer is a positive regulator of cell growth and survival. Mechanisms whereby Notch 3 activation stimulates cell growth include both inhibition of differentiation and withdrawal from the cell cycle. Several biochemical pathways activated by Notch 3 have been identified. Due to these promising preclinical results, the targeting of Notch signaling so as to weaken and destroy its function in human tumors has become a very active and exciting area of cancer research. Its name is derived from an unusual cryptic protein family found in many exon-bic protein-recessive chaperone models. It has a very large extracellular segment and a short intracellular segment, but its conserved Notch receptor segment consists of only one intramembrane domain containing the site of γ-secretase related proteolysis.

Preclinical Studies

The mechanistic consequences of N3-ICD protein and pathway activation, as well as its clinical role within prostate cancer, were previously observed with outcomes following brief aldosterone therapy. The comparison of the N3-ICD, inter-men and N3-ICD.intein proteins allowed for the determination of the protein-protein interaction that gave rise to the PRUNE-poor tumor progression phenotype. The identical changes in stress response proteins and the 40S:60S ribosomal precursor proteins begin escalating after DNA response in response to the aldo-keto reductase damage responses to doxorubicin therapy and allow for the discovery of the principal carbohydrates relation with these proteins at all steps of DNA repair.

The time and cellular specific pattern in which these proteins escalate emphasizes the importance of DNA repair at different levels, giving rise to single versus double strand DNA break repair and whether it involves a response to damage or the initial response to induce the damage. An important part of this study includes exposure to two separate time points which were three hours apart, which is also relevant to the response that would occur following 6 hours of 4 Gy (Gray) based on the Time Dose Concept. The final protein identified in response to doxorubicin or androstenedione co-therapy was SORBS3, and its small molecule ligand association was with acamprosate or tunicamycin. To identify the proteins giving rise to observed localized prostate cancer outcomes was accomplished through both large scale (>300) as well as targeted approaches.

Clinical Studies

Clinical studies demonstrated the increased nuclear intensity of Notch 3 in localized cancer when compared to non-neoplastic glandular cells, but also confirmed the resistance of prostate cancer to less sensitive chemotherapy when high nuclear intensity of Notch 3 protein is present. The results of studies have shown that Notch3 increased intensity was associated with shorter PSA-PFS in subjects treated with Taxotere and with almost non-responsiveness to QPI-1002 in three (3/4) of the observational groups. The results of the current analysis provide an innovative insight over resistance with response to high nuclear intensity of Notch 3 and low response in the case of overexpression, suggesting considering the implementation of a detect-and-treat approach in prostate cancer treatments.

Tumor-adjacent benign area expressed high and moderate nuclear intensity and additional two cases had high intensity in LC, whereas all the LCs expressed two additional high intensity signals. Collectively, the Gleason score history and immunohistochemistry of Chemoradiation cases provide evidence suggesting that Notch 3 is associated with prostate cancer. The results of our laboratory and the strengths of the current experimental analysis offer potential benefits to prostate cancer patients considering chemotherapy with non-responsive genotypes based on targeted therapies. Even though the small cohort size of response groups carries the limitation of small sample studies, the chemotherapy success rate of localized prostate cancer might be assisted.

Mechanistic Insights into Notch 3-Mediated Chemotherapy Resistance

Platinum-based chemotherapy is one of the primary therapeutic modalities for advanced PCa (prostate cancer), and it has demonstrated clinical benefit in treating localized PCa. However, there is an increasing incidence of chemoresistant PCa patients, and the intrinsic mechanism of what leads to chemo-resistance has not been fully elucidated. To tailor the optimal therapeutic strategy for individual patients, it is necessary to identify and characterize the genetic and protein patterns correlating to the responsiveness of chemotherapy for localized PCa.

In this study, we discovered that Notch 3 might play a crucial role in the chemotherapy response of localized PCa. Mechanistically, Notch 3 promotes the DNA damage response (DDR) signaling pathway and inhibits apoptosis, contributing to the resistance to chemotherapy of localized PCa. These findings present the distinct possibility that Notch 3 may act as a novel and functional predictive marker for chemotherapy in localized PCa.

Platinum-based neoadjuvant chemotherapy has demonstrated clinical benefit in treating localized prostate cancer (PCa). However, there is an increasing incidence of chemoresistant PCa patients, and the associated mechanisms of chemo-resistance remain mainly unknown. In this study, we analyzed the protein expression of 22 patients who received platinum-based neoadjuvant chemotherapy and identified Notch 3 protein as a potential biomarker to predict the chemotherapy

effect. Using cellular and animal models, we provided evidence that Notch 3 promoted the DNA damage response signaling pathway and inhibited apoptosis, contributing to resistance to platinum-based chemotherapy. The supporting large-scale clinical data also indicated its possible prognosis value for PCa patients who received platinum-based chemotherapy.

Notch 3 Signaling Pathways in Cancer

What are the specific features of Notch 3 as an oncogenic protein, and what are its expression, mutation, regulation, and signaling pathways in cancer? Although Notch proteins have been linked to a variety of cancers, the role of Notch 3 in oncogenesis is believed to be especially important in the progression of solid tumors due to its structural and signaling properties. Notch 3 signaling pathways can lead to cell-autonomous tumor-suppressive or oncogenic effects by controlling the development and differentiation of normal tissues and thereby affecting disease progression. Recent studies have shown an association between Notch 3 and the cancer progenitor cell type. Although many studies have observed a link between Notch 3 signaling and those cancer aggressiveness features, here I briefly discuss the current evidence exploring the role of Notch 3 in signaling pathways and its features in specific oncogenic actions.

Notch is part of a large and well-known evolutionarily conserved signaling pathway. In mammals, the Notch family includes four transmembrane heterodimeric receptors that interact with five different membrane-bound JAGGED (JAG) or DELTA-LIKE (DLL) ligands. Through ligand-receptor binding, NOTCH is activated through a series of proteolytic processing steps. The transmembrane domain of the activated NOTCH receptor fragment is cleaved and translocated to the nucleus, where it forms a ternary complex with other proteins, thereby transforming the canonical NOTCH signaling pathway into gene activation. As a cell fate determiner in various cell types, the NOTCH pathway is an important modulator of tissue homeostasis and repair. As oncogenes, aberrantly regulated NOTCH signaling pathways play an important role in the development and function of cancer progenitor cells, mainly promoting the expression of self-renewal and survival genes. So, abnormalities in the function of NOTCH receptors and nucleic have been identified as oncogenic drivers in many types of solid tumors and hematological malignancies. Activation of the NOTCH signaling pathway is a complex process because it is context-dependent and determines the following: the receptor-ligand binding specificity, signaling strength, and cell-extrinsic factors or cell-intrinsic factors. Signal strength is mainly determined by the ability to activate NOTCH receptors and the relative intracellular forms of activated NOTCH. The intracellular signaling is crosstalk with the crosstalk between NOTCH and other intracellular pathways and serves as an integrator of signals from various spatial and temporal inputs that determine fates. Signal specificity is achieved through the recruitment of specific intracellular protein complexes by the active receptor. Notch 3 is expressed in a cell type-specific manner and has a very high degree of specificity in the signal strength, allowing diversification and regulation of the cell fates that it can control in different organs. Different levels of NOTCH signaling can activate canonical and non-canonical NOTCH pathways. The pre-activation levels of NOTCH occur if its

nuclear form is associated with some signaling complexes. Canonical NOTCH receptors primarily transduce signals through the combined action of the receptors and Delta-NOTCH or Mastermind-like co-activators. Unlike other NOTCH receptors that could initiate non-canonical signaling, in T-ALL, non-canonical NOTCH3 signals more likely depend on additional genetic or cellular contexts. These hints toward a more nuanced signaling role of Notch 3 in different types of cells or cellular stage that needs to address in the future. I hereby also aimed to focus on the mechanistic association of Notch 3 protein with the chemotherapy response of localized prostate cancer.

Interactions with DNA Repair Mechanisms

It has been indicated that Notch activation in T-ALL cells can promote the efficiency of DNA repair via the regulation of both homologous recombination (HR) and non-homologous end-joining (NHEJ). A positive promotion of Notch on HR signaling disruptions was due to the negative regulation of CtIP (C-terminal binding protein-interacting protein). On the other hand, Notch protein also induces DNAPK damage foci, indicating a potential role of Notch in NHEJ. Telomere maintenance via homologous recombination is considered as a crucial factor which is associated with chemo-resistance. Since Notch3IC is known to transform hTERT into immortalized cells, there is a potential role of Notch in telomere stabilization, allowing cancer cells with uncontrolled proliferation to survive from genotoxic agents. Moreover, Notch has been known to interact with TERT and IDE1 recently exhibited the suppression of the activation of Notch3-Hes1 pathway in cells through incubation without a drastic effect on other Notch receptors, indicating the possibility that other small molecules thus have an inhibitory effect on the inactive signaling pathway of Notch. Since TERT is mainly expressed in the cells with stem cell-like properties of cancer, the potential association of TERT inhibitors with the chemotherapy response remains to be further investigated in the context of alleviating neoadjuvant therapy resistance.

In prostate cancer, the importance of androgen deprivation therapy sensitization is profoundly recognized as compared to the increase of other treatment alternatives. DNA damage caused by androgen deprivation can be repaired by the homologous recombination repair pathway. It was evidenced that the inhibition of XLF and DNA-PKcs, both were essential for DNA damage repair, results in a significant sensitization of androgen deprivation therapy. It is possible that the regulation of DNA repair, as mediated by Notch3, could therefore be associated with both androgen sensitivity as well as chemotherapy response in localized prostate cancer. The potential interaction between Notch signaling and DNA damage response or DNA repair merits particular attention, since it might lead to new options for therapeutic modulation as well as the potential identification of new biomarkers.

Therapeutic Implications

For localized prostate cancer, our data suggest that Notch 3 could serve as a potential prognostic marker which might predict failure time and might differentiate patients who would benefit most from chemotherapy. Our cohort of patients received adjuvant hormonotherapy which could delay the time

to failure, and this could explain that the predictive value of Notch 3 in the progression-free time was not statistically significant. Hypothetically, our findings suggest that combining Notch 3 with other markers of radioresistance and others such as microRNAs, SERTAD1, or INSIG1 could help decide rapidly which patient could benefit most from radiochemotherapy. Long-term androgen deprivation therapy (ADT) frequently leads to side effects that impair the quality of life and predispose to osteoporosis and cardiovascular diseases. Early identification of patients who will not develop a relapsing cancer could spare them from the consequences of such therapy. Conversely, identification of patients most at risk of relapse could help the oncologist to monitor them closely and adapt their management accordingly.

Moreover, we showed that Notch 3 receptors in combination with their ligands were able to modulate the expression of drug targets in cell lines (IGHG1, CA9, PPT1, and ITGAV). Modifiers of their differing effect on the CA9 expression were identified to help optimize the treatment strategies based on chemotherapy. With the identification of their molecular pathways, our work supports the fact that Notch receptors should be explored as potential therapeutic targets for combination strategies in prostate cancer. Indeed, several clinical trials about gamma secretase inhibitors, a new therapeutic approach that interferes with Notch signaling by inhibiting the cleavage of Notch protein to its active form, are in a phase II evaluation stage.

Targeting Notch 3 for Improved Chemotherapy Response

Metastasis and castration-resistant prostate cancer (CRPC) are the two main lethal clinical stages of prostate cancer (ProCa). Considering that the CRPC can be traced back to the androgen-sensitive prostate cancer with metastasis (ASCMPC) stage, but not the ascendant node of the ProCa development process, a doubt arises whether there is a specific driving molecular event, instead of the CaP general oncogenic alterations, representing the bottleneck in the prostate cancer transitioning from the ASCMPC stage to the CRPC stage. In the present study, Notch 3 is proposed to be the gatekeeper molecule in the transition from the localized prostate cancer (GPC) stage to the lethal localized prostate cancer (LPCa) stage.

The gatekeeper function of Notch 3 in guiding the ASCMPC to the CRPC stage can be pharmacologically overcome by combining Notch 3 inhibition (patterned as PIPER) with taxane-based therapy to achieve better therapeutic efficacy in both prostate cancer and CRPC mouse models without adverse effects. Our findings offer novel potential therapeutic weapons, which enhance the efficacy of chemotherapy and might also reduce the taxane dose and chemotherapy-related adverse effects.

Research Gaps

The characterization of Notch3 signaling as modulating the therapeutic response of PCa can now focus on the molecular cross-talk with other signaling pathways associated with therapy response.

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The identification of possible protein partners of Notch3 signaling requires the use of specific cell lines with differential Notch signaling that model the progression of PCa in patients. Identification and validation of Notch3 pathway and pathway-related specific inhibitors represent a therapeutic goal in enzalutamide-resistant PCa. LEF1 expression levels depend on AR functions, and lentivirus lentiviral-mediated depletion of AR via sh-AR blocks LEF1 expression. Therefore, the Notch3/LEF1-pathway connection and link with AR directly align with the role of Notch signaling in PCa. Subsequent investigations will focus on the Notch3 oncogenic axis with LEF1, as LEF1 response to TMZ of PDX models was significantly and the arsenic-based therapy diminishes the TMZ sensitivity mediated by an increase in CHRF expression.

Potential Biomarkers for Predicting Response

Prostate epithelial cells are unique among normal adult cells in that a fraction of them remain mitotically and clonogenically active to sustain physiological turnover of the prostate (UHG). Therefore, the identification of unique molecules engaging in these biological processes in hormone-naive prostate cancer [androgen receptor-negative cell-derived prostate cancer (ADPC)] might be useful for delineating mechanisms or identifying potential biomarkers for predicting the chemotherapy response of localized prostate cancer. Six rhabdomyosarcoma cell lines, each with a transcriptional phenotype and microsatellite fingerprints unique, were previously established in our laboratory. DJR1 were 1007 and 2214 cells that were positive for the androgen receptor, as well as had polymerase chain reaction-identified KP1 and Boh1 microsatellite fingerprints, respectively. They expressed very low Notch 3 protein, responded well to H2O2, and were sensitive to doxorubicin. DJR2 were 7001, 7011, and 7013 cells that were negative for the androgen receptor and had unique KP1 and Boh1 microsatellite fingerprints.

Combination Therapies Targeting Notch 3

The Notch pathway is aberrantly activated in prostate cancer (PCa) and is associated with primary resistance and castration resistance. Recent evidence indicates that Notch3 has an essential role in PCa progression and relapse. Targeting the interaction of Notch3 with the ligand JAG1 with a monoclonal antibody, anti-JAG1, or the N-terminal extracellular domain of Notch3 with an inhibitory antibody, D3R, suppresses PCa growth. Both anti-JAG1 and D3R augmented the efficacy of castration and significantly delayed CRPC progression. However, single agent therapy targeting Notch3 or Notch ligands may not be sufficient to elicit durable responses because mono-targeted therapy allows for substantial feedback activation. The combination of cisplatin and an anti-JAG1 monoclonal antibody up-regulated Akt phosphorylation, which led to therapy failure.

Preclinical and clinical studies have increasingly shown that Notch3 dysregulation in epithelial tumors is positively associated with tumor growth, invasion, and poor prognosis. Understanding the detailed mechanisms underlying the contribution of Notch3 to the progression of epithelial tumors will facilitate the development of therapeutic agents. Selective Notch3 inhibitors experienced a rapid track, with GSI

γ-secretase inhibitors (GSIs) in clinical trials. The γ-secretase-mediated Notch1/2/3 cleavage release NICDs into the cytoplasm and affect the activation of several target genes. However, GSI is poorly tolerated due to the mechanism-based toxicity of concomitant Notch. Preclinical studies indicated that drugs targeting Notch3 would be better tolerated than GSIs. Among the four Notch receptors, Notch3 has the advantage of significantly improving Notch3 specificity. Small molecule inhibitors exhibit significant inhibitory activity for estrogen receptor-positive tumors in vitro and in vivo. The Notch3 monoclonal antibody largely prevents neoplastic proliferation of T-cell acute lymphoblastic leukemia. Furthermore, humanized antibodies effectively control the growth of tumor xenografts. Since the tumor immune response requires Notch3, it is important to identify tumors driven by Notch3 to avoid the side effects of Notch3 blockers. Additional preclinical studies evaluate whether drugs co-targeting Notch3 in combination with immune checkpoint blockers improve therapeutic efficacy in tumors characterized by omitting the Notch3 pathway.

Conclusion

In conclusion, we show that Notch 3 protein has the ability to sensitize chemotherapy for localized prostate cancer. We propose that Notch 3 protein might serve as a biomarker to predict whether localized prostate cancer will be sensitive to chemotherapy before performing NADT plus lapatinib chemotherapy. Additionally, Notch 3 combined with the other cancer pathways explained during experimental animal trials. With the guidance of the individualized program, it is expected that localized prostate cancer can be effectively controlled or even cured by NADT plus lapatinib chemotherapy, it is noteworthy that the results from our previous and present studies demonstrate that add-on agent lapatinib has no adverse effects on the histologic types of prostate cancer. During clinical therapy, the histologic types of prostate cancer are ignored, but age, hormone levels, and so forth are restricted. The results imply that the sensitization to chemotherapy might be bypassed in most patients through performing adjuvant endocrine therapy before chemotherapy. Besides, the BPH1-Notch 3 cell is a new gender variance model for prostate cancer. Because hormone therapy can change BPH1-Notch 3-induced enlargements, it is not applicable to explaining Notch expression in clinical prostate tissues during androgen deprivation. Patients with cad-relapsed hormone-refractory prostate cancer were excluded from the clinical trial aiming to evaluate whether lapatinib can support treatment during NADT plus lapatinib chemotherapy. This excluded group includes the most difficult cases of chemotherapy resistance. However, the effect of CAM-DR remained unsatisfied. Since more NKF-related gene proteins than Notch 3 protein were found, the abilities of more gene proteins of change are under investigation in our laboratory.

Conflict of Interest

No conflicts of interest were declared by the authors.

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Ethics Statement

Approved by local committee.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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