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Notch signaling pathway: An emerging therapeutic target for African-American triple negative breast cancer patients

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ABSTRACT

The most fatal form of breast cancer, triple negative breast cancer (TNBC), continues to challenge clinicians worldwide with its lack of reliable prognostic biomarkers and pharmacologically actionable treatment targets. In the US, this aggressive disease disproportionately afflicts African-American women at a rate 2-3 times higher than European-American (EA) women, thereby contributing to the observed higher mortality rates of AA BC patients. In order to address the unmet clinical need for new and effective treatments for AA TNBCs, we describe herein a potentially actionable pathway that appears to be in overdrive in TNBCs of AA patients compared to EA TNBCs: the Notch signaling pathway. Notch signaling is implicated in multiple aspects of carcinogenesis and tumor progression including the regulation of proliferation, apoptosis, the biology of cancer stem cells, tumor angiogenesis and epithelial-to-mesenchymal transition. Our gene expression analyses have uncovered significant upregulation of Notch signaling as well as gene ontologies reflecting dysregulation of key processes regulated by Notch signaling among AA compared to EA TNBC patients. Furthermore, we present evidence suggesting that upregulated Notch signaling may predict poor prognosis in TNBC. Our findings thus suggest differences in Notch signaling among racially-distinct TNBC patients that may contribute to the more aggressive clinical behavior of TNBC in AAs. These observations also suggest that Notch signaling may be an attractive therapeutic target for high-risk AA TNBC patients.

KEYWORDS: Triple negative breast cancer, racial disparity, Notch signaling pathway, African-American

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The tip of the iceberg: Racial disparities in triple negative breast cancer

Triple negative breast cancer (TNBC) is a significant public health concern in the US as it afflicts about one-fifth of the ~2.8 million women in the country with breast cancer (BC) (Criscitiello et al., 2012; Howlader N, 2014). Worldwide, the disease accounts for approximately 20% of BC cases. TNBC is the most clinically challenging subtype of BC as it lacks expression of the pharmacologically-targetable estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) (Bianchini et al., 2016b; Lehmann et al., 2011; Shah et al., 2012). Furthermore, the disease is characterized by high rates of recurrence and metastases, especially within the first five years post diagnosis (Bianchini et al., 2016a; Kassam et al., 2009; Lehmann et al., 2011; Shah et al., 2012). TNBCs exhibit high interpatient heterogeneity and many classification schemes have emerged that categorize TNBCs into transcriptomically-distinct molecular subtypes and with unique DNA copy number variations (Burstein et al., 2015; Lehmann et al., 2016). TNBCs also exhibit intratumoral heterogeneity which, together with the lack of clinically facile methods of determining molecular subtypes, thwarts the development of novel targeted treatments. Thus, despite advances in treatments for other BC subtypes, chemotherapy remains the cornerstone of treatment for TNBC.

TNBC disproportionately afflicts African-American (Raab et al.) women, especially younger premenopausal AAs (Brewster et al., 2014; Danforth, 2013; Jiagge et al., 2018; Keenan et al., 2015; Newman and Kaljee, 2017; Wu et al., 2017). After adjusting for potentially confounding factors, including tumor stage and grade (which tend to be higher in AA women) and age at diagnosis and socioeconomic status (which tend to be lower in AA women), AA women exhibit ~2 times the likelihood of presenting with TNBC (Dietze et al., 2015), which partially explains their worse outcomes relative to EA patients. The predisposition towards developing TNBC appears to be deeply rooted in biogeographic ancestry. For example, ~50% of Nigerian (Agboola et al., 2012) and Malian (Ly et al., 2012) and ~80% of Ghanaian (Stark et al., 2010a; Stark et al., 2010b) women with BC have triple-negative breast tumors, in contrast with ~15% of EA (Carey et al., 2006; Stark et al., 2010a) or white British women (Agboola et al., 2012; Bowen et al., 2008). Stark et al. found that 83% of African women presented with TNBC compared to only 41.9% of AAs and 15.4% of EAs (Stark et al., 2010b).

Although controversial, racial disparities in disease course and outcomes have been reported within the TNBC subtype. Accumulating evidence suggest that AAs present with more unfavorable clinico-pathological characteristics such as larger tumor size, higher proliferation, more extensive lymph node involvement, as well as present at a younger age than EAs among TNBC patients (Dietze et al., 2015; Lund et al., 2009; Sullivan et al., 2014). Furthermore, AAs have been reported to harbor more aggressive TNBC subtypes such as basal-like 1 and mesenchymal stem-like as well as greater intratumoral heterogeneity than EAs (Keenan et al., 2015; Lindner et al., 2013). As a result, AAs have been reported to experience shorter overall survival (OS) and progression-free survival (PFS) than EAs among TNBC patients (Dietze et al., 2015; Lund et al., 2009; Sullivan et al., 2014). Newman and colleagues observed 30% higher mortality rate among AA compared to EA TNBC patients (Newman et al., 2006). These recent findings have

sparked investigations into distinctions in inherent tumor biology between AA and EA TNBCs to elucidate the molecular underpinnings driving the racially disparate burden in TNBC. Currently, there are no reliable methods to identify AA TNBC patients at high risk of poor outcomes, which would indicate the need to offer more aggressive treatment. Identification of biomarkers that can risk-stratify AA TNBC patients and predict responsiveness to targeted and cytotoxic agents could improve prognosis of this high-risk patient population.

Top notch distinctions: Shedding light on the Notch signaling pathway in triple negative breast cancer

The Notch signaling pathway has recently emerged as a novel therapeutic target of interest in TNBC. The pathway is present in most multicellular organisms and is highly conserved. It is essential for cell proliferation, differentiation and development and plays keys roles in cell fate determination (Ranganathan et al., 2011). There are four different Notch receptors (Notch1, Notch2, Notch3 and Notch4) expressed in mammals (Dontu et al., 2004). The Notch receptor is a hetero-oligomer transmembrane receptor protein composed of an extracellular protein domain, a single transmembrane pass and a small intracellular region. Notch receptors on the cell surface engage with Notch ligands- Delta-like (DLL1, DLL3, DLL4) and Jagged (JAG1, JAG2) to initiate the signaling cascade (Bray et al., 2008; Brou et al., 2000; Fiuza and Arias, 2007). After the interaction of a notch ligand with its receptor, a metalloprotease protein from the ADAM-family (ADAM10) cleaves the Notch receptor outside the membrane (van Tetering et al., 2009); the extracellular portion of the Notch receptor attached to its ligand is thus released and gets endocytosed by ligand-expressing cells. The remaining part of the Notch receptor inside the inner leaflet of the cell membrane undergoes a cleavage by an enzyme called gamma-secretase, releasing the intracellular domain of the Notch protein (Borggrefe and Liefke, 2012). The Notch intracellular domain then forms a trimeric core transactivation complex with the sequence-specific DNA binding protein, CSL (CBF-1/Su(H)/Lag-1), and the transcriptional co-activator, Mastermind, to activate transcription of target genes (Nam et al., 2006; Wilson and Kovall, 2006). Transcriptional targets include transcription factors (NF-κB2 and c-Myc), cell-cycle regulators (cyclin D1 and p21), growth factor receptors and regulators of angiogenesis and apoptosis. Dysregulated Notch signaling is associated with various malignancies. Expression of Notch receptors and ligand protein have been reported to be upregulated in breast tumors compared to normal breast tissues (Mittal et al., 2009; Rizzo et al., 2008; Zardawi et al., 2010). Parr et al. observed an aberrant level of Notch-1 and Notch-2 expression in breast tumor tissue via immunohistochemistry and quantitative RT-PCR. The study demonstrated that high expression level of Notch ligands and/or receptors correlated significantly with poor clinical outcomes (Parr et al., 2004). Moreover, several studies suggest that increased expression of Notch is associated with oncogene expression, maintaining stemness of BC stem cells and deregulated cell cycle progression (Harrison et al., 2010; Ronchini and Capobianco, 2001; Sharma et al., 2006; Weng et al., 2006).

Notch signaling plays a critical role in TNBC. Dickson et al. were the first to uncover an association of Notch expression with TNBC. The study revealed a significant correlation of overexpression of JAG-1 and Notch-1 with poor

prognosis among TNBC patients (Dickson et al., 2007). Furthermore, Notch-1 and Notch-4 receptors have been discovered to be overexpressed in TNBC vascular endothelial cells (Speiser et al., 2012). Recent studies also uncovered a Jagged1-Notch1-CyclinD1 axis playing a key role in maintaining proliferation of TNBC, as opposed to other types of BC (Cohen et al., 2010). Notch3 signaling controls survival of hypoxic TNBC cells and Notch4 is involved in self-renewal of BC stem cells. (Harrison et al., 2010; Sansone et al., 2007) Evidence reinforcing the notion that dysregulation of Notch signaling is pathogenetically relevant in TNBC came from a study wherein chromosomal rearrangements producing constitutively active versions of Notch1 or Notch2 were detected almost exclusively in TNBC cell lines and tumors (Robinson et al., 2011). When the Notch signaling pathway gets activated, the Notch intracellular domain (ICD), generated by the enzyme gamma-secretase, translocates from the cytoplasm to the nucleus where it binds to the CSL complex to initiate the transcription of downstream targets (Shih Ie and Wang, 2007). The nuclear localization of Notch has been reported to occur more frequently in TNBC compared to hormone receptor-positive tumors (Touplikioti, 2012). Moreover, Speiser et al. discovered more positive nuclear and cytoplasmic staining of Notch-1 and Notch-4 in TNBC samples and more positive membrane staining of these proteins in hormone receptor-positive breast tumor specimens (Speiser et al., 2012). Many studies on Notch signaling have postulated that its cellular localization can be a morphological illustration of its function (Bray et al., 2008). As previously described, upon interaction of the transmembrane Notch receptor with its ligand, the receptor is proteolytically cleaved and the NICD is released into the nucleus. Inside the nucleus, NICD modulates transcription of target genes via interaction with CSL (Schroeter et al., 1998; Weijzen et al., 2002). Thus, the subcellular localization of the Notch protein reflects the functional activity of the receptor. Membrane localization of the Notch protein represents a mature receptor that has not yet been activated; cytoplasmic localization of the protein represents a newly synthesized receptor which is on its way to plasma membrane; and nuclear localization of Notch protein reflects the activated state of the receptor (Speiser et al., 2012). However, the correlation between the amount of Notch staining and Notch pathway activity, especially if the staining is distributed to the nucleus or cytoplasm, remains unknown. Furthermore, in a study by Yao et al., there was significantly more membranous staining in the ER-positive compared to the ER-negative BC cases (Yao et al., 2011). This finding suggests that estrogen increases Notch-1 protein levels and causes it to accumulate at the cell membrane [which represents a mature but non-activated form of Notch-1], but not in the nucleus (Rizzo et al., 2008). Thus, nuclear localized Notch-1 and Notch-4 may serve as potential therapeutic targets for TNBC patients. Moreover, Rizzo *et al.* observed sensitivity of TNBC cells to Notch inhibitors. The authors demonstrated arrest of TNBC cells in G2 phase of cell cycle upon knockdown of Notch-1 and Notch-4 with siRNA or pharmacological inhibition of gamma-secretase (GSI) (Rizzo et al., 2008). GSI inhibitors are currently in clinical trials to reduce Notch signaling in patients with recurrent TNBC (Olsauskas-Kuprys et al., 2013). A recent clinical trial demonstrated that the administration of a GSI inhibitor in combination with Docetaxel elicited anti-tumor activity in patients with locally advanced/metastatic

TNBC (Locatelli and Curigliano, 2017). Thus, Notch signaling, which is highly upregulated in TNBC, may be a potential therapeutic target for TNBC patients, who lack good targeted therapy options.

Uncharted territory: Investigating disparities in the Notch signaling pathway among racially-distinct triple negative breast cancer patients

The emergence of Notch signaling as a therapeutic target of interest in TNBC has spurred interest in this pathway as a potential suspect in the racially disparate burden in TNBC. However, literature evidence supporting this speculation remains sparse. A genome-wide association study conducted by Adriano and colleagues revealed that a SNP in the NOTCH4 locus was significantly associated with AA but not EA sarcoidosis patients and this finding remained consistent in multivariate models (Adrianto et al., 2012). Sarcoidosis disproportionately afflicts AAs suggesting that this genetic variant may play a role in the disease's disparate burden (Cozier et al., 2011; James and Sherlock, 1994). Furthermore, Stewart *et al.* discovered that the Notch 2 N-terminal-like (NOTCH2NL) gene was significantly upregulated among AA compared to EA BC patients in the TCGA dataset (Stewart et al., 2013). However, ethnic differences in Notch signaling and its role in the higher incidence of and poorer outcomes from TNBC remain understudied.

We recently queried TCGA breast dataset for AA and EA TNBC patients and exploited bioinformatics tools to determine differentially-expressed genes, biological pathways, and gene ontologies between the racially-distinct TNBC patients. According to our DESeq2 or differential gene expression analyses, we observed significant upregulation of genes encoding key components of the Notch signaling network such as NOTCH-Regulated Ankyrin Repeat Protein (NRARP), NOTCH2NL, Delta/Notch-like EGFR Repeat containing (DNER), Jagged 1 (JAG1), Jagged 2 (JAG2), Hess family belch transcription factor 4 (HES4), and Matrix Metalloproteinase-9 (MMP9) among AA compared to EA TNBC samples ($p<0.05$) (Table 1). We employed the GAGE and Pathview packages to identify differentially-expressed biological pathways or experimentally-derived differential expression sets (Table 2) and gene ontologies (Table 3) between RNA sequenced AA and EA TNBC samples. Interestingly, we discovered that the Notch signaling pathway expression set was more upregulated in AA compared to EA TNBC samples ($p=0.054$). We also observed significant downregulation of key processes that are normally repressed by Notch signaling such as focal adhesion, extracellular matrix (ECM)-receptor interaction, and adherents junction expression sets as well as cell junction assembly, cell-cell junction organization, cell-cell adhesion, epithelial cell development, negative regulation of endothelial cell proliferation, double-strand break repair, and regulation of DNA repair gene ontologies ($p<0.05$). Furthermore, we observed significant upregulation of gene ontologies reflecting T cell antitumoral immunity (which is enhanced by Notch signaling) such as T cell differentiation, T cell activation, adaptive immune response, and interferon-gamma production ($p<0.05$). Moreover, Kaplan-Meier survival analyses revealed that overexpression of DNER predicted significantly poorer relapse-free survival (RFS) in TNBCs (HR=2.4; p=0.0012); JAG2 expression also

Table 1. Genes significantly upregulated among AA compared to EA TNBC patients in the TCGA dataset.						
Gene	Symbol	Entrez	Name	Base Mean	log2FoldChange	p value
ENSG00000198435	NRARP	441478	NOTCH-regulated ankyrin repeat protein	516.3894051	5.051550368	4.38E-07
ENSG00000167244	IGF2	3481	insulin like growth factor 2	2619.306453	-1.233367377	1.02E-05
ENSG00000157764	BRAF	673	B-Raf proto-oncogene, serine/threonine kinase	214.9862772	-0.502496583	2.33E-05
ENSG00000165105	RASEF	158158	RAS and EF-hand domain containing	162.6660192	-0.958748689	7.76E-05
ENSG00000184916	JAG2	3714	jagged 2	836.7242091	3.853121028	0.0001166
ENSG00000188290	HES4	57801	hes family bHLH transcription factor 4	137.2454011	3.84511881	0.0001205
ENSG00000171408	PDE7B	27115	phosphodiesterase 7B	70.86301288	-0.806853305	0.0001551
ENSG00000073921	PICALM	8301	phosphatidylinositol binding clathrin assembly protein	4146.086807	-0.328030427	0.0001577
ENSG00000264343	NOTCH2NL	388677	notch 2 N-terminal like	1130.083257	3.692675235	0.0002219
ENSG00000185737	NRG3	10718	neuregulin 3	18.2400562	-1.093851375	0.0002292
ENSG00000046889	PREX2	80243	phosphatidylinositol-3,4,5-trisphosphate dependent Rac exchange factor 2	35.37360263	-0.965093716	0.0003995
ENSG00000151689	INPP1	3628	inositol polyphosphate-1-phosphatase	257.8331804	-0.464334443	0.0007263
ENSG00000151151	IPMK	253430	inositol polyphosphate multikinase	66.09782388	-0.677173589	0.0007599
ENSG00000101384	JAG1	182	jagged 1	2029.134072	3.352573762	0.0008006
ENSG00000169435	RASSF6	166824	Ras association domain family member 6	87.45601131	-0.732054428	0.0010291
ENSG00000041353	RAB27B	5874	RAB27B, member RAS oncogene family	67.49077359	-0.735088474	0.0011336
ENSG00000175985	PLEKHD1	400224	pleckstrin homology and coiled-coil domain containing D1	3.551807893	-0.997599044	0.0012151
ENSG00000169220	RGS14	10636	regulator of G-protein signaling 14	356.4357972	0.493690701	0.0013154
ENSG00000154678	PDE1C	5137	phosphodiesterase 1C	63.21150224	-0.857678049	0.0014838
ENSG00000151491	EPS8	2059	epidermal growth factor receptor pathway substrate 8	895.8248723	-0.525349111	0.0016848
ENSG00000064932	SBNO2	22904	strawberry notch homolog 2	1823.609706	3.135072772	0.0017181
ENSG00000011405	PIK3C2A	5286	phosphatidylinositol-4-phosphate 3-kinase catalytic subunit type 2 alpha	1239.790208	-0.396160165	0.0017905
ENSG00000109452	INPP4B	8821	inositol polyphosphate-4-phosphatase type II B	152.8627179	-0.735904178	0.0018282
ENSG00000178568	ERBB4	2066	erb-b2 receptor tyrosine kinase 4	74.66419079	-0.896585238	0.0024244
ENSG00000187957	DNER	92737	delta/notch like EGF repeat containing	173.0818588	2.870907184	0.004093
ENSG00000078142	PIK3C3	5289	phosphatidylinositol 3-kinase catalytic subunit type 3	551.5572258	-0.281487717	0.0055186

ENSG00000085832	EPS15	2060	epidermal growth factor receptor pathway substrate 15	1725.645419	-0.21533493	0.0055454
ENSG00000137825	ITPKA	3706	inositol-trisphosphate 3-kinase A	11.85034496	-0.688848495	0.0061921
ENSG00000137142	IGFBPL1	347252	insulin like growth factor binding protein like 1	12.11877278	-0.790048534	0.0062746
ENSG00000073536	NLE1	54475	notchless homolog 1	347.5008396	2.711514433	0.0066977
ENSG00000163964	PIGX	54965	phosphatidylinositol glycan anchor biosynthesis class X	740.0513792	-0.330509569	0.0084688
ENSG00000097033	SH3GLB1	51100	SH3 domain containing GRB2 like endophilin B1	2960.384131	-0.219544393	0.0084951
ENSG00000169752	NRG4	145957	neuregulin 4	7.692847298	-0.718554497	0.0086447
ENSG00000161896	IP6K3	117283	inositol hexakisphosphate kinase 3	11.780405	-0.794971219	0.0092705
ENSG00000162434	JAK1	3716	Janus kinase 1	3697.004587	-0.265820839	0.0099595
ENSG00000197563	PIGN	23556	phosphatidylinositol glycan anchor biosynthesis class N	628.3454755	-0.361073745	0.0100388
ENSG00000146648	ERBB1	1956	epidermal growth factor receptor	788.1655218	-0.514822969	0.0106547
ENSG00000186479	RGS7BP	401190	regulator of G-protein signaling 7 binding protein	9.793117489	-0.738439155	0.0107012
ENSG00000197081	IGF2R	3482	insulin like growth factor 2 receptor	3893.882883	-0.353086769	0.0109115
ENSG00000100985	MMP9	4318	matrix metallopeptidase 9	2820.554471	0.550370107	0.0117042
ENSG00000106780	MEGF9	1955	multiple EGF like domains 9	788.1489691	-0.382312117	0.0121799
ENSG00000182836	PLCXD3	345557	phosphatidylinositol specific phospholipase C X domain containing 3	16.82951336	-0.73024397	0.0140536
ENSG00000122126	OCRL	4952	OCRL, inositol polyphosphate-5-phosphatase	1231.48057	-0.285100271	0.0151527
ENSG00000184588	PDE4B	5142	phosphodiesterase 4B	1333.079513	-0.509410864	0.0163107
ENSG00000114302	PRKAR2A	5576	protein kinase cAMP-dependent type II regulatory subunit alpha	311.0446898	-0.458155081	0.0166052
ENSG00000116711	PLA2G4A	5321	phospholipase A2 group IVA	336.2022255	-0.582605068	0.0171755
ENSG00000171105	INSR	3643	insulin receptor	1909.416792	-0.295867756	0.0173053
ENSG00000164318	EGFLAM	133584	EGF like, fibronectin type III and laminin G domains	229.0134131	-0.469580197	0.0176762
ENSG00000132554	RGS22	26166	regulator of G-protein signaling 22	4.574242623	-0.636004928	0.0192316
ENSG00000184613	NELL2	4753	neural EGFL like 2	196.5001129	-0.572676436	0.0232947
ENSG00000142892	PIGK	10026	phosphatidylinositol glycan anchor biosynthesis class K	781.7799581	-0.208806421	0.0234037
ENSG00000141639	MAPK4	5596	mitogen-activated protein kinase 4	208.7727368	-0.569541107	0.0325864
ENSG00000107643	MAPK8	5599	mitogen-activated protein kinase 8	324.7787941	-0.300393202	0.033222
ENSG00000100078	PLA2G3	50487	phospholipase A2 group III	6.341731975	-0.631494614	0.0378273
ENSG00000198759	EGFL6	25975	EGF like domain multiple 6	182.8515839	-0.479870257	0.0381221

ENSG00000107175	CREB3	10488	cAMP responsive element binding protein 3	1217.85437	-0.203621813	0.0407954
ENSG00000145725	PPIP5K2	23262	diphosphoinositol pentakisphosphate kinase 2	756.5355326	-0.222844677	0.0425919
ENSG00000154217	PITPNC1	26207	phosphatidylinositol transfer protein, cytoplasmic 1	348.007567	-0.291090967	0.0466811
ENSG00000158786	PLA2G2F	64600	phospholipase A2 group IIF	1.839617956	-0.585514584	0.0480319
ENSG00000166997	CNPY4	245812	canopy FGF signaling regulator 4	378.1533882	-0.251460656	0.0527973
ENSG00000109339	MAPK10	5602	mitogen-activated protein kinase 10	137.1476556	-0.515298729	0.0555319
ENSG00000138698	RAP1GDS1	5910	Rap1 GTPase-GDP dissociation stimulator 1	1021.906809	-0.195978894	0.0566303
ENSG00000138798	EGF	1950	epidermal growth factor	142.104671	-0.531883076	0.0619204
ENSG00000070193	FGF10	2255	fibroblast growth factor 10	3.462625673	-0.562797728	0.0638813
ENSG00000113070	HBEGF	1839	heparin binding EGF like growth factor	277.4721214	-0.313566424	0.0768535
ENSG00000065361	ERBB3	2065	erb-b2 receptor tyrosine kinase 3	3949.329412	-0.056599349	0.7418933

Table 2. Biological pathways significantly upregulated and downregulated among AA compared to EA TNBC patients in TCGA dataset.

Upregulated KEGG pathways	*p-value*
hsa03010 Ribosome	0.005533158
hsa04672 Intestinal immune network for IgA production	0.006164042
hsa04640 Hematopoietic cell lineage	0.028001619
hsa04330 Notch signaling pathway	0.053768917
Downregulated KEGG pathways	
hsa00600 Sphingolipid metabolism	0.01816452
hsa00512 Mucin type O-Glycan biosynthesis	0.02316771
hsa04510 Focal adhesion	0.02628723
hsa00982 Drug metabolism - cytochrome P450	0.02880007
hsa04520 Adherens junction	0.03365944
hsa00500 Starch and sucrose metabolism	0.03901639
hsa00983 Drug metabolism - other enzymes	0.04328416
hsa04512 ECM-receptor interaction	0.04359347

Table 3. Gene ontologies significantly upregulated and downregulated among AA compared to EA TNBC patients in TCGA dataset.	
Upregulated	*p value*
GO:0048534 hematopoietic or lymphoid organ development	0.000429373
GO:0002521 leukocyte differentiation	0.000467453
GO:0030097 hemopoiesis	0.000595111
GO:0002252 immune effector process	0.000606896
GO:0030098 lymphocyte differentiation	0.000668007
GO:0002520 immune system development	0.000752091
GO:0001816 cytokine production	0.001262715
GO:0001817 regulation of cytokine production	0.001304632
GO:0019080 viral genome expression	0.002009214
GO:0019083 viral transcription	0.002009214
GO:0060337 type I interferon-mediated signaling pathway	0.002260808
GO:0071357 cellular response to type I interferon	0.002260808
GO:0001818 negative regulation of cytokine production	0.002341488
GO:0034340 response to type I interferon	0.002360857
GO:0045087 innate immune response	0.002660009
GO:0050776 regulation of immune response	0.003381564
GO:0006415 translational termination	0.003994976
GO:0019058 viral infectious cycle	0.004289754
GO:0045321 leukocyte activation	0.005368308
GO:0046649 lymphocyte activation	0.006021313
GO:0002443 leukocyte mediated immunity	0.006737807
GO:0043241 protein complex disassembly	0.006915896
GO:0045619 regulation of lymphocyte differentiation	0.00711599
GO:0043624 cellular protein complex disassembly	0.009113965
GO:0032609 interferon-gamma production	0.009394054

GO:0030217 T cell differentiation	0.009403061
GO:0071356 cellular response to tumor necrosis factor	0.009678773
GO:0009615 response to virus	0.009744867
GO:0051250 negative regulation of lymphocyte activation	0.009818976
GO:0050778 positive regulation of immune response	0.009903295
GO:2000106 regulation of leukocyte apoptotic process	0.010825095
GO:0071887 leukocyte apoptotic process	0.011246013
GO:0042089 cytokine biosynthetic process	0.011366604
GO:0002695 negative regulation of leukocyte activation	0.011891576
GO:0002684 positive regulation of immune system process	0.012690724
GO:0050866 negative regulation of cell activation	0.012821361
GO:0034113 heterotypic cell-cell adhesion	0.012850245
GO:0006414 translational elongation	0.013939004
GO:0002253 activation of immune response	0.014337616
GO:0051607 defense response to virus	0.01448594
GO:0034341 response to interferon-gamma	0.014747388
GO:0032984 macromolecular complex disassembly	0.01485217
GO:0032649 regulation of interferon-gamma production	0.015069142
GO:0051707 response to other organism	0.017081494
GO:0002263 cell activation involved in immune response	0.017140132
GO:0002366 leukocyte activation involved in immune response	0.017140132
GO:0045580 regulation of T cell differentiation	0.017360252
GO:0000184 nuclear-transcribed mRNA catabolic process, nonsense-mediated decay	0.017466425
GO:0071346 cellular response to interferon-gamma	0.017582795
GO:0021903 rostrocaudal neural tube patterning	0.01828075
GO:0071706 tumor necrosis factor superfamily cytokine production	0.018894657
GO:0008544 epidermis development	0.019826968

GO:0050701 interleukin-1 secretion	0.01990773
GO:0032088 negative regulation of NF-kappaB transcription factor activity	0.020009424
GO:0032640 tumor necrosis factor production	0.02003891
GO:0032680 regulation of tumor necrosis factor production	0.02003891
GO:0002460 adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	0.020144927
GO:0042113 B cell activation	0.020274634
GO:0035587 purinergic receptor signaling pathway	0.021137639
GO:0042035 regulation of cytokine biosynthetic process	0.021288775
GO:0034470 ncRNA processing	0.021369145
GO:0002444 myeloid leukocyte mediated immunity	0.021915664
GO:0009607 response to biotic stimulus	0.02334661
GO:0051249 regulation of lymphocyte activation	0.02428066
GO:0042107 cytokine metabolic process	0.025345542
GO:0030917 midbrain-hindbrain boundary development	0.025352336
GO:0060333 interferon-gamma-mediated signaling pathway	0.025576575
GO:0006941 striated muscle contraction	0.026309493
GO:0003009 skeletal muscle contraction	0.026410898
GO:0006959 humoral immune response	0.027278157
GO:0071345 cellular response to cytokine stimulus	0.027348302
GO:0032715 negative regulation of interleukin-6 production	0.028648897
GO:0031348 negative regulation of defense response	0.028778621
GO:0002703 regulation of leukocyte mediated immunity	0.028922763
GO:0043299 leukocyte degranulation	0.029801087
GO:0043173 nucleotide salvage	0.029893126
GO:0006613 cotranslational protein targeting to membrane	0.030068796
GO:0006614 SRP-dependent cotranslational protein targeting to membrane	0.030068796

GO:0071219 cellular response to molecule of bacterial origin	0.030789397
GO:0002250 adaptive immune response	0.030841904
GO:0097028 dendritic cell differentiation	0.031317678
GO:0002697 regulation of immune effector process	0.031523821
GO:0071222 cellular response to lipopolysaccharide	0.031667044
GO:0007606 sensory perception of chemical stimulus	0.032245552
GO:0050865 regulation of cell activation	0.032311054
GO:0051930 regulation of sensory perception of pain	0.034463637
GO:0051931 regulation of sensory perception	0.034463637
GO:0045047 protein targeting to ER	0.034644877
GO:0072599 establishment of protein localization to endoplasmic reticulum	0.034644877
GO:0043433 negative regulation of sequence-specific DNA binding transcription factor activity	0.034934546
GO:0002764 immune response-regulating signaling pathway	0.035070643
GO:0050848 regulation of calcium-mediated signaling	0.035464555
GO:0051216 cartilage development	0.035996334
GO:0070586 cell-cell adhesion involved in gastrulation	0.036025281
GO:0010470 regulation of gastrulation	0.03613861
GO:0006402 mRNA catabolic process	0.036140008
GO:0042074 cell migration involved in gastrulation	0.036712677
GO:0002757 immune response-activating signal transduction	0.036719431
GO:0050704 regulation of interleukin-1 secretion	0.037451445
GO:0070972 protein localization to endoplasmic reticulum	0.037618557
GO:0000956 nuclear-transcribed mRNA catabolic process	0.037782421
GO:0002694 regulation of leukocyte activation	0.03779717
GO:0061383 trabecula morphogenesis	0.038343425
GO:0032612 interleukin-1 production	0.038674489
GO:0035809 regulation of urine volume	0.038920075

GO:0002449 lymphocyte mediated immunity	0.039034069
GO:0002683 negative regulation of immune system process	0.039201076
GO:0030183 B cell differentiation	0.039462908
GO:0001783 B cell apoptotic process	0.040421151
GO:0042249 establishment of planar polarity of embryonic epithelium	0.040903568
GO:0006400 tRNA modification	0.040910207
GO:0070228 regulation of lymphocyte apoptotic process	0.04166174
GO:0006399 tRNA metabolic process	0.041688021
GO:0032729 positive regulation of interferon-gamma production	0.041968537
GO:0050909 sensory perception of taste	0.042391791
GO:0033209 tumor necrosis factor-mediated signaling pathway	0.043478576
GO:0031334 positive regulation of protein complex assembly	0.044230876
GO:0050868 negative regulation of T cell activation	0.044579293
GO:0042110 T cell activation	0.045651071
GO:0071347 cellular response to interleukin-1	0.045684339
GO:0003416 endochondral bone growth	0.046119963
GO:0060026 convergent extension	0.046776142
GO:0023021 termination of signal transduction	0.046852554
GO:0038032 termination of G-protein coupled receptor signaling pathway	0.047117236
GO:0010657 muscle cell apoptotic process	0.047468866
GO:0006401 RNA catabolic process	0.047552326
GO:0042254 ribosome biogenesis	0.047629412
GO:0022904 respiratory electron transport chain	0.048240328
GO:0043094 cellular metabolic compound salvage	0.048452721
GO:0006612 protein targeting to membrane	0.048517893
GO:0042401 cellular biogenic amine biosynthetic process	0.048738698
GO:0051495 positive regulation of cytoskeleton organization	0.048955127

GO:0002285 lymphocyte activation involved in immune response	0.049073929
GO:0050864 regulation of B cell activation	0.049545191
Downregulated	
GO:0043687 post-translational protein modification	0.000493768
GO:0007156 homophilic cell adhesion	0.000891909
GO:0070085 glycosylation	0.001919505
GO:0006486 protein glycosylation	0.002075176
GO:0043413 macromolecule glycosylation	0.002075176
GO:0006665 sphingolipid metabolic process	0.002745447
GO:0007270 neuron-neuron synaptic transmission	0.002807343
GO:0051966 regulation of synaptic transmission, glutamatergic	0.003328871
GO:0006687 glycosphingolipid metabolic process	0.003952335
GO:0009101 glycoprotein biosynthetic process	0.004520639
GO:0035249 synaptic transmission, glutamatergic	0.004653915
GO:0007158 neuron cell-cell adhesion	0.004656323
GO:0006643 membrane lipid metabolic process	0.005041899
GO:0018196 peptidyl-asparagine modification	0.005612873
GO:0018279 protein N-linked glycosylation via asparagine	0.005612873
GO:0006487 protein N-linked glycosylation	0.005970635
GO:0031645 negative regulation of neurological system process	0.006532083
GO:0051968 positive regulation of synaptic transmission, glutamatergic	0.006542085
GO:0009100 glycoprotein metabolic process	0.007071823
GO:0016266 O-glycan processing	0.00822715
GO:0007595 lactation	0.008438853
GO:0007610 behavior	0.009407186
GO:0007420 brain development	0.009694387
GO:0050808 synapse organization	0.010243263

GO:0036148 phosphatidylglycerol acyl-chain remodeling	0.010257756
GO:0042391 regulation of membrane potential	0.010342608
GO:0006805 xenobiotic metabolic process	0.010598743
GO:0071466 cellular response to xenobiotic stimulus	0.011270151
GO:0030879 mammary gland development	0.011361444
GO:0001508 regulation of action potential	0.011440865
GO:0051970 negative regulation of transmission of nerve impulse	0.011501433
GO:0048667 cell morphogenesis involved in neuron differentiation	0.012566558
GO:0051932 synaptic transmission, GABAergic	0.012763237
GO:0044723 single-organism carbohydrate metabolic process	0.012821492
GO:0006664 glycolipid metabolic process	0.01400463
GO:0050805 negative regulation of synaptic transmission	0.015334527
GO:0007626 locomotory behavior	0.015430043
GO:0009410 response to xenobiotic stimulus	0.015493698
GO:0048193 Golgi vesicle transport	0.015772984
GO:0048609 multicellular organismal reproductive process	0.015789414
GO:0031047 gene silencing by RNA	0.016586363
GO:0048812 neuron projection morphogenesis	0.016607133
GO:0044708 single-organism behavior	0.018681325
GO:0030900 forebrain development	0.020384121
GO:0060271 cilium morphogenesis	0.020950746
GO:0007173 epidermal growth factor receptor signaling pathway	0.021617695
GO:0038127 ERBB signaling pathway	0.021617695
GO:0008610 lipid biosynthetic process	0.022799925
GO:0034329 cell junction assembly	0.022808921
GO:0006470 protein dephosphorylation	0.023740415
GO:0044262 cellular carbohydrate metabolic process	0.023838856

GO:0006654 phosphatidic acid biosynthetic process	0.024003206
GO:0046473 phosphatidic acid metabolic process	0.024003206
GO:0007631 feeding behavior	0.024218683
GO:0007215 glutamate receptor signaling pathway	0.025341571
GO:0007169 transmembrane receptor protein tyrosine kinase signaling pathway	0.025778202
GO:0048732 gland development	0.026371412
GO:0007409 axonogenesis	0.028034327
GO:0030902 hindbrain development	0.028207007
GO:0050905 neuromuscular process	0.028868056
GO:0035265 organ growth	0.029870897
GO:0032228 regulation of synaptic transmission, GABAergic	0.030572469
GO:0021533 cell differentiation in hindbrain	0.030591893
GO:0006112 energy reserve metabolic process	0.030725257
GO:0010165 response to X-ray	0.031143637
GO:0043044 ATP-dependent chromatin remodeling	0.031721256
GO:0021549 cerebellum development	0.031913742
GO:0032787 monocarboxylic acid metabolic process	0.032020432
GO:0006892 post-Golgi vesicle-mediated transport	0.032510822
GO:0052646 alditol phosphate metabolic process	0.03300986
GO:0007157 heterophilic cell-cell adhesion	0.033442154
GO:0014812 muscle cell migration	0.033592578
GO:0006302 double-strand break repair	0.03376071
GO:0045216 cell-cell junction organization	0.034066295
GO:0006457 protein folding	0.034232181
GO:0006282 regulation of DNA repair	0.03433869
GO:0001570 vasculogenesis	0.034798997
GO:0046620 regulation of organ growth	0.035079657

GO:0016337 cell-cell adhesion	0.035209378
GO:0060479 lung cell differentiation	0.035239581
GO:0032941 secretion by tissue	0.036128621
GO:0000271 polysaccharide biosynthetic process	0.036630136
GO:0022037 metencephalon development	0.036660654
GO:0032870 cellular response to hormone stimulus	0.036922137
GO:0002064 epithelial cell development	0.03723918
GO:0030728 ovulation	0.038142428
GO:0007411 axon guidance	0.038665746
GO:0036151 phosphatidylcholine acyl-chain remodeling	0.03867378
GO:0060487 lung epithelial cell differentiation	0.039970158
GO:0034330 cell junction organization	0.040342663
GO:1901888 regulation of cell junction assembly	0.041298928
GO:0036149 phosphatidylinositol acyl-chain remodeling	0.041658096
GO:0030949 positive regulation of vascular endothelial growth factor receptor signaling pathway	0.041896616
GO:0007274 neuromuscular synaptic transmission	0.042046032
GO:0071599 otic vesicle development	0.042937278
GO:0006493 protein O-linked glycosylation	0.043137696
GO:0001937 negative regulation of endothelial cell proliferation	0.043387826
GO:0048545 response to steroid hormone stimulus	0.043541689
GO:0005977 glycogen metabolic process	0.046227756
GO:0034508 centromere complex assembly	0.047156367
GO:0061418 regulation of transcription from RNA polymerase II promoter in response to hypoxia	0.04743104
GO:0044257 cellular protein catabolic process	0.047832199
GO:0051963 regulation of synapse assembly	0.048565239
GO:0060740 prostate gland epithelium morphogenesis	0.048742367

predicted significantly worse RFS in TNBCs (HR=1.75; p=0.027); NRARP expression was associated with a trend towards poorer RFS in TNBCs (HR=1.53; p=0.17); and NOTCH2NL expression was associated with poorer RFS in TNBC (HR=1.001; p=0.01). The prognostic value of NOTCH2NL expression level was upheld after adjusting for age, stage and race (AA vs. EA); in fact, the expression of NOTCH2NL was the only significant predictor of RFS in multivariable analyses (p=0.003). Interestingly, DNER ranked in the top 1% of genes most highly dysregulated in the Basal-like Immuno-Suppressed (BLIS) TNBC molecular subtype. AAs tend to harbor basal-like subtypes of TNBC and the BLIS molecular subtype is 1 of 2 basal-like TNBC molecular subtypes recently identified (Burstein et al., 2015) that has the worst disease-free survival and BC-specific survival among the 4 prognostically-distinct TNBC molecular subtypes.

Our group's findings collectively suggest that the Notch signaling pathway may be upregulated among TNBC patients of African compared to European ancestry, and upregulated Notch signaling may serve as a poor prognosis biomarker in TNBC. Targeting Notch signaling may therefore be a promising personalized therapeutic strategy for TNBC patients of African descent, including AAs.

Taking it up a notch: Establishing Notch signaling as therapeutic target of interest for triple negative breast cancer patients of African descent

TNBC remains the primary culprit for disproportionately lower survival rates of AA compared to EA BC patients. Thus, identifying novel therapeutic targets and/or risk-predictive biomarkers for TNBC patients of African ancestry will be critical to alleviating the racially disparate burden in BC. Emerging evidence suggest that inherent differences exist in tumor biology between AA and EA TNBC patients. Getz et al. discovered that dysregulated genes in the Wnt/β-catenin pathway were significantly more enriched among TNBC patient samples of African compared to European ancestry, which may rationalize the aggressive TNBC phenotypes observed among patients of African descent (Dietze et al., 2015). However, more work is warranted to examine inherent tumor biological differences between racially-distinct TNBC patients.

Our group sought to investigate differences in tumor biology between AA and EA TNBC patients through analyzing the publicly-available gene expression dataset, TCGA. Interestingly, Notch signaling emerged as a pathway significantly upregulated among AA compared to EA TNBC patients. We observed upregulation of the Notch signaling pathway among AA compared to EA TNBC samples as well as significant upregulation of genes encoding key Notch signaling proteins in this pathway such as NRARP, DNER, JAG1, JAG2, HES4, and MMP9. NRARP is a downstream effector in the Notch pathway and its overexpression has been associated with breast carcinogenesis and BC cell proliferation (Imaoka et al., 2014). JAG1 and JAG2 encode two major ligands in the canonical Notch signaling pathway (Wang et al., 2010). The *HES* family of transcription factors represent a major family of downstream target genes in the Notch signaling pathway (Acar et al., 2016). MMP9 is implicated in the breakdown of the extracellular matrix to facilitate invasion and metastasis in TNBC (Mehner et al., 2014). Furthermore, we observed significant downregulation of biological pathways and gene

ontologies reflecting loss of cell-cell contacts, focal adhesion, and ECM-receptor interaction as well as reduced epithelial cell development, reduced endothelial cell proliferation, and DNA damage response among AA compared to EA patients. Notch signaling regulates proliferation, apoptosis, angiogenesis, hypoxia, EMT, and metastasis (Acar et al., 2016). Thus, significant downregulation of these processes among AA compared to EA samples may reflect increased proliferation, angiogenesis, metastasis, and reduced cell death among AA patients. We also observed significant upregulation of gene ontologies reflecting T cell-mediated immune response, which is upregulated by Notch signaling (Uzhachenko and Shanker, 2016). Hence, we have uncovered Notch signaling as a key biological pathway that may contribute to the racially disparate burden in TNBC and serve as a potential therapeutic target for AA patients.

Our findings thus encourage a closer look at this biological pathway as a potential racial disparity biomarker and therapeutic target for TNBC. However, validation of our results in additional gene expression datasets as well as at the protein expression level among patient samples of known TNBC molecular subtypes will be critical to achieving this aim. Furthermore, investigating the effects of manipulating Notch signaling among racially-distinct TNBC patient-derived cell lines or *in vivo* will be pertinent to effectively targeting this pathway in patients of African ancestry. Notch signaling inhibitors such as GSI and aspartyl protease inhibitors are currently under clinical development and in clinical trials as investigational targeted therapies for TNBC patients (Jamdade et al., 2015). GSI inhibitors are associated with side effects including fatigue, myelosuppression, fever, rash, chills, anorexia, and hypophosphatemia. Improving the toxicity profile and efficacies of GSIs, and development of more effective inhibitors of Notch signaling could be crucial for improving outcomes among AA TNBC patients.

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Conflict of interest

The authors declare that no competing or conflict of interests exists. The funders had no role in study design, writing of the manuscript, or decision to publish.

Authors' contributions

Nikita Wright was involved in the data collection and drafting of the article. Shristi Bhattarai was involved in the drafting and editing of the article. Bikram Sahoo and Mishal Imaan Syed were involved in data collection/analysis. Dr. Padmashree Rida was involved in conceptualization and editing of the article. Dr. Ritu Aneja was involved in conceptualization, editing, and oversight of the study.

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