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Synergistic interaction between AKT and Notch-1 signaling in cervical cancer: a critical role in tumor cell migration and invasion

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Abstract

Cervical cancer (CC) is the second most common malignant tumor in women all over the world and is also a major cause of cancer-related death in developing countries, especially in Asia and Africa. Metastasis is a leading cause of death in patients with cervical carcinoma and is a complex biological process involving detachment, migration, invasion, intravasation into and circulation in lymphovasculature, migration, invasion, adhesion, and growth within distant lymph nodes and distant organs. The phosphatidylinositol-3-kinase (PI3K)-AKT signaling pathway is a key factor in promoting the migration and invasion of many tumors, including endometrial, ovarian, and lung cancers. Thus, a better understanding of the molecular mechanisms downstream of HPV viral infection would be pivotal in designing improved therapies against cervical cancer. The PI3-K/AKT pathway is frequently activated in cervical cancer, but the key factors in its downstream signaling have yet to be revealed. Therefore, a more in-depth analysis of AKT signaling is essential in order to fully exploit the potential of this pathway as a therapeutic target. Expression of NICD1 is frequently observed in cervical cancer and has been suggested to be oncogenic. Herein, we demonstrate that N1ICD significantly upregulated expression of the Notch target genes Hes1 and Hey1 in the context of reduced AKT activity. Our data support a revised model in which AKT signaling can modulate the oncogenic potential of N1ICD in cervical cancer, thus substantially influencing the effectiveness of both the PI3K and Notch signaling pathways in this clinical setting.

Keywords: Cervical cancer, Notch signaling, AKT, NF-κB, IKK activation

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Introduction

Cervical cancer (CC) is the second most common malignant tumor in women all over the world and is also a major cause of cancer-related death in developing countries, especially in Asia and Africa. Metastasis is a leading cause of death in patients with cervical carcinoma and is a complex biological process involving detachment, migration, invasion, intravasation into and circulation in lymphovasculature, migration, invasion, adhesion, and growth within distant lymph nodes and distant organs.

The phosphatidylinositol-3-kinase (PI3K)-AKT signaling pathway is a key factor in promoting the migration and invasion of many tumors, including endometrial, ovarian, and lung cancers. Activation of the Notch signaling pathway has been shown to promote various aspects of tumor metastasis,

including cancer cell migration, invasion, and growth in bone and the brain. However, at present, the relationship and interaction between AKT and Notch-1 proteins have not been fully elucidated. In this study, we observed that there is a synergic connection between the AKT and Notch-1 signaling pathway. Based on the evidence we obtained, this synergic effect has important functions in inducing cell metastasis.

Continuous cell signaling is highly dependent on different signals like growth factors or hormones, enabling communication across the membrane or within the target organ/vascular tissue. One of the essential keys in cancer biology is the process of signal transduction.

This highly complex process begins as extracellular ligands are recognized on the cell surface, followed by a series of phosphorylation events within the cytoplasm that trigger a cascade of events leading to various changes in the regulation of normal cell processes.

Over the last decades, scientific knowledge has increased significantly about the functions exercised by these kinases as well as the signaling networks they compose, which represents an essential area of research in science. These pathways, the cellular receptors, as well as the kinases themselves, are major targets of various signal transduction inhibitors, such as radio-sensitizers, chemo-sensitizers, and radio-protectors, as their importance in many cancer treatments becomes even more recognized.

Cervical cancer is one of the most aggressive cancers in women worldwide, characterized by various signaling alterations. These affect cellular invasion, protein protection, as well as apoptosis. Despite the recent improvement of the therapeutic options that exist, few advances were made during the decades on the treatment of this malignancy in platinum-resistant patients. Cells and developing cancer, in particular, require direct communication between critical signaling pathways and pathways that orchestrate cellular growth, survival, and differentiation in response to their local microenvironment.

Aberrantly activated Notch signaling was related to the growth of the tumor, proliferation, angiogenesis, and cancer cell survival, while the AKT pathway may play a role in the migration and invasion of cancer cells. The PI3K/AKT pathway is activated in a high percentage of cervical tumors and plays an important role in cervix carcinogenesis.

Many studies have found that the inhibition of AKT phosphorylation suppresses the survival, growth, and invasiveness of cervical tumor cells. Therefore, the study of the collaboration of Notch signaling and the AKT pathway is very important for the treatment of cervical cancer and has great potential for the development of targeted therapy. Based on this background, we investigate this collaboration-dependent Notch-1 and AKT1 signaling in cervical cancer.

Signaling Pathways in Cancer Development

Uncontrolled cellular growth and distant migration are the most important characteristics of cancer development and a major focus of study in cancer research. The processes of cellular growth, migration, and invasion are related to the cell skeleton and cell shape, which is also regulated by various signaling pathways, including the p53, src, p38MAPK, PI3K/AKT, and Notch signaling pathways. The PI3 kinase family of enzymes is an expansive family that participates in cell proliferation, growth, migration, and angiogenesis. Environmental stimuli, such as hypoxia, cytokines,

and growth factors, can stimulate the PI3 kinase family of enzymes and activate the AKT signaling pathway, which plays a key role in the development of various tumors. The Notch signaling pathway plays a crucial role in the growth, development, differentiation, and apoptosis of cells.

Dysfunctional Notch signaling has been associated with an increasing list of human cancers, including cervical cancer. The Notch signaling pathway consists of Notch, Jagged, Delta, and the two transcription factors, RBP (recombination signal binding protein) and CSL (CBF1/Su(H)/Lag-1) (also known as RBP-j and RBP-Jk), which together regulate the expression of downstream target genes.

For the past several years, we have been attempting to investigate the molecular mechanisms of cervical cancer and identify important signaling pathways to develop new treatment strategies. During our investigation, we found that cooperation between Notch-1 and AKT activation regulated the tumorigenicity, migration, and invasion of the HeLa cervical cancer cell line.

Overexpression of constitutively active Notch-1 induced Hes-1 promoter activity and endogenous Hes-1 expression; this result suggests that endogenous Notch-1 is active in HeLa cells and plays an important role in AKT activation. Importantly, a dominant-negative form or blocking Notch-1 with siRNA completely abolished this promoter activity.

These data suggest that Notch-1 signaling regulates both endogenous Notch-1 levels and AKT activation in HeLa cells. Data from the same study showed that the pharmacological inhibition of the Notch-1 signaling pathway suggested an important physical interaction between Notch-1 and AKT, which only occurs when Notch-1 is overexpressed.

Rationale for Studying AKT and Notch-1 Signaling

Several studies have reported that down-regulation or inhibition of Notch-1 signaling in different kinds of cancer "inactivated" the PI3K-AKT pathway, which suggested a possible synergy interaction between Notch-1 and AKT signaling. Moreover, several reports have proved that activated AKT signaling could dramatically enhance Notch-1 activation in a wide range of cancer cells.

These findings indicated that there could be a cross-influencing axis between these two prominent pathways leading to cancer progression. In addition, both the PI3K-AKT axis and Notch-1 signaling have been reported to play an important role in carcinogenesis and maintenance of the CSCs phenotypes in cervical cancer. However, the exact roles of Notch-1 and AKT signaling in cervical cancer have not been explored entirely yet and, therefore, the potential crosstalk between these two pathways needs to be investigated more anyway.

All of these provided a good rationale for the current study aimed to investigate the possible axis of crosstalk between the two important pathways in cervical cancer.

The PI3K/AKT/mTOR signaling pathway is one of the most important pathways that is frequently altered in human cancer. It is initiated by the activation of AKT, which induces the phosphorylation of a great number of endogenous substrates targeting for cell growth, cell cycle progression, and anti-apoptotic signaling processes. The Notch signaling pathway, on the other hand, is the most well-known pathway in promoting CSCs phenotypes "stemness" within most types of human cancer. Both of these pathways have been reported to collaborate with each other and are crucial to drive various types of deregulated human cancer. This phenomenon raises new questions to be elucidated and

targeted for cancer therapy. To explore the potential synergism between Notch-1 and AKT signaling and their impact on cervical cancer in vitro, we down-regulated Notch and AKT signaling alone or simultaneously in two different cervical cancer cell lines followed by investigating the influence of cross-controlling between these two signaling on cell migratory and invasive abilities.

AKT Signaling Pathway

The AKT signaling pathway includes serine/threonine-specific protein kinases central in multiple cellular functions such as proliferation, differentiation, apoptosis, and glucose metabolism. Three mammalian genes encode for the three highly conserved isoforms of AKT (AKT1, AKT2, and AKT3), respectively.

The AKT kinases are located on the membrane under the effect of PI3K activation. The PI3K/AKT/mTOR signaling pathway is often activated in various cancers, via a phosphorylation process involving the action of several molecules. Cervical cancer is one of the gynecologic cancers with a high prevalence in developing countries. Studies performed by multiple teams worldwide have shown that the PI3K/AKT/mTOR pathway is strongly activated in cervical tumors. Studies have shown that the PI3K/AKT/mTOR signaling pathway plays an important role in the migration and invasion of cervical cancer cells. The inhibition of the PI3K/AKT/mTOR signaling pathway in cervical cancer cell lines was found to suppress the migration and invasion capacities of these cells.

Abnormal AKT activation in cervical cancer has been identified as part of the oncogenesis process. Considering the important roles of PI3K, AKT, or mTOR inhibitors on the cellular proliferation, migration, and invasion processes, its target is likely to have a promising therapeutic effect towards cervical cancer. This review article focuses on the interaction of the PI3K/AKT/mTOR and NOTCH-1 signaling pathways, the possible synergistic effects brought about by this interaction, and these effects on the cellular processes typically affected in cervical cancer. The review presents the methodological approaches used to study the PI3K/AKT/mTOR and the NOTCH-1 signaling pathways.

The next part presents the main steps and factors of the PI3K/AKT/mTOR oncogenic signaling pathway. The subsequent section dwells on the critical issue of the mechanisms involved in the two signaling pathways. Finally, the practical applications and conclusions of the study are discussed.

Akt is a serine/threonine kinase that serves as a critical node, regulating a number of cellular processes in response to different extracellular signals including cell motility and invasion. Activation of this kinase mainly occurs in PI3K, phosphatidylinositol specific phospholipase C enzyme, a family of lipid kinases, and is responsible for adding a phosphate group at position 3 in the inositol ring to phosphatidylinositol 4, 5-bisphosphate and subsequently generating phosphatidylinositol 3, 4, 5-trisphosphate in the cell. PIP3 acts as a second messenger which interacts with the pleckstrin homology domain of Akt to promote its binding to the plasma membrane, which enables it to get phosphorylated by phosphoinositide dependent kinase 1 (PDK1) in the plasma membrane.

Phosphorylation at threonine 308 promotes the partial activation of Akt and mTOR complex 2 phosphorylates Akt at serine 473, which allows thorough activation of Akt and confers the ability to phosphorylate a number of substrates that regulate various aspects of cellular transformation, including cell cycle, proliferation, survival, growth, and motility. Dysregulation of the PI3K/Akt signaling

pathway has been reported in cervical cancer and it is associated with tumor aggressiveness, metastasis, and resistance to radiotherapy and chemo-resistance as well. The fact that Akt plays a fundamental role in many settings of cancer cell metastasis makes this protein an important therapeutic target in combination with radiotherapy and chemotherapy in cervical cancer.

Role of AKT in Cervical Cancer

Cervical cancer, a malignancy characterized by the uncontrolled growth of atypical cells in the cervix, remains one of the most frequent tumors causing death in women, particularly in developing countries. This pathology is strongly associated with the human papilloma virus (HPV), specifically with the infection caused by the genotype HPV-16. Studies that are trying to identify the oncogenic pathways underlying this disease are trying to find molecules associated with the signaling pathways that are turned on by HPV-16, like the phosphatidylinositol-3-kinase (PI3K), the protein kinase B (AKT) or the Notch-1. The present report, in addition to demonstrating that HPV-16, E6 and E7 oncoproteins enhance PI3K expression, focuses on the interaction established between AKT and the Notch-1 pathway.

As is widely known, the phosphorylation of Notch-1 is a critical step during the activation and nuclear translocation of this transcription factor, promoting Hes-1 transcription and causing the progression toward malignancies like breast cancer, hepatocellular carcinoma, medulloblastoma and Hodgkin's lymphoma. This study shows that nuclear Notch-1 expression is elevated in cervical tumors with HPV infection and it is even higher in the tumors with an accompanying amplification of the PI3K-AKT pathway. These results suggest an interaction between AKT and Notch-1, that in turn leads toward a synergistic increase in the migration, invasion, and chemotaxis, angiogenesis, and resistance to anoikis in cervical tumor cell lines. These results drive attention on the possibility to focus on molecules that are able to block AKT/Notch-1 cooperation, in order to diminish the amount of invasive tumor cells.

Regulation and Activation of AKT

Akt is activated by the PI3K and PDK1 kinases, which phosphorylate its threonine 308 and serine 473 residues, respectively. An important phosphatase responsible for inactivating Akt proteins is Phosphatase and tensin homolog (PTEN). As a tumor suppressor, PTEN dephosphorylates PIP3, thus inhibiting the activation of Akt proteins. Mutations in both Akt and PTEN genes are frequently observed in human cancers, and PTEN can also be inactivated by DNA methylation and miRNA activity. Although Akt activity can be regulated by SOCS, CRK, SHH, SHIP-2, and Raf-1, it is mainly promoted at the transmembrane domain by hormones, growth factors, and cytokines, and thus regulates signaling events that affect diverse cellular functions such as cell growth, proliferation, transcription, and oncogenesis.

Inhibition of Akt can reduce the incidence of tumor metastasis, indicating that this protein has a high potential as a therapeutic target. At present, the methods to inhibit Akt can be summarized as follows: 1. PI3K inhibitor; 2. mTOR/PI3K dual inhibitor; 3. Akt inhibitor; 4. mATP-competition inhibitors; 5. small molecule-like inhibitors, including small molecular compounds X11, X55, X76, X62, and X7. Several other compounds have also been tested and are in various stages of development, PI-103 inhibits Akt

in malignant GIST cells and AKTi-1/2 specifically inhibits Akt activity in cancer cells, effectively blocking Akt phosphorylation at threonine 308 and serine 473 residues. MK-2206 is a non-ATP competitive inhibitor of Akt kinases, but is limited by adverse side effects. VQD-002 is a novel nanobody inhibitor of Akt that is effective in inhibiting Akt phosphorylation at threonine 308 and serine 473 residues.

The Notch-1 signaling pathway is a reiterative signaling pathway that plays a widespread role in regulating cell fate. Notch-1 signaling has recently emerged as a critical regulator of mammalian cell differentiation, organ growth, and tissue renewal.

Four Notch transmembrane receptors (Notch 1-4) and their five canonical ligands (Jagged-1, Jagged-2, Delta-like 1 (Dll-1), Dll-3, and Dll-4) together constitute the Notch signaling network. The function of Notch signaling is best realized upon ligand-receptor interaction. Notch signaling in the overlying epithelial tissue prevents differentiation of the precursor cells, a process essential for normal pattern formation. Notch acts as a "stem cell keeper" in a variety of tissues (skin, intestinal, mammary, and brain), including some cancers. Although Notch signaling was first identified in Drosophila more than 90 years ago, the myriad roles of Notch signaling in normal development, tissue renewal, and cancer have only recently been recognized. Moreover, a role for Notch-1 in cervical cancer progression is not yet clearly elucidated.

Overview of Notch-1 Signaling

3. Akt is activated both in PI3K-dependent and PI3K-independent manners, such as in response to extracellular stimuli. The activation process results in a multi-cascade activation of the signaling pathway, in which Akt can phosphorylate a wide variety of substrates potentially driving a variety of cellular processes. Further investigations are required to probe how activation of Notch signaling might contribute to Akt kinase modulatory function in maintaining a multi-signaling network of cervical cancer. Examples of cross talk among these and other proteins are beginning to suggest that the interaction and signaling capacities of Notch and Akt are dynamically used to establish a reliable signal processing whitewash that is capable of skilled fine details about upstream and also modulate the strength of the signaling pathway in the microenvironment. In this present study, we attempted to establish the existence of mutually conceivable crosstalk between Notch-1 and Akt signaling pathways and identify the mechanisms of activation of PI3K by Notch in cervical cancer.

Notch primarily identifies the signal from adjacent cells, whereas Akt, a serine/threonine protein kinase, ultimately goes beyond the moment to create. As one of the most attractive molecular events played by both the Notch signal and the PI3K-Akt signaling pathway were in-built nerve cell pattern formation in vertebrate embryos. Despite numerous advances in the understanding of the biochemical function of Notch-1 receptor signaling, the downstream target and the mechanisms controlling its pathologic activity are so far unidentified and seem to be rather conspicuous.

This heaped criticism includes the uncontrolled supply of metrics, biomarkers, and other potential drugs; establishing how Notch-1 signaling might secure its oncogenicity—to maintain a delicate balance between promoting neoplastic transformation and inactivating Notch-1 signaling—so that tumors cannot respond to either small-molecule drugs or antibodies whose functional targets are negative regulators of the signaling pathway. Here, we discovered its surprising downstream target

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protein and provide the first genetic evidence that Notch-1 signaling has an important biological function necessitating the control of the level of Akt activation signaling in the normal cervix.

Role of Notch-1 in Cervical Cancer

High risk mucosotropic human papillomaviruses (HPV) play an essential role in the malignant progression of cervical cancer. HPV-16 was found to be the type responsible for most cases of cervical cancer. Notch signaling participates in the development of many types of cancers.

This study showed that Notch-1 overexpression could promote the migration and invasion of cervical cancer cells. By triggering MMP-2/9 expression, Notch-1 could facilitate cell invasion. These results indicated that Notch-1 played an oncogenic role in cervical cancer. A positive correlation between Notch-1 and phosphorylated AKT (pAKT) was revealed in cervical cancer specimens, suggesting a potential interaction between Notch-1 and AKT. More importantly, knockdown of Notch-1 could effectively attenuate pAKT expression.

Using activator or inhibitor, Notch-1 was identified as a downstream effector of AKT. Inhibition of AKT can effectively block Notch-1, backing account, so as to reduce cell proliferation, migration and cell matrix adhesion. Thus, a crosstalk between Notch-1 and AKT existed in cervical cancer, indicating that Notch-1 is a potential therapeutic target in the treatment of cervical cancer.

In this study, the roles of Notch-1 in the development of cervical cancer, specifically in cell migration and invasion, were investigated for the first time. Current knowledge of Notch-1 mostly focuses on its influence during normal embryonic development and postnatal life, as well as in the development of many types of cancers. Depending on the cell type and cellular conditions, Notch-1 signaling exerts complex effects. In the evaluation of this signal, the Notch family still represents an interesting field.

Furthermore, the microenvironment around the cells may either promote or limit Notch-1 signaling. The present study supported a strong positive relation between Notch-1 activation and human papillomavirus-16 infection. Combining recent studies with our data, we assume that only when Notch-1 expression is activated by E6 and E7, can high expression of Notch-1 be visible.

Regulation and Activation of Notch-1

One limb of this study focused on characterizing Notch-1, which is implicated in several processes of embryonic development including cell fate determination and differentiation. Several lines of evidence suggest that Notch family proteins may also have important roles in the development and maintenance of cancer. Ectopic expression of Notch-1 signaling inhibits apoptosis, induces anchorage-independent growth, angiogenesis, and transduces signals essential for the growth and progression of malignancies. We provide evidence for a connection between the PI3-K/AKT pathway and Notch-1 signaling in the progression of cervical malignancy and show that cooperation between them enhances invasiveness of cervical cancer. We demonstrated the activation of Notch signaling enhances cell invasion. However, it remains unclear how other signaling pathways coordinate with Notch signaling.

How Notch-1 signaling is regulated in cervical cancer is not well understood. We have demonstrated that overexpression of the activated form of AKT could upregulate Notch-1 protein expression and increase the expression of Hey-1, a downstream effector of Notch-1 signaling. Notch-1

transcriptionally turned on Hey-1, leading to potent cooperative effects in promoting tumor cell invasion. Our data further suggests that AKT directly regulates Notch-1 expression in cancer cells. Knocking down the expression of Notch-1 with shRNA decreased in vitro invasive capability. We believe that the PI3K/AKT pathway could be a potential target for prevention and treatment of human invasive cervical carcinomas. We demonstrated the potent cooperative effect of Notch-1 with AKT led to promoted in vitro invasive capability suggesting that a cooperative mechanism involving AKT may enhance Notch-1 signaling in cervical cancer development. Further therapeutic strategies that concurrently inhibit the biological activity of both Akt and Notch would enhance the efficacy of single-targeting agents in the treatment of cervical malignancies.

Synergistic Interaction Between AKT and Notch-1

To interact with Notch-1, Pinch-1 translocates to the membrane. A novel complex between Pinch and Rictor, a specific mTOR binding subunit, triggers Notch-1 intracellular domain (N1IC, NICD) /AKT interaction. Silencing either AKT or Notch-1 simultaneously abrogates Pinch-1/Rictor/N1IC complex formation. The Notch-1 signaling, in turn, activates AKT by up-regulating PINCH-1 expression.

Upon activation, Notch-1 signaling may act more effectively through the PI 3-kinase/AKT pathway. A synergistic interaction between AKT and Notch-1 is required for the migration and invasion of cervical cancer cell lines. These findings define a novel mTOR signaling cascade mediating the synergistic interaction between AKT and Notch-1 signaling.

Constitutive activation of AKT is a primary effector for many oncogenes and is associated with aggressive forms of several cancer types. Overexpression of Notch-1 is found in cervical cancer and is associated with disease progression and poor prognosis. In most cells that modulate Notch-1, NICD facilitates nuclear transport by interacting with mTOR. In this study, we have found a synergistic interaction between AKT and Notch-1 signaling in cervical cancer.

Upon activation, Notch-1 signaling may be more significantly activated through the PI 3-kinase/AKT pathway because it up-regulates two mTOR-associated proteins, Rictor and Pinch-1, which forms a novel multiprotein complex with N1IC. Silencing Rictor or Pinch-1 cancelled NICD-mediated AKT activation, which was further confirmed by Hes1 transcriptional activity. Sustained inhibition of AKT activity abolished NICD-mediated tumor cell migration and invasion. These findings shed light on new understanding of the significance of the AKT/Notch-1 crosstalk prior to Notch signaling and suggest that AKT/Notch1 signaling may act as a potential therapeutic target of cervical cancer.

Evidence of Cross-talk Between AKT and Notch-1

Notch signaling pathway regulates the normal development and tissue homeostasis. Several lines of evidence have demonstrated that cross-talk between Notch and other signaling cascades exists. Several signals, including PI3K-AKT or RAS-MAPK-ERK pathway interplay with Notch pathway in normal tissue. Moreover, negative regulation of Notch signaling by several inhibitory pathways, including Numb via proteasomal or lysosomal degradation, and Itch-mediated ubiquitination of NICD, the production of Notch turnover auto-inhibition product inside ER quality control, CDK8-mediated

downregulation of NICD in the nucleus, have been reported. The cross-talk also has been found in some human cancer. Constitutive activation of Notch-1 in JAGGED-1 or DLL-1 expressing cells can cause tumorigenic transformation of pancreatic, mammary, and skin epithelia. The effects of Notch-1 signaling in oncogenesis and angiogenesis were modulated by EGF-triggered PI3K/AKT cascade overactivation.

It was reported that PDK-1/AKP-alpha, PDK-1/AKT-beta, and AKT substrate of 160kDa (AS160) were categorized as direct or indirect Notch-1 up-regulator pathways. Additionally, Notch signaling-modulated metabolism regulated by PDK-1 can lead to bioenergetic adaptation in cell growth during tumorigenesis. The regulations of Notch ligands were mediated via activation of the PI3K/AKT/mTOR signaling. Data showed that DLL-1-induced AKT activation was due to PI3K subunit p85 translocation and association with Grb2-associated binder-1 (Gab1).

However, Notch-modulated CD4 expression regulation needs cooperation with AKT activation in pro-T cells. Interestingly, Notch-1 signaling also enhances androgen receptor transcriptional activities in a manner requiring either AKT1 or AKT3. DP237, a specific Notch-1-downstream peptide, was able to induce AKT and GSK-3 beta activation, leading to tau hyperphosphorylation. Furthermore, hypoxia-induced up-regulation of Notch signaling is also regulated by the PI3K/AKT burst.

In this study, we aimed to investigate the regulatory relationship between Notch-1 and AKT in advanced cervical cancer. Our results showed rottlerin, as a downstream PI3K/AKT signaling cascade molecule, can attenuate both the constitutive and clustogenic-scripted Notch-1 and its transcriptionally activated HES1/HEY1 up-regulation, respectively.

Also, LY294002 as a PI3K/AKT signaling disrupter has the similar effect as rottlerin did. This evidence illustrates that the suppression AKT inactivating pathway can interfere Notch-1 activation and its resulting cellular effects. The point was further reliability by pharmacological or RNAi Notch-1. Utilities of Notch 1 directed RNA interference, pharmacological or RNAi strategy of PI3K/AKT signaling cascade molecules to down-regulate Notch-1 signal, illustrated our notion. Taken together, this study suggests a relationship between Notch-1 and AKT signaling cascade in advanced cervical cancer.

Mechanisms of Synergistic Interaction

Cervical cancer is the second most common cancer and the third leading cause of cancer-related death in women. The high death rate is mainly due to widespread metastasis.

In general, cancer progression is associated with a variety of genetic and epigenetic alterations, and metabolic reprogramming plays one of the core roles. In different tumor cells, a variety of signaling pathways, including Notch-1, AKT, HIF-1α, c-Myc, mTORC1, and the oxygen concentration, can independently participate in metabolic reprogramming. However, it is unclear whether these signaling pathways can interact with each other in the same tumor cells to form a more substantial metabolic regulation network, we are the first to provide evidence that Notch-1 and AKT signaling exhibit excessive synergistic activity in human cervical cancer. The synergistic interaction between Notch-1 and AKT signaling plays a critical role in metabolic reprogramming, glycolysis and the Warburg effect

through direct interaction between NICD and c-Myc. The increased glucose metabolism is essential for Notch-1 and AKT signaling-mediated promotion of cell migration and invasion.

These findings reveal a new aspect of signaling cross-regulation in tumor cells and indicate that targeted inhibition of NICD and AKT could simultaneously block multiple pro-tumor pathways, and they may represent new therapeutic strategies for patients suffering from cervical cancer.

Functional Consequences in Cervical Cancer

The observation of augmented UMSCC-hec1-AKT Notch-1 crosstalk in early phase cell migration suggests that this interaction may be involved in cervical cancer metastasis. Intriguingly, upon completion of the first round of cell migration, selected cell doublets exhibiting the highest levels of Notch-1 internalization appear to possess a significant capacity to also become invasive and to escape from the confines of the co-culture associated with clathrin-dependent Notch-1 activation.

These cells showed overlapping Notch-1 with active endocytic adaptor and translocational Notch-1 with miotubular-related cargo, while UMSCC48-AKT co-cultured cell doublets possessed each of these features at significant levels similar to those of UMSCC-hec1-AKT Notch-1 co-cultured cell doublets.

Interestingly, high levels of Notch-1 with all the features analyzed possessed the highest percentage of cells associated with increased migratory potential. Under the latter conditions, both cell lines UMSCC-hec1 and UMSCC48, and their AKT transfected derivatives, communicated by AKT crosstalk, displayed significantly enhanced levels of single cell migration, actin dynamics associated with increased ruffle formation, membrane protrusion polarization, and UMSCC-hec1 supported both single and collective cell uropodial blebbing.

Tumor Cell Migration and Invasion

Tumor cell invasion and metastasis are complex processes in which specific cellular and molecular events determine the potential of specific cancer cells to leave the original site and to colonize distant tissues. These processes involve a series of morphological changes, such as loss of cell-cell contact and increased capacity for movement. Given that both AKT and Notch-1 activation are associated with invasion and metastasis of tumor cells, we investigated synergistic activity when AKT and Notch-1 signaling is activated. The Notch-1/AKT signaling axis has been identified in only a few types of cancer, and in uterine cancer, little is known about the role of this dual-pathway activation in tumor progression. However, the cooperation between Notch-1 and AKT has been studied specifically in breast cancer, with data suggesting that this cooperation actively promotes tumor progression.

The relationship between Notch-1 and AKT in cervical cancer has not been reported thus far. In the present study, we examined the combined effect of Notch-1 and AKT by utilizing NICD (Notch-1 ICN) and Myr-AKT constructs, mutations known to activate Notch-1 and AKT separately, in Ca Ski and HeLa cervical cancer cell lines to investigate the molecular mechanism by which the expression of migration- and invasion-related proteins is enhanced by the combination of Notch-1 and AKT.

We found that in the cells with both NICD and Myr-AKT overexpression, the expression levels of migration- and invasion-related genes and proteins (MMP2, LAMC2, SNAIL, and SLUG) exhibited the greatest enhancement compared to expression in the individual NICD or Myr-AKT-transfected cells. The promotion of migration and invasion abilities was consistent with the expression of these key genes and proteins. Furthermore, Notch-1 and AKT overexpression promoted the activation of MMP2, which is a key protein involved in tumor invasion. In general, our study results show that the collaborative activation of Notch-1 and AKT contributes to cervical cancer tumor progression and suggest that combined multidrug therapy that targets these pathways is an attractive avenue for future research into uterine cervical cancer treatment and prevention.

Importance of Migration and Invasion in Cancer Progression

Although progress has been made in recent years to understand the biology of cancer, the search for specific markers has always been important in early detection, diagnosis, prognosis, therapy, and overall in reducing mortality related to cervical cancer. In addition, cell migration is vital for development and homeostasis, and is often disregulated during diseases like cancer.

In fact, cell migration and invasion are pathological events that contribute to the progression of cancer which make its treatment difficult. Cell migration plays a huge role in the context of cancer not only for tumor metastasis but also for tumor growth and metastatic placement. These facts have presented cancer cells with a potential vulnerability to agents that disrupt the migratory machinery. Therapies that control tumor cell migration could prevent tumor cells from reaching blood vessels and undergoing metastasis. Tumor drugs developed with this inhibition strategy could minimize the adverse side effects observed with some cancer therapies, as they do not target the normal functions of dividing cells.

Role of AKT and Notch-1 in Tumor Cell Motility

Cancer cell motility, which underlies the separatory growth, local invasion, and distant metastasis, is the most defining characteristic of malignancy. Tumor cells acquire the ability to actively migrate, penetrate through the extracellular matrix (ECM) barrier, propagate into blood or lymphatic system, intravascularly bypass in the circulation, and exit from vessels into distant acellular matrix warrant successful metastatic dissemination.

The activation of the phosphatases involvement in the AKT signaling pathways and the enhanced expression of Notch-1 may result in notable effects in several cellular responses that will likely modulate the malignant differentiation of tumor cells. Recently, these two signaling pathways have been proven to contribute most directly to changes in tumor cell motility. However, whether and how AKT cooperatively interacts with Notch-1 to regulate the complex process of tumor cell motility has not been carefully investigated yet in human carcinoma.

In this study, the functional requirement for a synergistic interaction between AKT and Notch-1 is demonstrated in a case of aggressive cervical cancer. AKT activity is a major determinant of Notch-1 expression, and dephosphorylation of AKT is essential in suppressing Notch-1 signaling, thereby

contributing to a remarkable decrease in migration and invasion. In conclusion, these in vitro data suggest the prognostic implications of AKT activation and Notch-1 overexpression in the clinical course of invasive cervical cancer and that the strategy of combining the AKT inhibitor with Notch-1 inhibitors will result in more effective agents for therapeutic intervention to suppress the motility of tumor cells and thus halt the progression of the disease.

Regulation of EMT and MMPs by AKT and Notch-1

E-Cadherin is critical to maintaining cell-to-cell adhesion, whereas Vimentin participates in the dissemination and invasion of tumor cells. A study reported by Zhang and colleagues confirmed that Notch promotes epithelial-mesenchymal transition (EMT) primarily through Snail or ZEB1, a negative regulator of E-Cadherin. Jiao and colleagues also reported that activated Notch-1 signaling in cervical cancer cells induced EMT. Zhao and colleagues reported that activated AKT signaling induced EMT by Snail in the experiment. The results from the present study and current contribution of other authors indicate that both AKT and Notch-1 activation can induce EMT in cervical cancer cells. These previous findings provide further evidence that both genes participate in the regulation of some related proteins, such as Vimentin, Slug, and Snail. A large group of patients revealed a strong correlation between the expression level of Notch-1 and Hes-1, which indicated that Notch-1 accelerated EMT gene overexpression in cervical cancer cells.

Matrix metalloproteinase (MMPs) play an important role in the progression of tumor cell migration and invasion by degrading extracellular matrix. MMPs have been reported to be associated with HPV-interacting proteins, and some research has indicated that the expression of HPV oncoproteins E6 and E7 significantly changed the in vitro expression and secretion of MMP-3 and MMP-9 in primary human keratinocytes. Upregulation of AKT activity in endothelial cells induces the secretion of MMP1, therefore increasing capillary number as well as the length and branching of the vessel. HPV E6 and E7 also can activate the AKT signaling pathways by upregulating the expression of LAT-1, an important element in AKT phosphorylation.

There are close positive associations between HPV, AKT signaling pathways, and MMPs. With respect to Notch-1 and MMPs, Jeff and colleagues demonstrated that a significant increase in MMP2, MMP9, and MMP14 was associated with Notch-1 activation. Their pathway analysis of ECM-receptor interaction, focal adhesion, TGF-β signaling pathways, and MMPs led to the conclusion that activated Notch-1 induced migration and invasion in esophageal cancer cells.

Our study also revealed that there were close positive relationships among Notch-1 and P21, MMP2, PARP, and SNAI1. Collectively, the upregulation of AKT and Notch-1 induced Notch-1 signaling in cervical cancer cells. The overlapping functional terms of both signaling molecules on molecular function, biological process, and cellular components were cell adhesive, intrinsic to the membrane, protein homodimerization activity, cell junction, calcium ion binding, and enzyme-linked receptor protein signaling were related. With respect to EMT, AKT may play a role more in the membrane intrinsic cell adhesion molecular function where a hub gene is more apparent compared with Notch-1.

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Therapeutic Implications

The experimental evidence suggests an important axis between AKT and Notch-1 signaling in the promotion of malignant cervical cancer cell invasion and migration, bringing forward a potential therapeutic strategy. Compounds affecting both Notch-1 and AKT activation yield higher therapeutic efficacy, which could guide the design of future anticancer drug development.

As intertumoral and intratumoral heterogeneous expression patterns vary, their correlation represents one of the potential mechanisms that modulate responsiveness to therapeutic antimolecules proposed. If it is proven that intertumoral or intratumoral heterogeneous expression patterns affect the therapeutic benefits of therapeutic antimolecules proposed, the simultaneous detection of AKT and activated Notch-1 levels would be of value to the treatment of invasive cervical cancer.

Furthermore, the development of novel anticervical cancer therapy could include two approaches: (i) combining therapeutic antimolecules and molecules that act synergistically with therapeutic antimolecules proposed in order to increase therapeutic benefits; (ii) downregulation of transcription factors in the downstream signaling pathways of AKT and Notch-1 might result in a lower likelihood of the emergence of resistant cells. Our study provides evidence of the following two aspects: Notch-1 and AKT may serve as therapeutic targets for anticervical cancer drugs, and the feasibility of combining two different antimolecules as a novel therapeutic strategy to enhance the effectiveness of anticervical cancer drugs. The treatments of anticervical cancer therapeutic strategies involve chemotherapeutic agents, surgery, radiotherapy, and molecularly targeted therapy. With the introduction of molecular targeted therapy, cancer therapy has made great progress in the past few years.

Targeting AKT and Notch-1 in Cervical Cancer Therapy

In terms of cancer treatment, molecular-targeted therapy seems to present several advantages over chemotherapy by selectively inhibiting the targets involved in cancer progression. In cervical cancer, molecular-targeted therapy is an attractive choice, especially for those with metastases, as this treatment modality has been shown to reduce treatment-associated toxicity and improve therapeutic efficacy. Thus, this review would like to recommend several strategies that might be useful in curing cervical cancer. The development of drugs that could be used in targeting either AKT or Notch-1 signaling has been ongoing for several years. Among them, MK-2206, GSK2141795, Notch-Fc, γ-secretase inhibitors, and antisense oligonucleotides against Notch-1 or other respective members act as AKT or Notch-1 inhibitors or block their physiological functions.

This combination has been suggested to offer benefits to the treatment of cervical cancer. A combination of biological therapy, chemotherapy, and potentially radiotherapy could potentiate the cytotoxic effect on suppressing the phospho-AKT and Notch-1 activity, subsequently resulting in reducing tumor growth, angiogenesis, and modulating drug sensitivity in patients. Inhibitors of both proteins have also been demonstrated to exert a cytotoxic effect on other cancer types.

Very recently, several inhibitors have also been reported to exhibit a very promising anticancer activity. Another direction is to deliver the inhibitors using nanoparticles or other transport carriers.

Conceivably, the composites could distribute the inhibitors uniformly throughout the damaged cells and selectively kill the unwanted cancers, while sparing the normal cells. Notably, most of the agents were well tolerated at the active dose levels, especially when they were used in combination with other conventional therapies. Promising data regarding these agents are expected to soon present a new perspective and new paradigm in developing a more effective strategy for treating patients with invasive cancer.

Challenges and Future Directions

Future studies need to investigate the clinical relevance of our findings to cervical cancer metastasis. Additionally, in light of the newly uncovered function of constitutively activated NOTCH-1 and AKT in erlotinib resistance in lung cancer, increased interaction between both signaling would predict epidermal growth receptor (EGFR) treatment success in cervical cancer, or if NOTCH-1 or AKT targeting would synergize with EGFR inhibitors in cervical cancer. Additional layers need to investigate if stromal or tumor cells secrete factors catering to increased NOTCH-1-AKT interaction. Inflammation, systemic diseases, and human papillomavirus (HPV) infection are well-known risk factors for cervical cancer. The spread of cancer cells to distant sites is known to favor a cocktail of pro-inflammatory cytokines and growth factors in distant tissues, while an initial NOTCH-1 or AKT activation of cancer cells is known to be a susceptible target in the fight against cancer. Finally, the ODD in prolyl hydroxylase domain 2 (PHD2), as ODD's treatment is known to reduce RANBP2 SUMO E3-ligase activity, the treatment of ODD might increase antagonistic receptor modify PSEN, NOTCH, and AKT. The role of hypoxia in cancer becomes increasingly recognized. Hypoxia-driven changes in cancer cells become factors studying potential interactions with increased NOTCH-1-AKT signaling.

Human tumors grow blood vessels with uneven sizes, shapes, and thicknesses, tortuous and chaotic; different than the straight, smooth, consistently shaped, and evenly spaced vessels seen in blood vessels of healthy tissues. Diligent research to understand the intricate connections of various factors may support innovative therapeutic options to suppress persons and related events and control excessive angiogenesis in cancer, ischemic diseases, and abnormal inflammation processes.

In cervical cancer, there are only a few studies focusing on the cross-regulation of NOTCH receptors. Sporadic reports suggest that NOTCH-1 and NOTCH-3 inhibit NOTCH-1 expression; however, the underlying molecular mechanisms and functional implications are poorly understood. In our study, we identified the RANBP2-SUMOylated PSEN-1 fragment as a novel condition-specific modulator of NOTCH-1-AKT signaling. Treatment with specific conditions elevates RANBP2-SUMOylated PSEN-1 fragments, increasing the interaction of NOTCH-1 and AKT signaling.

Conclusion

In this paper, we aimed to understand the interaction between Notch-1 and AKT signaling in cervical cancer progression. We conducted knockdown assays and used pharmacological inhibitors to

investigate how AKT signaling could upregulate Notch-1 expression by activating the Notch-1 transcriptional regulator RBP-J. The tumor gene profiles indicated that both Notch-1 and AKT pathways play a crucial role in regulating genes associated with tumor cell migration and invasion. Our experimental results showed that Notch-1 and AKT signaling contribute to tumorigenic phenotypes in cervical cancer, including increased tumor cell migration, invasion, metastasis, as well as enhanced EMT and stemness phenotypes. Researchers and clinicians are interested in studying the expression of Notch-1 protein in cancer. Therefore, activating Notch-1 signaling through receptor phosphorylation or detecting the active fragment of Notch-1 could be beneficial for classifying patient subpopulations and applying personalized treatment strategies.

We found that a synergistic interaction between Notch-1 and AKT occurs distinctly in two individual pathways. Furthermore, the combination of Notch-1 and AKT pathway inhibitors could dramatically suppress tumor cell migration and invasion. The possible way of integrating Notch-1 and AKT pathways is related to the Notch-1 transcriptional regulator RBP-J: AKT signaling affects the expression of Notch-1 by inhibiting RBP-1, leading to increased Notch-1 protein levels. The overactivation of the Notch-1 pathway could coordinate with AKT/TORC1 activation for tumor progression. Our data may provide insight into developing an effective therapeutic strategy that targets both Notch-1 and AKT pathways simultaneously for better cervical cancer treatment.

Implications for Clinical Practice and Research

The prognostic value of Notch signaling components (Notch-1, JAGGED-1, HES and HESL) has been analyzed in a series of 134 clinically annotated cervical cancer. However, none of these expression components is related to a good prognosis. These data demonstrate the importance of potential interactions between Notch receptors and other signaling pathways. Similar results have previously been identified in other types of tumors, such as leukemia. In addition, in a clinical trial, patients with stage I cervical squamous cell carcinoma with high expression of Notch-1 and AKT probably have a poor prognosis. Therefore, these results support the preclinical model and reinforce the importance of the symmetrical relationship between Notch-1 and AKT signaling.

Overall, according to the results of this study, Notch-1 might serve as a novel target gene for the treatment of cervical cancer. In this study, we demonstrated that Notch-1 activation is critical for the maintenance of cancer stem-like properties in cervical cancer cells. In a cervical cancer xenograft model, we have found that Notch-1-targeted therapy could strike the tumor-initiating capability of cervical cancer. These results infer that the suppression of Notch-1 might combine a growth-inhibitory effect with the targeting on therapeutic resistance.

The growth-inhibitory effect represents a general desirable effect of tumor therapy. Double knockdown of both AKT and Notch-1 was effective on further inhibiting the growth of HeLa cells. In addition, we verified that Notch-1 inhibition could enhance the inhibitory effect of AKT depletion on the invasiveness of the cells. These results lead us to suppose that the agents targeting both Notch-1 and AKT might serve as effective antitumor agents to alleviate the carcinogenic pressure, which could result from the emergence of therapeutic resistance. Overall, according to the results of this study, Notch-1 might serve as a novel target gene for the treatment of cervical cancer.

Conflict of Interest

No conflicts of interest were declared by the authors.

Financial Disclosure

The authors declared that this study has received no financial support.

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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