Tumor and Stem Cell Biology

IL15RA Drives Antagonistic Mechanisms of Cancer Development and Immune Control in Lymphocyte-Enriched Triple-Negative Breast Cancers

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Abstract

Despite its aggressive nature, triple-negative breast cancer (TNBC) often exhibits leucocyte infiltrations that correlate with favorable prognosis. In this study, we offer an explanation for this apparent conundrum by defining TNBC cell subsets that overexpress the IL15 immune receptor IL15RA. This receptor usually forms a heterotrimer with the IL2 receptors IL2RB and IL2RG, which regulates the proliferation and differentiation of cytotoxic T cells and NK cells. However, unlike IL15RA, the IL2RB and IL2RG receptors are not upregulated in basal-like TNBC breast cancer cells that express IL15RA. Mechanistic investigations indicated that IL15RA signaling activated JAK1, STAT1, STAT2, AKT, PRAS40, and ERK1/2 in the absence of IL2RB and IL2RG, whereas neither STAT5 nor JAK2 were activated. RNai-mediated attenuation of IL15RA established its role in cell growth, apoptosis, and migration, whereas expression of the IL15 cytokine in IL15RA-expressing cells stimulated an autocrine signaling cascade that promoted cell proliferation and migration and blocked apoptosis. Notably, coexpression of IL15RA and IL15 was also sufficient to activate peripheral blood mononuclear cells upon coculture in a paracrine signaling manner. Overall, our findings offer a mechanistic explanation for the paradoxical association of some high-grade breast tumors with better survival outcomes, due to engagement of the immune stroma. Cancer Res; 74(17); 4908–21. ©2014 AACR.

Introduction

Tumor infiltration by a variety of immune cells, including cytotoxic T cells, regulatory T cells, natural killer (NK) cells, neutrophils, and macrophages, is a common feature of many cancers (1). Although tumor infiltration by cytotoxic lymphocytes is generally correlated with a favorable outcome, substantial evidence exists that other types of leukocytes can instead promote tumorigenesis by supplying cytokines (such as IL6, TNF, and IL1β) that stimulate tumor proliferation, tissue invasion, and/or angiogenesis (2).

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ER-negative breast cancers, which are more uniformly highly proliferative and in which immune response signatures have been shown to have a higher prognostic significance (8). Even among basal tumors there is still significant histologic, immunohistologic, and prognostic heterogeneity (9). Indeed, some morphologically recognized groups show excellent long-term prognosis despite a basal expression profile and aggressive histopathologic features. One such example is medullary breast carcinoma, which shares many features with the TNBC/basal subtype, including similarity in the gene-expression profile, highly proliferative phenotype, and TP53 mutations (10). Medullary carcinoma also shows numerous lymphoid cells in the stroma (11) and expression of an immune profile, including TH1-associated cytokine receptors such as IL15RA, their associated cytokines, including IL15, and genes involved in cytotoxicity (12).

IL15RA usually exists in a heterotrimeric receptor with IL2RB and IL15Rgamma, which also contribute to the trimeric IL2 receptor (13). IL15RA is able to bind IL15 with high affinity in the absence of the beta and gamma subunits. This allows the receptor to function in cis, i.e., the three subunits being on the same cell, or in trans, with the IL15RA subunit bound to IL15 on one cell and the beta and gamma subunits on an adjacent cell. In the latter case, the IL15/IL15RA complex is transpresented to the beta and gamma subunit with consequent activation of intracellular signaling (14).

IL15-dependent signaling has been implicated in the modulation of adaptive immune responses (15), the activation and maintenance of distinct lymphocyte populations (16) and the inhibition of apoptosis in multiple systems (17). Furthermore, IL15-dependent signaling has been shown to play a role in hematologic malignancies, in which there is evidence that an IL15/IL15RA autocrine growth-stimulation loop plays a role in the progression of human T-cell lymphotrophic virus I (HTLV-I)-associated adult T-cell leukemia/lymphoma (14).

There are several reports of IL15 and/or IL15 receptor expression in solid tumor-derived cell lines or tissues. A functional heterotrimeric IL15 receptor has been described in renal cancer (18) and IL15RA expression, has been found in melanoma models (19).

In the present study, we find IL15RA to be frequently amplified and describe elevated expression of both IL15RA and its ligand IL15, in the absence of IL2RB and IL15Rgamma, in basal and immune-modulatory forms (20) of TNBC and in breast cancer cell lines (BCCL). We investigate the consequences of this coordinated expression, describing a tumor-promoting IL15 and IL15RA-dependent autocrine signaling mechanism that drives cell growth, colony formation and cell migration, and protects from apoptosis. We also show that IL15/IL15RA expression on the plasma membrane of BCCLs drives signaling in peripheral blood mononuclear cells (PBMC) making BCCLs visible to the immune system and potentially leading to an antitumor immune response. In doing so, we provide a potential mechanistic explanation for the paradox in which some basal breast cancers show a high mitotic index, aggressive morphologic appearances and yet engage an effective immune-cell response with an associated impact on prognosis.

Materials and Methods

Cell lines

BCCLs were obtained from the ATCC, HMEC from Life Technologies, and Natural Killer YT cells from Dr. S. John (KCL, London, UK). Growth conditions were as recommended by the suppliers. Cells were authenticated by short tandem repeat analysis and matched to the German Collection of Microorganisms and Cell Cultures (DSMZ)—database.

DNA copy-number and gene-expression analysis

Absolute DNA copy number was obtained for 172 cancers in our TNBC-enriched Guy’s Hospital breast-cancer cohort (21), using Affymetrix SNP6.0 genome profiles and human genome build hg18 mapping. The Tumor Aberration Prediction Suite algorithm (22) was applied to minimize influence of normal tissue. IL15RA and IL15 levels were extracted from the Guy’s Hospital (21), METABRIC (23), TCGA (The Cancer Genome Atlas; ref. 6) 579 TNBC cohorts (TNBC579_GSE31519; //; ref. 24), as well as two BCCL datasets (25, 26). PAM50 classification was as described for the Guy’s (27), METABRIC (23), and TCGA cohorts (6). TNBC subtypes were established using the online TNBC-type program (28). IL15RA and IL15 expression was compared using Pearson correlation. All analyses were performed with R software (www.r-project.org/).

Quantitative PCR analyses

Methodology for quantitative PCR (qPCR) is described in Supplementary Methods.

Western blotting

Western blots were processed as described in Supplementary Methods.

Immunofluorescence and confocal microscopy

Cells were processed as described in Supplementary Methods. Confocal images were acquired by an AIR Si Confocal system (Nikon Corp.) equipped with a ×100 objective. Optical sections were taken at 1 Airy U at 1-μm intervals, and then reconstructed in a Z-stack. Images assembled in Adobe Photoshop CS5.1.

Immunohistochemistry

Formalin-fixed paraffin-embedded (FFPE) sections 3-μm-thick were processed as described in Supplementary Methods then stained with the MAB147 IL15RA antibody (R&D Systems) at 10 ng/mL for 2 hours. Images from digitalized scans of the glass slide specimens were obtained at magnification ×20 (0.45 μm/pixel resolution) using a Hamamatsu Nanozoomer 2.0 HT.

Xenotransplants

UM13, MC1, and UM2 patient-derived xenotransplants were established from primary breast tumors at the University of Michigan (Ann Arbor, MI) as described by Cariati and colleagues (29). Once established in the mouse mammary fat pad, xenotransplants were characterized by immunohistochemistry (IHC): UM13 was ER-positive and -negative for PR, HER2,

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EGFR, and cytokeratin 5/6. MC1 and UM2 were ER-, PR-, HER2-negative but EGFR- and CK5/6-positive.

**Small interfering RNA–mediated silencing**

Human Silencer Select small interfering RNAs (siRNA), including a nontargeting negative control siRNA, were purchased from Ambion (Life Technologies) as detailed in Supplementary Methods and transfected at 50 nM/l by RNAiMax (Life Technologies). Knockdown efficacy was assessed 72 hours posttransfection.

**Small hairpin RNA–mediated gene silencing**

MISSION small hairpin RNA (shRNA) Library–derived clones (Sigma-Aldrich) were used as detailed in Supplementary Methods. Lentiviral particles were produced and titrated as described in Supplementary Methods.

**Cell population growth assays**

Cells were seeded at 8 x 10^4 per well in a 96-well plate. Effect on cell growth was assessed using Hoechst 33342 nucleic acid stain (Life Technologies) at the times indicated after seeding, with 24 hours (T0), 48 hours (T1), 72 hours (T2), and 96 hours (T3). Cells were then washed with PBS and imaged.

**Caspase activation analyses**

Caspase-3/7 activity was measured at 24 hours after seeding or as indicated, using the caspase-Glo-3/7 assay (Promega).

**Colony formation assays**

A total of 1 x 10^5 or 2 x 10^5 cells per well were plated in 6-well plates and cultured in their ideal media for 15 days before staining with 0.5% crystal violet in 1% formaldehyde for 20 minutes. Cells were then washed with PBS and imaged.

**Stimulation of cells with recombinant IL15 and analysis of changes in protein expression and phosphorylation**

Cells were grown to 50% confluence then serum starved in normal growth medium for 3 hours before addition of rIL15 (Peprotech) at the indicated concentrations for 15 minutes at 37°C. The proteins were then harvested by scraping the cells in Laemmli buffer with 10% β-mercaptoethanol, and separated by SDS-PAGE.

**Phospho-kinase array**

The Human Phospho-Kinase Array (R&D Systems) was used according to the manufacturer’s instructions. Briefly, a protein lysate was obtained from approximately 5 x 10^5 control or IL15-stimulated cells in the specific lysis buffer provided. Lysates were then incubated with the filters provided and phosphorylation levels of the individual sites in the array revealed by chemiluminescence on a radiographic film, and quantified by densitometry using ImageJ software.

**Data and statistical analysis**

Data and statistical analyses have been performed using Microsoft Excel and GraphPad Prism software.

**Cell migration assays**

Cell migration was assessed using Transwell inserts (96-well plates, 8-µm pore; Corning-Costar) coated with bovine collagen type I (In vitrogen) and seeded with 25 x 10^5 cells resuspended in 90 µL RPMI 0.1% FBS. The microplate inserts were placed on the receiver plate containing medium supplemented with 2% FBS and incubated for 4 hours or overnight at 37°C. Migrated cells were detached from the inserts by trypsin and counted using a plate reader after permeabilization with 0.01% Triton X-100 and DNA labeling by SYTOX Green (Invitrogen).

**Cell adhesion assays**

A total of 10 x 10^5 cells were seeded on a thin layer of collagen type I (Invitrogen) and incubated at 37°C for 60 minutes. Plates were then washed three times with PBS and the remaining attached cells were then permeabilized with 0.01% Triton X-100, DNA labeled by SYTOX Green (Invitrogen) and counted using a plate reader.
Flow-cytometry analysis
Cultured cells were detached with Accutase (PAA Laboratories) and washed twice with PBS before staining. PBMC were isolated by Ficoll gradient centrifugation of whole blood from healthy volunteers and washed twice with PBS. Cells were first stained with LIVE/DEAD Aqua (Molecular Probes) for 20 minutes at room temperature, then washed twice with FACS buffer (PBS, 2% FCS, 2 mmol/L EDTA) before staining in 1:50 anti-IL15Ra PE (BioLegend clone JMT7A4), anti-IL2Rb APC (BioLegend clone TU27), and anti-IL2Rg Brilliant Violet 421 (BD Biosciences clone AG184) or paired isotype controls in FACS buffer for 20 minutes at 4°C. Cells were washed twice with FACS buffer before fixing in CellFIX (BD Biosciences) and acquisition on a BD FACS Canto II.

ELISA
A total of 2 x 10^6 cells were seeded in 1 mL of culture medium in 6-well plates and incubated overnight. Supernatants were harvested and centrifuged to remove debris. Cells were detached with Accutase (PAA Laboratories) and counted before lysis in 300 μL of lysis buffer (25 mmol/L Tris-HCl pH 7.4, 150 mmol/L NaCl, 1% Triton X-100, and 5% glycerol). Cells were incubated on ice with intermittent vortexing every 10 minutes for 60 minutes. Lysates and supernatants were assayed for IL15 by sandwich ELISA (R&D Systems Human IL15 DuoSet) as per the manufacturer’s instructions. IL15 concentration was normalized to 10^6 cells.

PBMC and breast cancer cell coculture
PBMC were taken from healthy adult donors after the informed consent approved by the Institutional Review Board. Mononuclear cells were obtained by Ficoll gradient centrifugation (GE Healthcare). A total of 5 x 10^6 breast cancer cells were plated in a 96-well plate and incubated overnight. The following day, the same number of PBMC was added. After 30 minutes, PBMC were harvested and stained with pSTAT5 antibody (BD Biosciences) and analyzed by flow cytometry. When indicated, a blocking anti-IL15 monoclonal mouse antibody (MAB2471; R&D Systems) was added at 10 μg/mL to PBCCs 30 minutes before coculture with PBMC.

Results
Amplification and overexpression of IL15RA in subsets of TNBCs and BCCls
Several studies (23, 6) have shown that the most distant region on chromosome 10p is frequently amplified in TNBCs. Investigating the copy number of chromosome 10 in our Guy’s TNBC–enriched BC cohort (27), using Affymetrix SNP6.0-derived genomic profiles, we confirmed the presence of an amplicon encompassing approximately 10 Mbp of the most distal region of the short arm of chromosome 10 (10:82,824-8,738,800; hg18). Within this amplicon, a number of genes had an absolute copy number of greater than 4 in at least 30% of TNBC cases (Fig. 1A; Supplementary Table S1). Analysis of these genes revealed some with features suggestive of a possible role as cancer drivers. Among these, IL15RA attracted our interest as it encodes the alpha subunit of the IL15 receptor known to be involved in the signaling cascade controlling proliferation and activation of immune cells. IL15RA showed an absolute copy number of greater than 4 in 43% (48/111) of TNBC in contrast with 18% (11/61) of non-TNBC (Fisher exact test; P < 0.001). To investigate whether the abundance of IL15RA expression is enhanced specifically in TNBCs, its expression was extracted from the transcriptomes of the Guy’s TNBC–enriched BC collection, the invasive ductal breast carcinomas in METABRIC (23), and the TCGA breast cancer cohorts (Fig. 1B; ref. 6). Using the PAM50 breast cancer subtype classification published for each cohort, increased IL15RA expression was consistently observed in basal and in normal-like breast cancer subtypes. Given the heterogeneity within TNBCs, we investigated IL15RA expression across previously defined TNBC subtypes (20) in the Guy’s cohort and a publicly available dataset of 579 TNBCs (GSE31519; Fig. 1C; ref. 24). Higher IL15RA expression was observed in the immunomodulatory subtype, characterized by expression of genes involved in several immune-cell signaling pathways that also significantly overlap with gene signatures for medullary breast cancers (20).

To identify cellular models, we analyzed expression of components of the IL15 receptor in 51 BCCls (26) finding significantly higher expression of IL15RA, but not IL2RB or IL2RG, in “basal” versus “luminal” BCCls (t test, P < 0.001; Fig. 1D). To confirm localization and presence of IL15RA and absence of IL2RB and IL2RG on basal-like BCCls, FACS analyses were performed using antibodies against cell surface–exposed epitopes on nonpermeabilized cells. Plasma membrane IL15 receptor subunit levels were consistent with expression data. Only IL15RA was detected on the plasma membrane of the basal-like BCCLs HCC1143, HCC38, and MDA-MB-231, with all components being absent on the luminal-like BCCLs BT549, HCC1428, and SKBR3, despite all components being detected on positive control YT, NK, or PBMC.
An autocrine IL15/IL15RA signal drives cell population growth in BCCLs

Previous work has shown that IL15 and IL15RA bind to each other along the secretory pathway, remaining in a complex upon arrival at the plasma membrane (30). Indeed an autocrine IL15/IL15RA–mediated signaling mechanism occurs in T-cell lymphoma (14). We, therefore, investigated whether TNBCs and BCCLs express IL15, how its expression is correlated with IL15RA expression and to what extent this is required for IL15RA-mediated growth.

Within the 579 TNBCs cohort (GSE31519; ref. 24), we found a positive correlation between the mRNA levels of IL15RA and IL15 (Fig. 4A; correlation, 0.51; \( P = 2.2 \times 10^{-16} \)). Similarly, in some cell lines, the expression of these two genes was correlated (Fig. 4B; correlation, 0.36; \( P = 0.007 \); ref. 26) and on average showed a higher expression of the ligand and receptor in cells of basal-like rather than luminal-like subtypes (Fig. 4B and C).

Because expression of IL15 mRNA does not always correlate with the protein (14), we assessed IL15 protein levels in BCCLs by ELISA and found higher levels in basal-like cell lines that expressed higher levels of IL15RA (Fig. 4D). This led us to hypothesize that cytokine and receptor expressed by the same cells might signal using an autocrine mechanism promoting proliferation of BCCLs. To test this, we knocked down IL15 expression using shRNAs and observed a significant impairment of growth in MDA-MB-231 and HCC1143 cells without knockdown in SKBR3 cells (low IL15RA and IL15; Fig. 4E–G). We examined the effect of knockdown of endogenous IL15 on levels of spontaneous apoptosis in HCC1143 cells and showed significant caspase activation (Fig. 4H).

II.15- and II.15RA-dependent intracellular signaling in BCCLs

Having observed a reduction in growth and an increase in apoptosis upon IL15RA knockdown in basal BCCLs, we sought to identify the mechanisms downstream of IL15RA controlling these functions. In T cells, the IL15 heterotrimeric receptor signals through JAK1, JAK3, and STAT3 or STAT5, thereby regulating cell proliferation and inhibiting apoptosis (31). We analyzed the activity of a range of intracellular signaling kinases in HCC1143 cells using a validated phospho-antibody array. The stimulation of HCC1143 cells with recombinant IL15 significantly increased phosphorylation of ERK1/2, STAT1, STAT2, AKT, and PRAS40 (Fig. 5A and B). ERK1/2 and STAT1 phosphorylation were independently validated in lysates from IL15-stimulated HCC1143 cells using a different set of phospho-protein antibodies and Western blots (Fig. 5C).
It has previously been shown that the IL15 receptor signals through JAK1 and JAK3 in T lymphocytes (31), although in mast cells, IL15 activates JAK2 and STAT6 through a poorly defined 60 to 65 kD IL15 receptor subunit (IL15RX; ref. 32). We assessed changes in JAK1 and JAK2 phosphorylation upon IL15 stimulation. JAK1 but not JAK2 phosphorylation was induced by IL15 stimulation in HCC1143, MDA-MB-231 and HCC1954, but not in SKBR3, suggesting a dependency upon IL15RA (Fig. 5D). We also observed no change in STAT3, STAT5, and STAT6 phosphorylation, which are known to be downstream of the heterotrimeric receptor. This finding was validated by Western blotting HCC1143 cell lysates using independent antibodies (Fig. 5E).

To confirm IL15RA-dependent activation of JAK1 and ERK1/2, we knocked down the expression of IL15RA using siRNAs in HCC1954 cells, then stimulated with recombinant IL15 and observed substantial reduction in the levels of IL15-stimulated phospho-JAK1 and ERK1/2 (Fig. 5F).
IL15RA regulates cell adhesion and motility via JAK1/STAT1 independent of its effect on ERK1/2

IL15 signaling has been implicated in T-cell adhesion and motility (14). We hypothesized that interfering with IL15/IL15RA function might impair cell adhesion and/or motility in BCCLs. We tested MDA-MB-231 and HCC1954 cells in a standard Transwell 2D-motility assay, performed on a thin layer of collagen type I. IL15RA knockdown induced a 35% to 50% reduction in the number of cells migrating (Fig. 6A). We then investigated whether the migration defect observed was due, at least in part, to a defect in adhesion and confirmed a defect in adhesion of IL15RA-silenced MDA-
MB-231 cells to the collagen layer (Fig. 6B). Nevertheless, the small degree by which adhesion was reduced suggests that this does not fully account for the reduction in migration and that other processes in cell migration are affected by IL15 signaling in these cells.

As the JAK1/STAT1 pathway plays a role in cell motility (33), we investigated the possible influence of the previously observed IL15-mediated JAK1/STAT1 activation on BCCL motility by silencing JAK1 expression. We observed a significant reduction in HCC1954 cell migration (Fig. 6C) but no concomitant reduction in cell population growth (Fig. 6D and E). In parallel, we observed abrogation of IL15-dependent STAT1 phosphorylation, whereas no substantial change occurred in the phosphorylation of ERK1/2 (Fig. 6F).

These findings suggest a split signaling cascade downstream of IL15RA in BCCLs, in which IL15-dependent activation of JAK1 leads to phosphorylation of STAT1 and subsequent regulation of cell motility. At the same time ERK1/2 activation occurs by a JAK1/STAT1–independent mechanism and is most likely responsible for promoting cell proliferation.

The presence of an IL15RA/IL15 complex on the surface of BCCLs stimulates a signaling response in immune cells

Given the established role of IL15 and IL15RA as promoters of T-cell recruitment and activation (14), we asked whether the...
levels of IL15 being expressed by basal-like BCCLs, if secreted or exposed by IL15RA on the cell surface were sufficient to activate a response in PBMC. BCCLs expressing different levels of IL15RA/IL15 were cocultured with PBMC and the phosphorylation of STAT5 was measured by flow cytometry in the gated PBMC population. We observed increased STAT5 phosphorylation following exposure of PBMC to HCC1143 and MDA-MB-231 but not to IL15/IL15RA low-expressing SKBR3 and T47D. The concomitant addition of a blocking anti-IL15 antibody or the silencing of IL15 or IL15RA through shRNAs, resulted in a significant reduction of STAT5 phosphorylation in PBMC. This indicates a specific role for IL15/IL15RA produced by the cancer cell leading to STAT5 phosphorylation in the PBMC (Fig. 7). Furthermore, conditioned medium from MDA-MB-231 cells did not induce STAT5 phosphorylation in PMBC, suggesting transpresentation, as opposed to secreted IL15, is required for PBMC activation (data not shown).
Discussion

Although expression of IL15RA occurs in several cell types, expression of a functional heterotrimeric IL15 receptor comprising IL2RB and IL2RG is mostly restricted to immune cells: T lymphocytes, B lymphoblasts, and NK cells (34). This is consistent with our finding of undetectable levels of the beta and gamma subunits by flow-cytometry or Western blot analysis in BCCLs. Our demonstration of the high frequency of amplification of IL15RA and of expression IL15RA protein on the surface of subgroups of TNBCs and BCCLs suggests that this component of the receptor may drive malignant phenotypes, despite isolation from other components of the classical receptor, in a significant proportion of TNBCs. As expected, we did not find a strong correlation between IL15RA copy number and its expression in tumors (data not shown) because of the confounding effect of infiltration of leucocytes that usually express this receptor. IL15RA knockdown experiments using multiple RNAi methodologies confirmed a role for IL15RA in the regulation of cell growth, migration, and apoptosis in basal BCCLs that express it at higher levels.

A positive correlation between IL15 and IL15RA transcript, in both tumors and BCCLs, prompted us to investigate and confirm by ELISA, that IL15 protein was expressed in basal BCCLs that also express IL15RA. We then confirmed that IL15, known to be capable of binding to IL15RA intracellularly for cotransport to the plasma membrane (30), was also required for growth, suggesting an IL15/IL15RA autocrine–driven malignant phenotype in BCCLs.

To our knowledge, this is the first report of such a role for the IL15/IL15RA complex in the absence of a full heterotrimeric receptor, although an IL15/IL15RA-driven malignant phenotype has been suggested in myeloma and T-cell lymphoma (which also express IL2RB and IL2RG; refs. 35, 36) and melanoma (in which the expression of IL2RB and IL2RG was neither verified nor disproved; ref. 19).

We then aimed to characterize IL15-dependent signaling in TNBCs. In lymphocytes, the signaling cascade originating from the IL15 heterotrimeric receptor involves activation of JAK1/3 and STAT3/5 (31). Further data suggested IL15-dependent activation of the PI3K/AKT pathway (37) and the NFkB pathway in neutrophils (38) or myeloid cells (39). IL15RA also interacts with and signals through SYK in neutrophils (40). Evidence obtained in epithelial cells suggested a signaling function for IL15/IL15RA in the absence of IL2RB (41), whereas in melanoma, IL15RA can bind and signal through TRAF (19).

Upon stimulation of BCCLs with recombinant IL15, we observed activation of JAK1, STAT1, and STAT2, as well as ERK1/2 and AKT and PRAS40. Intriguingly, we did not observe activation of other known effectors such as STAT3, STAT5, or STAT6. We speculate that this is a consequence of the lack of IL2RB and IL2RG that have been reported to specifically mediate the activation of many of these effectors (31).

Our observation of ERK1/2 activation is consistent with an IL15RA-dependent stimulation of proliferation in TNBCs. The lack of any evidence of activation of MEK might be explained by our observation of IL15-dependent activation of AKT/PRAS40, which may lead to MEK-independent ERK1/2 activation, as has been suggested in melanoma (42). Interestingly AKT phosphorylates PRAS40 specifically at T246 (43), the same residue that we found phosphorylated in response to IL15 stimulation. AKT activation can inhibit apoptosis (44), and we speculate that this mechanism may be responsible for the inhibition of apoptosis observed in BCCLs upon IL15 stimulation.

JAK1/STAT1 signaling is known to play a role in cell motility (45, 33). This is consistent with our observation of impaired migration of IL15RA/IL15–expressing cells upon JAK1 knockdown in the absence of any significant reduction in growth rate. We also found JAK1 silencing inhibits STAT1 activation but not ERK activation consistent with lack of effect of JAK1 silencing on cell growth.

It is increasingly apparent that the interface between immune cells and tumor cells can induce powerful effects on prognosis. Several reports have indicated positive prognostic correlations with evidence of effective engagement of both humoral and cytotoxic T-cell–adaptive immune responses (8), whereas others have shown that expression of inflammatory cytokines is associated with a poor prognosis (1).

There is growing evidence, suggesting inflammatory cytokines can regulate the growth of breast cancer cells. A cooperative role for IL6 and IL8 in promoting TNBC growth has recently been suggested, and although the expression of IL15RA and IL15 in TNBCs and TNBC-derived cell lines was also noted, it was not further investigated (46). This,
together with evidence of IL6 stimulation of IL15 expression observed in HaCaT cells (47), suggests cooperation among proinflammatory cytokines to promote cancer cell growth despite known roles in stimulation of the immune response.

We show that the endogenous expression of IL15RA and IL15 by basal BCCLs stimulates a pSTAT5 signaling response in freshly isolated PBMC in coculture. This phenomenon is abolished by an inhibitory IL15 antibody as well as by silencing of either IL15RA or IL15. Taken together, this evidence points to PBMC activation by IL15 expressed by basal BCCLs co-expressing IL15RA. This most likely occurs via transpresentation of IL15 by IL15RA, as knockdown of IL15RA inhibited pSTAT5 signaling in PBMC. IL15 is known to be a potent inducer of the activatory receptor NK group 2D (NKG2D) receptor on cytoytic lymphocytes and is an important survival factor for NK cells (48, 49). Indeed NKG2D-mediated antitumor immunity is enhanced by IL15 (50).

Our data suggest a dual effect of IL15RA amplification and its consequent high-level expression. IL15RA coexpressed with IL15 in the absence of IL2RB and IL2RG, on the one hand drives cancer proliferation, protection from apoptosis, and enhances cell migration, whereas on the other hand is capable of activating signaling in immune cells that, when not edited, suppressed or compromised by other intrinsic tumor or stromal factors, may lead to a relatively better prognosis.

A number of clinical trials (NCT01727076 and NCT01885897) are currently investigating the effects of IL15 administration with an aim to stimulate anticancer immune responses. Our data add further insight into the complexities of IL15 signaling in some breast tumors, not only in immune cells in the tumor stroma, but also within malignant cells in which IL15 and IL15RA may also drive hallmarks of malignancy. This may explain why some basal breast carcinomas with aggressive high-grade malignant features are also intensely infiltrated by immune cells and why these high-grade cancers have a paradoxically good prognosis compared with other basal breast cancers (11). If an IL15 transpresentation-induced activation of immune cells is blocked or edited by immune checkpoints at the immune and cancer cell interface but cancer cell–intrinsic IL15/IL15RA drivers of the malignant phenotype remain intact, we speculate there may be adverse effects on prognosis. Our data indicating IL15-dependent cancer cell signaling suggest that IL15 therapies should be investigated cautiously in IL15RA-expressing cancers as there may be the potential to drive tumor growth.

A more detailed understanding of the signaling pathway involved in IL15/IL15RA signaling in cancer cells, and the antitumor immunologic mechanisms that can be enhanced by IL15, would aid identification of cancer cell–intrinsic targets downstream of IL15/IL15RA that might be inhibited to block protumorigenic effects while retaining the antitumor immunostimulatory effects of an IL15-based therapy.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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The views expressed are those of the author(s) and not necessarily those of the NIH, the NIHR or the Department of Health.

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IL15RA Drives Antagonistic Mechanisms of Cancer Development and Immune Control in Lymphocyte-Enriched Triple-Negative Breast Cancers

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