



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

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Abstract

Basal-like and claudin-low are two major subgroups of triple-negative breast cancers (TNBC). The subtype claudin-low is represented by MDA-MB 231 cells. The Notch and NF- κ B 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- κ B through nuclear IKK α . We also showed that Notch-1 can activate the estrogen receptor alpha (ER α), also through nuclear IKK α , and the IKK signalosome in T-LL cells. However, the mechanism(s) of IKK α activation downstream of Notch remain unknown. Notch1 is highly expressed in TNBC, and Notch ligands Jagged1 and 2 correlate with poor prognosis. IKK α (CHUK) is expressed in all subtypes of TNBC. We investigated whether Notch-1 can activate NF- κ B in TNBC cells of claudin-low and basal like subtypes, whether this requires IKK α and how IKK α is activated.

Introduction

TNBC are a group of heterogeneous, clinically aggressive tumors (1). These tumors lack expression of the nuclear receptors for estrogen and progesterone and do not overexpress HER-2/neu. The standard of care for these cancers is chemotherapy, with variable response rates (2). Recurrent tumors tend 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 (4,5). 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 Among recurrent TNBC, tumors with high Notch-1 expression have significantly poorer survival (Hicks, unpublished). The Notch and NF- κ B signaling pathways regulate proliferation, differentiation, and apoptosis and are known to cross-talk (9,10). Nuclear IKK α acts downstream of Notch-1 to activate NF- κ B (11) and ER α (12). However, it is unclear how Notch-1 activates IKK α . IKK α is phosphorylated by Akt (reviewed in 11). Akt is commonly phosphorylated in TNBC (12). Notch activates the PI3-kinase (PI3K)-Akt pathway in a variety of cells (13-16). Therefore, we investigated whether Notch-1 activates NF- κ B in PTEN^{wt} TNBC cells via a PI3K-Akt-IKK α pathway.

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Objectives

- To investigate whether Notch -1 activates NF- κ B in TNBC cells and whether this activation requires IKK α
- To investigate whether Akt mediates the effects of Notch-1 on NF- κ B 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 nucleus 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 C-20 or IgG –Rabbit Immunoblot for P85 α , p110 α .
- Immunoblot:** 30-100 μ g of sample was prepared and loaded into 7% precast gels
- Xenografts** 10⁶ 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 (30 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 RNAiMAX.

Results

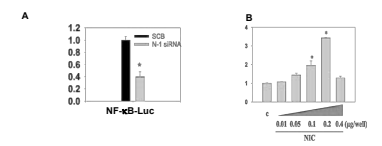


Figure 1: Notch-1 maintains and activates NF- κ B transcriptional activity in MDA-MB231 cells. Knockdown of Notch-1 decreases NF- κ B dependent transcription activity (A). Over-expression of Notch-1 IC (NIC) induces a dose dependent activation of NF- κ B (B).

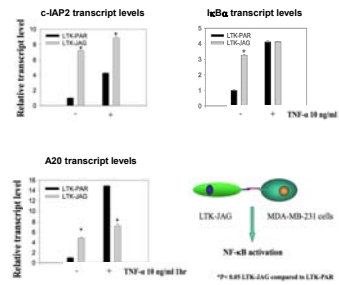


Figure 2: Notch-1 activation increased expression of NF- κ B transcriptional target genes. Co-culture of MDA-MB-231 cells with Jagged-1-expressing LTK fibroblasts showed increased transcription of NF- κ B target genes: c-IAP2, IkB α , and A20 when induced by TNF- α , a known activator of NF- κ B.

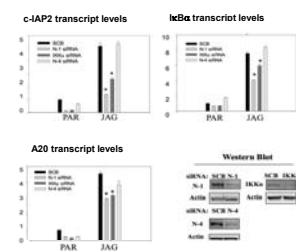


Figure 3: Notch-1 is necessary for the transcriptional activity of NF- κ B. NF- κ B target genes c-IAP2, A20, and IkB α decreased with knockdown of Notch-1 and IKK α . Target genes were unchanged with the knockdown of Notch-4.

Results

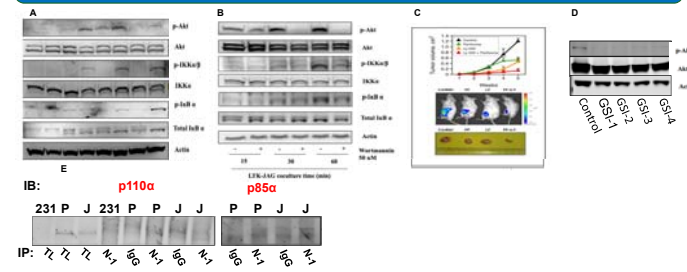


Figure 4: Notch activation is necessary for Akt phosphorylation; Inhibition of Akt or Notch decreases IKK α phosphorylation; Combined Akt and Notch inhibition is effective in vivo; Notch-1 complexes with PI3K upon Notch activation. Notch activation causes phosphorylation of Akt at S473 and IKK α at S180 (A). Wortmannin reduces IKK α 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 μ M, D). Activated Notch-1 complexes with PI3K subunits, p85 and p110, after 30 minutes of co-culture of MDA-MB 231 cells with Jagged-1 expressing LTK cells (E).

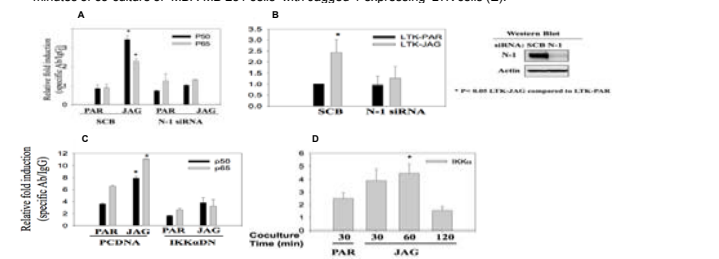
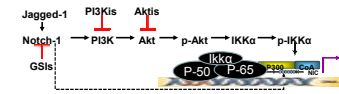


Figure 5: Notch-1 is required for NF- κ B chromatin recruitment and is recruited to the c-IAP2 promoter; IKK α kinase activity is required for NF- κ B chromatin recruitment; IKK α is recruited to the c-IAP2 promoter after Notch activation. Notch-1 knockdown prevents cIAP2 promoter binding of p50 and p65 (A). Notch-1 is recruited to the same site (B); IKK α kinase activity is required for accumulation of p50 and p65 at the c-IAP2 promoter (C). IKK α is recruited to the c-IAP2 promoter with a peak at 60 min after Notch activation (D).



Conclusions

In PTEN^{wt} MDA-MB231 cells, we describe a Notch-PI3K-Akt-IKK α -NF- κ B pathway that leads to expression of NF- κ B 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 the what described by Sade et al. in T-cells. Our data suggest that Notch complexes with PI3K subunits, p85 and p110, after ligand-mediated activation. Inhibition of Notch activity through GSIs (γ -secretase inhibitors) leads to decreased phosphorylation of Akt. Our data support the investigation of combined use of Notch inhibitors and inhibitors of the Akt pathway in PTEN^{wt} triple negative breast cancers.