A NOVEL NOTCH - Akt - NF-κB AXIS IN TRIPLE NEGATIVE BREAST CANCER CELLS

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Abstract

Basal-like and claudin-low are two major subtypes of triple-negative breast cancers (TNBC). The subtype claudin-low is represented by MDA-MB 231 cells. The Notch and NF-κB signaling pathways regulate proliferation, differentiation, and apoptosis by regulating the transcription of target genes. Both pathways are commonly active in TNBC. There is strong evidence for cross-talk between these pathways. We previously showed that in cervical cancer cells, Notch-1 can activate NF-κB through IKKα. We also showed that Notch-1 can activate the estrogen receptor alpha (ERα), also through nuclear (IKKα) and the PI3K signalosome in T47D cells. However, the mechanism(s) of IKKα activation downstream of Notch remains unknown. Notch-1 is highly expressed in TNBC, and Notch ligands, Jagged-1 and 2 correlate with poor prognosis. IKKα (CHUK) is expressed in all subtypes of TNBC. We investigated whether Notch-1 can activate NF-κB in TNBC cells of claudin-low and basal-like subtypes, whether this requires IKKα and how IKKα is activated.

Introduction

TNBC is a group of heterogeneous, clinically aggressive tumors (1). These tumors lack expression of the nuclear receptors for estrogen and progesterone and do not overexpress HER2/Neu. The standard of care for these cancers is chemotherapy, with variable response rates (2). Recurrence tends to exhibit poor overall survival rate and disease free survival (3). There is currently no standard targeted therapy for these tumors. TNBC express high levels of Notch-1 (4). Notch is an evolutionary conserved pathway; pathway prediction and network modeling confirmed that Notch receptors and genes involved in the Notch signaling pathway interact with genes containing SNPs associated with risk for breast cancer (6). High expression of Notch-1 and Jagged-1 mRNA correlates with poor prognosis in breast cancer (7,8). Among recurrent TNBC, tumors with high expression of Notch-1 have significantly poorer survival (Hicks, unpublished). The Notch and NF-κB signaling pathways regulate proliferation, differentiation, and apoptosis and are known to be required for survival in TNBC (9-13). Nuclear IKKα activation downstream of Notch (35). However, it is unclear how Notch-1 activates IKKα downstream of Notch-1 to activate NF-κB.

Objectives

• To investigate whether Notch-1 activates NF-κB in TNBC cells and whether this activation requires IKKα
• To investigate whether Akt mediates the effects of Notch-1 on NF-κB in TNBC cells
• To investigate whether Akt pathway inhibitors and gamma secretase inhibitors (GSIs) can be used in combination in TNBC models

Methods

Cell culture: MDA-MB-231 cells were obtained from ATCC and grown in DMEM supplemented with 10% FBS and 1% Penicillin/Strep.
Chromatin immunoprecipitation (CHIP): MDA-MB-231 cells were cross linked with 1% formaldehyde. Cells were lysed in nuclease lysis buffer. The lysate was sonicated on ice at 95% total power for six cycles of 12 pulses each.
Co-culture: Mouse fibroblast: LTK-Parental (P) and LTK-Jagged (J) plated onto MDA-MB 231 cells to induce Notch activation.
Co-immunoprecipitation (coIP): MDA-MB-231 cells co-cultured. Immunoprecipitated with Notch C20 or IgG -Rabbit Immunoglobulin for PRKAs, p110α
Immunoblot: 30-100 μg of sample was prepared and loaded into 7% precast gels
Xenografts: 1x10^6 cells were injected in the mammary fat pad of nude mice (n = 4). Mice were treated with LY411,575 (2.5 mg/kg, 3 days a week), perifosine (20 mg/kg every other day) or both. Tumor growth was monitored by bioluminescence with an IVIS Caliper instrument and by Vernier caliper.
Reverse Transcriptase Polymerase Chain Reaction (RT-PCR): was used to quantify relative transcript levels.
RNA interference: MDA-MB-231 cells were transiently transfected at 30-50% confluency with Lipofectamine RNAmax.

Results

Figure 1: Notch-1 maintains and activates NF-κB transcriptional activity in MDA-MB231 cells. Knockdown of Notch-1 decreases NF-κB-dependent transcription activity (A). Over-expression of Notch-1 IC (NIC) induces a dose dependent activation of NF-κB (B).

Figure 2: Notch-1 activation increased expression of NF-κB transcriptional target genes. Co-culture of MDA-MB-231 cells with Jagged-1 expressing LTK fibroblasts showed increased transcription of NF-κB target genes c-IAP2, IκBα, and A20 when induced by TNF-α, a known activator of NF-κB.

Figure 3: Notch-1 is necessary for the transcriptional activity of NF-κB. NF-κB target genes c-IAP2, A20, and IκBα decreased with knockdown of Notch-1 and IKKα. Target genes were unchanged with the knockdown of Notch-4.

Figure 4: Notch activation is necessary for Akt phosphorylation; Inhibition of Akt or Notch decreases IKKα phosphorylation; Combined Akt and Notch inhibition is effective in vivo; Notch-1 complexes with PI3K upon Notch activation. Notch activation causes phosphorylation of Akt at Ser473 and S450 at S18 (A). Wortmannin reduces IKKα phosphorylation (B). Akt inhibitor perifosine and Notch inhibitor GSI LY411,575 in combination reduce tumor volume in MDA-MB231 xenografts (C). Four chemically different clinical GSIs inhibit Akt phosphorylation in MDA-MB231 at clinically achievable concentration (10 μM). D. Activated Notch-1 complexes with PI3K substrates, p85 and p110, after 30 minutes co-culture of MDA-MB 231 cells with Jagged-1 expressing LTK cells (E).

Figure 5: Notch-1 is required for NF-κB chromatin recruitment and is recruited to the c-IAP2 promoter; IKKα kinase activity is required for NF-κB chromatin recruitment; IKKα is recruited to the c-IAP2 promoter after Notch activation. Notch-1 knockdown prevents c-IAP2 promoter binding of p60 and p65 (A). Notch-1 is recruited to the same site (B). IKKα kinase activity is required for accumulation of p60 and p65 at the c-IAP2 promoter (C). IKKα is recruited to the c-IAP2 promoter with a peak at 60 min after Notch activation (D).

Conclusions

In PTEP® MDA-MB231 cells, we describe a Notch/Pi3K-Akt-IKKα-NF-κB pathway that leads to expression of NF-κB target genes such as c-IAP2. Akt activation is mediated through a non-canonical Notch signaling pathway that operates within minutes of ligand binding, similar to what described by Soke et al. in T-cells. Our data suggest that Notch complexes with PI3K substrates, p85 and p110, after ligand-mediated activation. Inhibition of Notch activity through GSIs (10 μM) decreases NF-κB phosphorylation (A). Our data support the investigation of combined use of Notch inhibitors and inhibitors of the Akt pathway in PTEP® triple negative breast cancers.