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Mini review

Emerging roles for IL-11 signaling in cancer development and progression: Focus on breast cancer

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ABSTRACT

Interleukin (IL)-11 is a member of the IL-6 family of cytokines that is defined by the shared use of the GP130 signal transducing receptor subunit. In addition of its long recognized activities as a hemopoietic growth factor, IL-11 has an emerging role in epithelial cancer biology. Through the activation of the GP130-Janus kinase signaling cascade and associated transcription factor STAT3, IL-11 can confer many of the tumor intrinsic 'hallmark' capabilities to neoplastic cells, if they express the ligand-specific IL-11R α receptor subunit. Accordingly, IL-11 signaling has recently been identified as a rate-limiting step for the growth tumors arising from the mucosa of the gastrointestinal tract. However, there is less appreciation for a potential role of IL-11 to support breast cancer progression, apart from its well documented capacity to facilitate bone metastasis. Here we review evidence that IL-11 expression in breast cancer correlates with poor disease outcome and discuss some of the molecular mechanisms that are likely to underpin these observations. These include the capacity of IL-11 to stimulate survival and proliferation of cancer cells alongside angiogenesis of the primary tumor and of metastatic progenies at distant organs. We review current strategies to interfere with IL-11 signaling and advocate that inhibition of IL-11 signaling may represent an emerging therapeutic opportunity for numerous cancers.

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1. Introduction

Interleukin (IL)-11 is a member of the IL-6 family of cytokines that comprises nine secreted soluble ligands; IL-6, IL-11, leukemia inhibitory factor (LIF), oncostatin-M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC), interleukin-27 (IL-27) and interleukin-31 (IL-31) [1–10]. Each ligand interacts with a specific non-catalytic transmembrane receptor or receptors, commonly referred to as the receptor alpha chain. Importantly, the family is defined by their shared use of the ubiquitously expressed transmembrane protein glycoprotein-130 beta-subunit (GP130, also known as IL6ST or CD130) [4,11,12].

IL-11 was purified as 19 kDa soluble factor in supernatants from a stromal cell line that promoted the proliferation of a plasmacytoma cell line that was otherwise dependent on IL-6 [10]. Although crystallization of the 178 amino acid human IL-11 protein revealed the characteristic type 1 four-helix bundle, its structure shows some distinct differences to that of its closest relative IL-6 [13]. Meanwhile the 7 kb human *IL11* gene comprises five coding exons and is localized to chromosome 19q13.3–19q13.4 [14].

Traditionally, IL-11 is recognized for its capacity to promote maturation of platelet producing megakaryocyte progenitors *in vitro* and in the bone marrow *in vivo* [15,16]. In order to boost platelet production, numerous clinical trials have been conducted with recombinant IL-11 in patients with breast cancer to reduce thrombocytopenia associated with chemotherapy [17,18]. This culminated in the recent FDA approval of Oprelvekin, as a modified more stable form of IL-11, to reduce chemotherapy-induced thrombocytopenia at lower doses [19,20]. Despite these striking activities within the hematopoietic system, studies in knockout

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mice have revealed that IL-11 is not essential for hematopoiesis, but instead is critical within the endometrial tissue of the pregnant adult female, as genetic deficiency of the IL-11 receptor subunit prevented formation of a decidua and hence resulted in abortion of 5 day old mouse embryos [21]. More recently, the use of *Il11ra*-null mice has revealed an unprecedented role for IL-11 signaling as a “gate keeper” for the growth of adenomas and possibly more advanced tumors derived from the gastrointestinal mucosa [22], and these findings have recently been reviewed [1]. Here we will focus on emerging insights into the role of IL-11 in the development and progression of breast cancer.

2. IL-11 signaling

IL-11 binds to its specific transmembrane receptor, IL-11 receptor alpha (IL-11R α) [23]. It is believed that the IL-11/IL-11R α dimeric complex interacts in turn with GP130 potentially as a tetrameric complex [24] (Fig. 1). In turn, and in analogy to IL-6 signaling, it is believed that these complexes transition into a high affinity ligand-binding and signaling-proficient hexameric complex comprising a 2:2:2 ratio of ligand, IL-11R α and GP130 [25]. The formation of this larger order complex initiates signaling through juxtapositioning of the intracellular Janus (JAK) family tyrosine kinases JAK1, JAK2 and TYK2, which are constitutively associated with a proline-rich intracellular domain of GP130, and enables kinase activation in response to *trans*-phosphorylation [12]. Activated JAK kinases in turn phosphorylate the various cytoplasmic tyrosine residues in GP130 to provide docking sites for the signaling molecules signal-transducer and activated of transcription-3 (STAT3), STAT1, SHP2 and the negative regulator suppressor of cytokine signaling (SOCS3), in addition to JAK-mediated tyrosine phosphorylation of the former molecules [12]. Genetic evidence suggests that activation of STAT3 is the most important event for the transduction of a majority of biological responses to GP130-family cytokines [26] (Fig. 2). While most of these effects depend on transcriptional regulation of target genes upon the binding of tyrosine-phosphorylated STAT3-dimers to regulatory DNA sequences, non-canonical activities of serine-phosphorylated STAT3 also appear to promote its oncogenic capacity by facilitating glycolysis and oxidative phosphorylation in the

mitochondria [27]. However, most of tumor-cell intrinsic ‘Cancer Hallmark’ activities elicited by STAT3 depend on its canonical role as a transcriptional modulator of target genes that affect cell proliferation, survival, motility and invasion [28,29] (Fig. 2). A key STAT3-induced target gene encodes the SOCS3 protein, which terminates GP130 signaling. SOCS3 binds to a membrane-proximal phosphotyrosine residue in GP130 to mediate formation of an E3 ligase scaffold with elongin BC and a cullin protein resulting in ubiquitination of the receptor complex and its proteasomal degradation [30].

Besides activation of STAT3, and to a lesser extent also STAT1, engagement of GP130 also triggers signaling through the RAS–RAF–ERK pathway following GP130 association and subsequent JAK-dependent phosphorylation of the tyrosine phosphatase SHP2/PTPN11 [31]. Finally, GP130 has also the capacity to activate the phosphatidylinositol 3' kinase (PI3K)–AKT–mTORC1 pathway, although in contrast to engagement of the STAT and SHP2/ERK signaling cascades, the former does not require tyrosine phosphorylation of GP130 [32] (Fig. 2).

3. Expression of IL-11 and relationship to outcome in breast cancer

3.1. IL-11 expression

Carcinomas comprise neoplastic epithelial cells intermingled with various non-transformed stromal cell types (Fig. 1). Individually, and in concert with one another, these non-transformed cell types collectively comprise the tumor microenvironment, and influence most if not all aspects of cancer cell behavior. In turn, tumor cells influence the composition and function of the tumor microenvironment [33,34]. Although expression of IL-11 has not been evaluated as extensively as that for IL-6 in whole breast tumors, most information regarding cell-type specific expression can be extrapolated from studies of other cancer types [35], or from analysis of human breast cancer cell lines [36–41]. In the majority of primary tumor lesions of all breast cancer sub-types and stages, IL-11 expression is elevated when compared to adjacent normal breast tissue, (Fig. 3A, B). Furthermore, analysis of expression

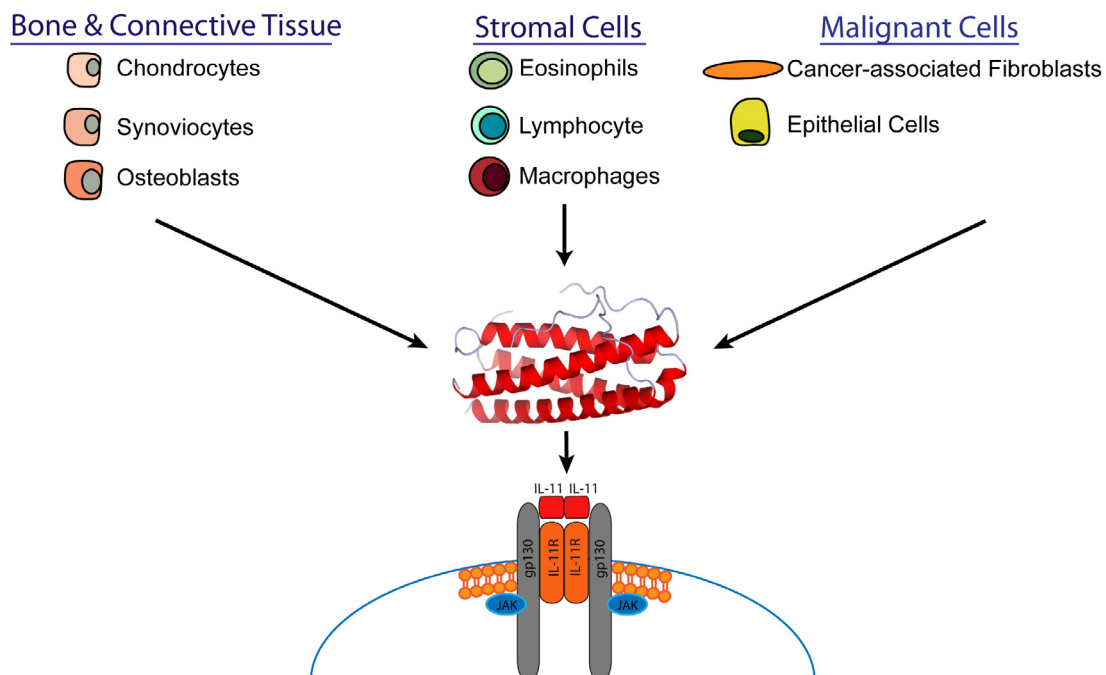


Fig. 1. Schematic representation of the major cellular sources of IL-11, which forms a hexameric 2:2:2 signaling complex comprising IL-11, IL-11R α and GP130.

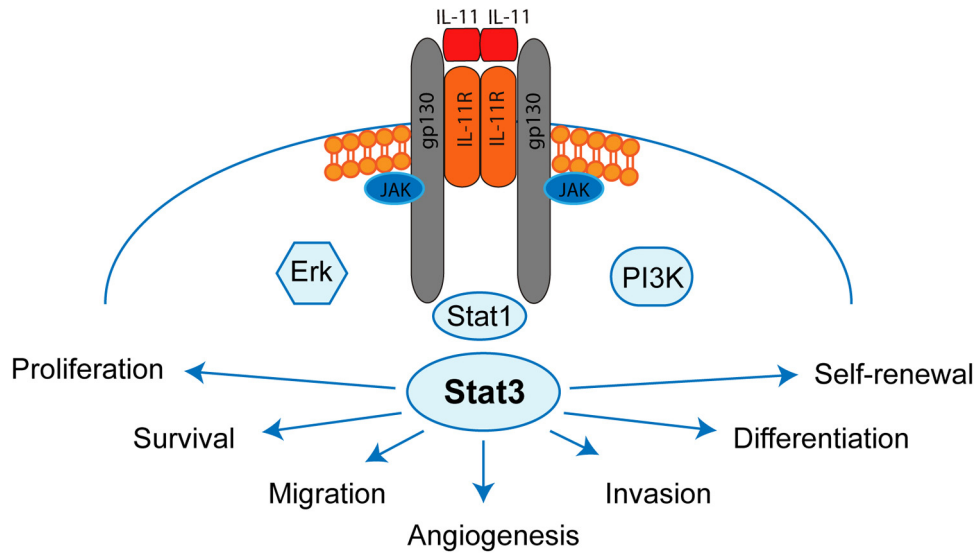


Fig. 2. Schematic representation of the pro-tumorigenic activities elicited through the IL-11/GP130/STAT3 signaling cascade.

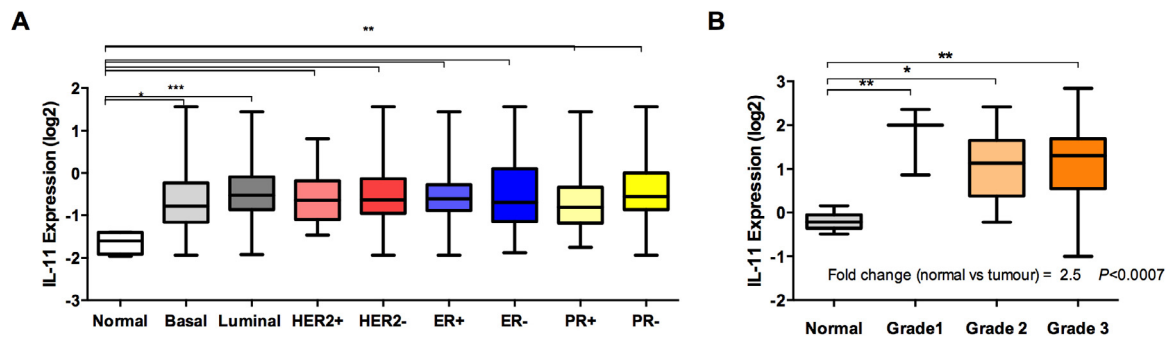


Fig. 3. IL-11 is elevated in breast cancer tissue irrespective of grade or hormone receptor status. (A) 'Gluck' [119] and (B) 'Finak' [120] Oncomine data is presented. * $P < 0.05$, ** $P < 0.001$, *** $P < 0.001$.

profiling data from The Cancer Genome Atlas (TCGA) and from the Curtis datasets through the Oncomine portal (<http://www.oncomine.org>, [42]) revealed elevated IL-11 transcript levels in both primary ductal and lobular breast adenocarcinoma (Table 1), suggestive of a potential role in breast carcinogenesis [43,44].

In the healthy mouse, IL-11 mRNA is detected at low abundance in thymus, spleen, heart, gastrointestinal tract, kidney, brain, testis, uterus, ovaries and bones [45,45], where production increase in response to infection, injury and inflammation [46]. Subepithelial myofibroblasts are a major source of IL-11 [47], as well as the gastrointestinal epithelium and both cell types are likely to

contribute IL-11 to cancers alongside the carcinoma cells, tumor-associated macrophages (TAMs) and T cells [2] (Fig. 1). In a neo-adjuvant trial of breast cancer patients undergoing chemotherapy, administration of epirubicin plus cyclophosphamide plus docetaxel enhanced IL-11 expression in primary breast tumors (Table 2), although no further investigations were carried out to determine the cellular origin of IL-11 [48]. However, this observation was not confirmed in a similar larger chemotherapy neo-adjuvant trial [49]. In response to chemotherapy, expression of IL-6, but not IL-11, was induced in thymic endothelial cells in a mouse model of Burkitt lymphoma and promoted survival of

Table 1

Expression of IL-11 and IL-11R α mRNA in normal breast and breast cancer: an analysis using Oncomine data.

| Gene | Type | F.C. | P | Sample size | Platform | Refs. |
|--------|----------|-------|-----------|-----------------------------|----------|--------------|
| IL11 | I.D.C. | 1.75 | 5.80E-11 | Normal (61), cancer (389) | RNA-Seq | TCGA [43] |
| IL11 | I.L.C. | 1.46 | 1.32E-4 | Normal (61), cancer (36) | RNA-Seq | TCGA [43] |
| IL11 | I.D.C. | 1.04 | 1.43E-7 | Normal (144), cancer (1556) | Array | Curtis [44] |
| IL11 | I.L.C. | 1.02 | n/s | Normal (144), cancer (148) | Array | Curtis [44] |
| IL11 | Mucinous | 1.01 | n/s | Normal (144), cancer (46) | Array | Curtis [115] |
| IL11RA | I.D.C. | -3.87 | 2.35E-51 | Normal (61), cancer (389) | RNA-Seq | TCGA [43] |
| IL11RA | I.L.C. | -2.69 | 1.24E-17 | Normal (61), cancer (36) | RNA-Seq | TCGA [116] |
| IL11RA | I.D.C. | -3.33 | 9.47E-117 | Normal (144), cancer (1556) | Array | Curtis [44] |
| IL11RA | I.L.C. | -2.49 | 2.25E-54 | Normal (144), cancer (148) | Array | Curtis [44] |
| IL11RA | Mucinous | -3.81 | 2.20E-21 | Normal (144), cancer (46) | Array | Curtis [44] |

P values were determined using Student's *t*-test.

F.C., fold change; Refs., references; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; TCGA, The Cancer Genome Atlas.

Table 2

Association of IL-11 mRNA levels in primary breast ductal tumors with patient outcome and other clinical parameters: an analysis using OncoPrint data.

| Gene | Parameter | F.C. | P | N | Platform | Refs. |
|-------------|-----------|------|---------|--------------------------------------|----------|----------------|
| <i>IL11</i> | Neo-chemo | 2.61 | 7.43E–8 | Control (32), treated (25) | Array | Stickeler [48] |
| <i>IL11</i> | 3 yr RFS | 1.77 | 4.23E–5 | No relapse (124), relapse (32) | Array | Desmedt [63] |
| <i>IL11</i> | 3 yr DMFS | 1.77 | 2.00E–4 | No metastasis (138), metastasis (19) | Array | Desmedt [63] |
| <i>IL11</i> | 5 yr RFS | 1.53 | 0.002 | No relapse (105), relapse (47) | Array | Desmedt [63] |
| <i>IL11</i> | 5 yr DMFS | 1.44 | 0.022 | No metastasis (123), metastasis (29) | Array | Desmedt [63] |

P values were determined using Student's *t*-test.

F.C., fold change; N, sample size; Neo-chemo, Neo-adjuvant chemotherapy; RFS, relapse-free survival; DMFS, distant metastasis-free survival; yr, year; Refs., references.

residual tumor cells in the thymus [50]. IL-6 was also induced in doxorubicin-treated human umbilical vein endothelial cells, suggesting that the vascular endothelium may also be a potential source of IL-6 related cytokines in certain clinical contexts.

In colorectal tumors, carcinoma-associated fibroblasts (CAFs) appear to be the principal source of IL-11, where *IL11* gene expression was activated in response to exposure to tumor cell-derived TGF- β [35]. The latter is consistent with the identification of two AP-1 motifs in the 5' region of the *IL11* gene that are essential for TGF β 1-induced transcriptional activation. Meanwhile, additional *cis*-regulatory elements in the gene promoter comprise, among others, binding sites for SP-1, STAT3, CTF/NF-1 and possibly NF κ B thereby confirming a role for IL-11 during inflammatory processes and both autocrine and paracrine enforcement of STAT3-dependent signaling [10,51].

3.2. IL-11R α expression

Interrogation of microarray data reveals relatively broad expression of IL-11R α mRNA in breast cancer cell lines (Johnstone C.N., unpublished observation), which we also observe in human breast tissue biopsies (Fig. 4). Published investigations at the protein and transcript level showed minimal IL-11R α expression in normal mammary gland, although expression of IL-11R α alongside its ligand were increased in a subset of primary breast cancers [40,52]. However, expression of IL-11R α was down-regulated in the ductal, lobular and mucinous histotypes of human breast cancer (Table 1). The former comprises the most common histological subtype and is further divided into the estrogen receptor alpha (ER α)-positive, HER2-positive and triple-negative (negative for ER α , progesterone receptor (PR) and HER2) clinical subtypes [44]. A recent meta-analysis of 21 breast cancer gene expression datasets proposed that triple-negative tumors can be further divided [53]. This includes a mesenchymal stem cell-like (MSL) subgroup, which is characterized by IL-11R α expression as an identifying marker [53]. This subtype has undergone epithelial-to-mesenchymal transition (EMT) and is particularly aggressive with a poor patient outcome [53,54]. Since EMT often correlates

with therapy resistance, it has been suggested that the breast cancer prognostic marker miRNA-30c targets IL-11 expression via an actin binding protein [55]. High miRNA-30c expression inversely correlated with low IL-11 expression and improved survival in breast cancer patients [55]. Surprisingly, despite accumulation of SOCS3 transcripts [36,37], triple negative tumors show very low levels of SOCS3 protein [56], possibly resulting in sustained JAK-STAT signaling. Thus, similar to observations in other epithelial cancers, IL-11R α expression is down-regulated in most primary breast tumors with the notable exception of elevated expression in the MSL-like subgroup of triple-negative cancers.

3.3. IL-11 signaling and patient outcome

Levels of IL-11 transcripts, rather than of IL-11R α transcripts, generally correlate positively with breast cancer progression. These associations are reminiscent of reports for hepatocellular carcinoma [57], gastric carcinoma [58] and renal cell carcinoma [59], and more recently IL-11 has also been implicated in poor survival of patients with non-small cell lung adenocarcinoma [60]. Similarly, there is also a trend toward higher IL-11 expression in tumors from patients with local or distant recurrence, as well as in patients who died from breast cancer, although these associations did not reach statistical significance possibly due to the small sample size [52]. Meanwhile, Sotiriou et al. [61] reported in a cohort of 89 patients a statistically highly significant association between IL-11 expression in primary tumors and an increased risk for development of bone metastasis. In support of this, IL-11 protein levels in the serum and primary tumors were significantly higher in patients with bone metastasis compared to those without distant metastasis [62]. Moreover, in the cases with bone metastasis, serum IL-11 levels were positively associated with reduced disease-free survival [62]. Similarly, higher IL-11 transcript levels have been observed in breast cancer patients that relapsed 3–5 years after initial diagnosis when compared to a relapse-free cohort [63]. While there is less compelling evidence between IL-11 expression and breast cancer patient outcome using

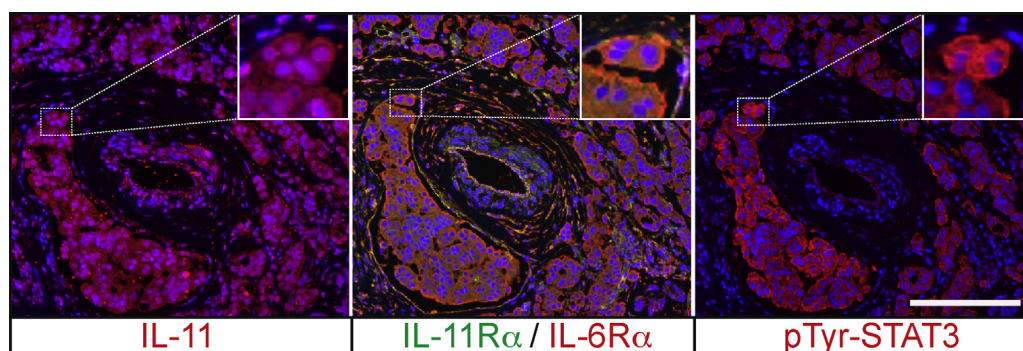


Fig. 4. IL-11 is produced by a range of cell populations in human primary breast tumors (left panel), with IL-6R and IL-11R α expression (middle panel) primarily associated with mammary epithelial cells and STAT3 activation (right panel). Scale bar = 100 μ m.

Table 3

Association of IL-11 and IL11RA mRNA levels in primary breast tumors with patient outcome and other clinical parameters: Kaplan–Meier analyses.

| Gene | Subgroup | Dataset | Survival | H.R. | 95% C.I. | N | P | Refs. |
|---------------|--------------------------|-------------|-------------------|--------|---------------|------|--------|----------------------------|
| <i>IL11RA</i> | L.N. metastasis negative | 26 datasets | DFS | 0.7961 | 0.6433–0.9852 | 1183 | 0.0357 | Madden [64] |
| <i>IL11</i> | All | Cardiff | DFS | NR | NR | ? | 0.0100 | Hanavadi [52] |
| <i>IL11</i> | All | UNC337 | RFS ^a | 2.3763 | NR | 337 | 0.0002 | Bockhorn [55], Prat [117] |
| <i>IL11</i> | All | Oxford | DMFS ^b | 2.6620 | NR | 210 | 0.0210 | Bockhorn [55], Buffa [118] |
| <i>IL11</i> | All | UC | PFS ^b | 1.7524 | NR | 44 | 0.0375 | Bockhorn [55] |

H.R., hazard ratio; C.I., confidence interval; N, sample size; Refs., references; L.N., lymph node; DFS, disease-free survival; RFS, relapse-free survival; DMFS, distant metastasis-free survival; PFS, progression-free survival; NR, not reported.

^a Gene expression values were dichotomized and allocated into high and low groups split at the median.

^b Gene expression values were used as a continuous variable.

the BreastMark meta-analysis algorithm (<http://glados.ucd.ie/BreastMark/index.html>) to survey survival data from 26 datasets on 12 different microarray platforms [64], such data may be limited by the frequent incorrect annotation of IL-11/IL-11R α on some microarray platforms. Nevertheless, BreastMark analysis revealed an association between higher IL-11R α expression and prolonged patient survival specifically in lymph node metastasis-negative patients (Table 3).

4. Molecular activities of IL-11 in breast cancer

4.1. Proliferation and Apoptosis

IL-11 acts as a potent growth factor for various hematopoietic progenitors, and the growth of tumors of the gastrointestinal mucosa is fueled by IL-11 [22], consistent with the capacity of STAT3 to transcriptionally induce genes that promote cell cycle progression (Fig. 2). Likewise, a second cancer hallmark activity of STAT3, namely to promote cellular survival through both the induction of Bcl-2 survival proteins as well as, possibly through indirect mechanisms, suppression of their BH3-only protein antagonists, is a prominent IL-11 activity [22]. Indeed we have identified the latter as the major mechanism that confers resistance of the colonic epithelium to experimentally induced acute colitis, as well as a mechanism by which IL-11 antagonists suppress the growth of gastrointestinal tumors in mice [22]. Consistent with these observations, Bockhorn et al. [55] also found a role for IL-11 in chemotherapy-induced apoptosis of MDA-MB-231 triple-negative breast cancer cells. Specifically, siRNA-mediated knockdown of IL-11 sensitized the cells to paclitaxel-induced cell death, while anti-IL-11 antibody added to culture medium increased doxorubicin-induced apoptosis. Indeed higher IL-11 expression was associated with reduced progression-free and overall survival in a cohort of 25 patients treated with doxorubicin, suggesting that IL-11 may also antagonize pro-apoptotic pathways *in vivo* [55]. Whether this is limited to the cell intrinsic apoptotic pathway or may also affect the tumor necrosis factor (TNF) superfamily dependent extrinsic pathway, remains to be fully elucidated.

4.2. Tumor hypoxia and angiogenesis

Cancers require a vascular network to both provide nutrients for growth and to remove harmful by products of proliferation and cellular metabolism. Indeed, stimulation of new blood vessels (angiogenesis) is required if tumors are to reach a size greater than a few mm³ [65]. Hypoxia near the center of actively growing tumors results in stabilization of the transcription factor hypoxia-inducible factor-1 α (HIF-1 α), which in concert with STAT3 activates expression of the key angiogenic growth factor vascular endothelial growth factor (VEGF) to stimulate angiogenesis [66]. Interestingly, IL-11 rather than IL-6 was induced by hypoxia in a range of cancer cell lines [67]. Induction by hypoxia was mediated by the HIF-1 α and AP-1 transcription factors. Similarly,

in vitro experiments showed that IL-11 expression enhanced the tumorigenicity of PC-3 prostate cancer cells under hypoxic, but not normoxic, conditions and this was associated with STAT1 rather than STAT3 phosphorylation [67]. Consistent with this, short-hairpin RNA (shRNA)-mediated stable knockdown of IL-11 attenuated the growth of PC-3 xenografts [67]. Furthermore, loss of IL-11 increased apoptosis of tumor cells primarily at early time points, consistent with a key role for IL-11 in the enhanced survival of tumors while emerging in a hypoxic microenvironment. Interestingly, while these investigators did not observe differences in microvessel density in IL-11 silenced tumors, others reported that exclusion of the IL-11-producing tumor subclone in a polyclonal xenograft was associated with a reduction of vessel growth [68]. Human umbilical vein endothelial cells express IL-11R α and respond to stimulation with IL-11 [69], thus raising the possibility that IL-11 may have direct effects on tumor endothelium.

4.3. Cancer stem cells and tumor heterogeneity

An emerging concept in breast cancers and tumors of other origins is the dynamic equilibrium between cancer stem cells (CSCs) and their non-cancer stem cells (NCSCs) progenies [70]. CSCs have the ability to initiate *de novo* tumors when transplanted, whereas NCSCs do not [70]. Since CSCs often divide more slowly than the bulk population of the tumor, they may escape chemotherapy-induced cell death and enable tumor recurrence at a later time point. At the molecular level, breast cancer stem cells are defined as bearing a CD44⁺CD24⁻ surface marker phenotype [71], or being positive for the enzyme aldehyde dehydrogenase (ALDH1) [72], and both of these populations form tumors in mouse models. Phenotypically, breast CSCs have been described as undifferentiated, or possessing a mesenchymal phenotype [73], and thus might be generated from NCSCs by epithelial-to-mesenchymal transition [74]. Indeed, Iliopoulos et al. [75] have shown that the conversion of NCSCs to CSCs is enhanced by IL-6. Several other studies identified critical roles for the JAK-STAT pathway and canonical STAT3 signaling in the maintenance of the CSCs population in breast cancer [76]. The STAT3 inhibitors LLL12 or STATTIC, for instance, reduced the viability of breast CSCs and inhibited the growth of xenograft tumors in mice that were formed by an inoculum enriched in CSCs [77]. Complementary studies have shown that IL-6 signaling mediates stem cell maintenance through STAT3-dependent mechanisms [78,79]. Polyak and colleagues delineated an IL-6/JAK2/STAT3 pathway in breast CSCs where the JAK2-specific inhibitor NVP-BSK805 caused tumor regression in triple-negative tumor xenografts in mice [79]. Meanwhile in the HER2+ breast cancer subtype, resistance often emerges to the standard-of-care anti-HER2 antibody (Trastuzumab) adjuvant therapy through mutational activation of the PI3K/AKT signaling pathway [80]. Since the latter results in increased production of IL-6, IL-8 and CCL5 [81] it is likely to also induce expression of IL-11, although this was not experimentally confirmed. Elevated IL-6 secretion stimulated the expansion of

the CSC population in several human breast cancer models, through engagement of the downstream JAK/STAT3 pathway, and is also likely to be associated with NF κ B signaling, which is an inducer of IL-6. Importantly, growth of Trastuzumab-resistant tumors was entirely dependent on sustained IL-6 signaling, as administration of the anti-IL-6R antibody Tocilizumab eliminated tumor growth *in vivo* [81].

Tumor heterogeneity and clonal cooperation are important concepts in our understanding that within a lesion the cancer cells may behave as a community and collectively confer increased aggressiveness to polyclonal tumors. Using a candidate approach of overexpressing cancer-promoting factors in individual subclones of MDA-MB-468 triple-negative breast cancer cells, Marusyk and colleagues identified IL-11 and vascular endothelial growth factor-D (VEGF-D) as the two factors that reproducibly conferred metastatic behavior to the corresponding polyclonal xenografts [68]. Furthermore, the IL-11 expressing subclone(s) acted as non-cell autonomous driver(s) of xenograft growth, most likely also promoting expansion of the VEGF-D producing clone to ensure sufficient (lymph-)angiogenesis. Indeed, the continuous presence of IL-11 appeared to ensure that all subclones maintained a certain equilibrium, which collectively retained aggressiveness of the xenograft. Removal of the IL-11 producing clone, on the other hand, prevented further growth of the xenografts and often resulted in their necrotic collapse. Surprisingly, neither IL-11 (nor VEGF-D) acted directly on the cancer cells. These data suggest a hitherto unrecognized capacity whereby IL-11 in the tumor microenvironment may not only promote tumor progression by maintaining an equilibrium among the most aggressive subclones, but also controls tumor angiogenesis, which may occur by both direct and indirect mechanisms [82].

4.4. Aromatase expression

Aromatase (*CYP19A1*) belongs to the cytochrome P450 group of enzymes and catalyzes the biosynthesis of estrogens. While in premenopausal women, the ovaries have the highest aromatase expression [83], in post-menopausal women with breast cancers, aberrant transcription of the *CYP19A1* gene in CAFs increases intratumoral estrogen levels and promotes tumor growth [84]. Accordingly, aromatase inhibitors are currently used to treat Tamoxifen-resistant ER α -positive tumors. While the mechanism for enhanced *CYP19A1* transcription in response to tumor-secreted factors involves the PII gene promoter normally utilized for ovarian transcription [85], the adipose tissue-specific PI.4 promoter contributes to local *CYP19A1* expression in the tumor microenvironment [86]. PI.4-mediated transcription is stimulated by TNF α and, in synergy with the activated glucocorticoid receptor, the GP130 cytokines IL-11 and oncostatin-M [87]. Consistent with this the PI.4 promoter contains specific GAS sequence to facilitate STAT1/3 binding, and its close proximity to a glucocorticoid response element molecularly underpins the synergistic effect between dexamethasone and IL-11 stimulated aromatase expression [87–89]. It remains to be established whether this mechanism also contributes to the breast cancer risk posed by chronic and obesity-associated inflammation, because accumulation of CD68+ macrophages in breast adipose tissue of obese women correlates with increased aromatase levels [90]. Thus, tumor-associated macrophages, *via* the secretion of cytokines such as oncostatin-M and IL-11, may contribute to elevated aromatase activity in breast cancer.

4.5. Metastatic dissemination

While IL-11 is likely to be only a lesser driver of cancer cell proliferation [35,39,91,92], evidence has accumulated for IL-11 to

facilitate metastatic dissemination of cancer cells to distant sites. As mentioned above, clinical data strongly implicates IL-11 in metastasis to bone. IL-11 is produced by bone marrow stromal cells where it can stimulate osteoclast development from progenitor cells [93] (Fig. 1). Unlike in prostate cancer, where the bone lesions are osteoblastic, breast cancer bone metastases are usually osteolytic, resulting in net bone destruction through breast cancer cell-released mediators, including IL-11 and parathyroid hormone-related protein (PTHrP), which activate osteoclasts [94].

Osteoclast-mediated bone remodeling in turn releases bone matrix-associated TGF- β and other growth factors into the local environment, and TGF- β readily induces expression of IL-11 and other osteoclast differentiation factors in breast cancer cells thereby further increasing the rate of bone loss [91]. Accordingly, ablation of the TGF- β -signaling node SMAD4 attenuated the capacity of breast cancer cells to produce IL-11 and to metastasize to bone [95]. IL-11 over-expression alone failed to increase the rate by which MDA-MB-231 cells formed bone metastases, suggesting that IL-11 may not be involved in the homing of disseminated cancer cells to bone. In this model, however, ectopic IL-11 expression co-operated with over-expression of the chemokine receptor CXCR4 to drive experimental osteolytic metastasis [39].

Advanced colorectal cancer has a high propensity to metastasize to the liver *via* the portal vein. Primary colorectal tumors produce high levels of TGF- β [35], though unlike breast cancer cells they mostly lack the ability to respond to TGF- β signaling owing to frequent mutational inactivation of components of the TGF- β pathway [96]. Calon and colleagues identified a pro-metastatic pathway involving TGF- β mediated induction of IL-11 expression in the CAFs associated with primary tumors. They further proposed that paracrine stimulation of cancer cells with IL-11 activates STAT3 to facilitate metastatic colonization mainly through evasion of apoptosis, and this was inhibited by silencing GP130 expression on cancer cells. Although it was not formally proven that the colorectal cancer cells express IL-11R α , it is reminiscent of findings demonstrating that the growth of primary colonic adenomas is impaired in bone marrow chimeras where the host, but not the bone marrow, lack IL-11R α expression [22]. It remains unclear whether TGF- β can induce IL-11 expression in CAFs irrespective of the site of the emerging micrometastasis. Interestingly, while colorectal cancer cell lines engineered to produce IL-11 displayed enhanced spontaneous metastasis from the orthotopic site in the cecal wall to multiple organs, ectopic over-expression of IL-6 only marginally enhanced metastasis [35]. The latter is most likely attributable to the low abundance of IL-6R α on these cells, and also suggests the absence of significant IL-6 *trans*-signaling, whereby cells lacking the trans-membrane IL-6R α can respond to a preformed soluble complex comprising IL-6 and the cleaved form of the extracellular domain of IL-6R α [97].

An alternative, and not mutually excluding mechanism suggests that TGF- β may also elevate IL-11 levels by stabilizing its mRNA through induction of the long non-coding RNA lncRNA-ATB in hepatocellular cancer cells. The latter appears to also sequester miR-200 family microRNAs [92]. Inhibition of miR-200 family miRNAs promoted epithelial-to-mesenchymal transition and cell invasion, while the increased IL-11 production resulted in autocrine STAT3 activation, although without modulating invasion. Similar to the aforementioned study from Calon et al., Yuan et al. [92] also observed that lncRNA-ATB expression in two different models of experimental metastasis, promoted tumor cell survival during the early phases of metastatic colonization though an IL-11/STAT3-dependent mechanism. Finally, IL-11 mRNA levels are significantly higher in portal vein tumor thrombi than in matched primary liver cancers from the same patient, which

coincides with the capacity of hepatocellular carcinomas to metastasize intra-hepatically *via* the portal vein.

Finally, it is worthwhile to consider that IL-11 may also promote metastasis indirectly owing to its capacity to stimulate platelet production [98,99]. Platelets can coalesce with circulating tumor cells in the bloodstream to both protect them from the immune system as well as assist cancer cell extravasation into tissue parenchyma [100].

5. Therapeutically targeting IL-11 signaling in cancer

To date, only a few studies have been published that document therapeutic targeting of IL-11 signaling in pre-clinical models of cancer. The viability and largely normal physiological response of adult *Il11ra*-null mice, with the lack of decidua formation in pregnant females being the most obvious defect [101], suggests that targeting of IL-11 signaling in cancer patients is likely to avoid major deleterious systemic side effects [102]. Likewise, prolonged treatment of mice with an antagonistic form of IL-11, designated mIL-11 Mutein and possessing over 20-fold higher affinity for mouse IL-11R α than wild-type mIL-11 [103], did not result in a drop in blood platelets or affect blood coagulation [22].

IL-11, rather than IL-6, was shown to drive the growth of gastrointestinal cancers in mouse models [22]. In a genetically engineered model of inflammation-associated gastric cancer, IL-11 acted directly on tumor cells to drive STAT3 activation, cellular proliferation and invasion. Moreover, administration of mIL-11 Mutein reduced gastric tumor burden in these mice coinciding with reduced proliferation and enhanced apoptosis of tumor cells and a reduction in both tumor-associated inflammatory cells and cytokine levels [22]. Treatment with mIL-11 Mutein also reduced tumor size and multiplicity in a mouse model of carcinogen-induced sporadic colorectal cancer and attenuated growth of DLD-1 colorectal cancer xenografts [22]. Inhibition of tumor growth using this strategy is likely to require sustained administration of the therapeutic as the growth of gastric tumors rebounded following withdrawal of mIL-11 Mutein. It is possible that combination therapy using mIL-11 Mutein in conjunction with standard chemotherapy may result in complete tumor remissions, although this has not been formally evaluated experimentally.

Several antibodies that bind to and inhibit IL-11 [104], IL-11R α (Putoczki T.L., unpublished observations) or GP130 have been developed and shown efficacy in various mouse models. Likewise, antibodies targeting human IL-11 (R&D Systems), IL-11R α (Putoczki T.L., unpublished observations) or GP130 [105] are being developed for preclinical evaluation, on the back of the clinical success of antibodies targeting IL-6 (Siltuximab) or IL-6R α (Tocilizumab). Indeed, targeting IL-11 or IL-11R α may prevent unwanted side-effects arising from targeting GP130, given the embryonic lethality of *gp130* knockout mice [106], although GP130 epitopes have been identified that allowed for the development of GP130 antibodies that specifically inhibit IL-11 signaling [107]. The IL-11R α has also been used to exploit chimeric antigen-receptor (CAR) strategies to mediate cytotoxic T-cell killing of a human osteosarcoma cell line [108].

Using *in vivo* phage display on patient-derived prostate cancer tissue, Pasqualini and colleagues identified peptides with ability to bind to tumor vascular endothelium [109,110]. One peptide bound to IL-11R α expressed on endothelial cells [110] because it mimicked a motif in IL-11 that binds the receptor. Conjugation of this peptide motif to an apoptosis-inducing peptide sequence yielded a peptidomimetic, which targeted bone metastases and showed promising anti-tumor activity in pre-clinical models of prostate cancer and osteosarcoma [111,112]. While these studies used cell surface IL-11R α as means for delivering therapeutics to tumors, they do not address the exact nature of the IL-11

responsive cell type(s) that serve(s) as the Achilles heel for the growth and survival of the tumors. However, these studies did suggest the involvement of IL-11 signaling in tumor angiogenesis and in light of the aforementioned insights from the study by Marusyk et al. [68], this is likely to be a mechanism by which inhibition of IL-11 signaling in breast cancer may confer clinical benefit.

A more generic approach of interfering with GP130-mediated signaling is afforded by the various small molecule kinase inhibitors currently being either in advanced clinical trial or following repurposing of the FDA-approved JAK2 inhibitors for the treatment myeloproliferative and other hematological diseases [113]. It is likely that development of additional novel JAK tyrosine kinase inhibitors that better distinguish between the four highly related family members are likely to reduce the dose-limiting toxicity, including thrombocytopenia that is often observed with the current compounds [114].

6. Conclusions

Analysis of the recent literature strongly indicates a complex multi-faceted pro-tumorigenic role for IL-11, including for breast cancer. IL-11 has now been implicated in several unrelated aspects of tumor biology. These include the promotion of angiogenesis, survival under hypoxic conditions, apoptosis and chemoresistance, as well as growth and survival of early micro-metastatic colonies in bone and soft tissues including liver and lung. Further investigations are required to pinpoint the precise mechanisms by which IL-11 confers each of these activities.

Based on the observations summarized here, blockade of IL-11 signaling either through targeting of the ligand or of its cognate receptor, and in a more general approach through one of the many small molecule JAK tyrosine kinase inhibitors currently in clinical trials, is likely to generate collateral interference with processes that govern tumor homeostasis and progression. Key to this will be the careful assessment of the effect of anti-IL-11 therapeutics on primary tumors and distant metastasis in well-characterized xenograft and syngeneic mouse models of breast cancer. Eventual translation of this approach to the clinical setting will require selection of patients most likely to benefit from anti-IL-11 therapy. Since IL-11 can promote cancer progression through both direct action on cancer cells and indirectly *via* effects on the tumor microenvironment, screening of patients for IL-11/IL-11R α expression or other appropriate surrogate markers may need to be considered in the fullness of time.

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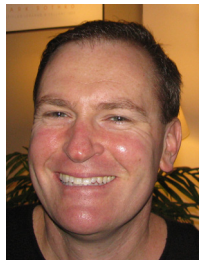
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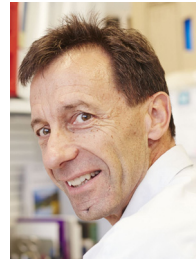


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