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Mini-review

Viewing the Eph receptors with a focus on breast cancer heterogeneity

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ABSTRACT

Aberrant expression of different family members of the Eph/ephrin system, which comprises the Eph receptors (Ephs) and their ligands (ephrins), has been implicated in various malignancies including breast cancer. The latter presents as a heterogeneous disease with diverse molecular, morphologic and clinical behavior signatures. This review reflects the existing Eph/ephrin literature while focusing on breast cancer heterogeneity. Hormone positive, HER2 positive and triple negative breast cancer (TNBC) cell lines, xenografts/mutant animal models and patient samples are examined separately as, in humans, they represent entities with differences in prognosis and treatment. EphA2, EphB4 and EphB6 are the members most extensively studied in breast cancer. Existing research points to the potential use of various Eph/ephrin members as biomarkers for assessing prognosis and selecting the most suitable therapeutic strategies in variable clinical scenarios, also for overcoming drug resistance, in the era of breast cancer heterogeneity.

1. Introduction

Breast cancer is the most common malignancy in women and the second most common in both sexes worldwide, showing 25% and 15% incidence and mortality rates, respectively [1]. The American Cancer Society reported it as the most commonly diagnosed malignancy for women, with an estimated 252,710 new cases and 40,610 deaths for 2017 in the US alone. However, due to the latest breakthrough developments in research, early diagnostics and therapeutics, the mortality rate has declined by 38% between 1989 and 2014, resulting in 297,300 less deaths [2].

Eph receptors (Ephs) make up the largest receptor of the tyrosine kinase (RTK) family. In humans, 14 Ephs are divided into two classes, A and B, based on their protein sequence homology and binding affinity to their eight ligands (ephrins): nine class A Ephs (EphA1 to EphA8, EphA10) preferentially bind to five class A ephrins (ephrin-A1 to ephrin-A5) and five class B Ephs (EphB1 to EphB4, EphB6) to three class B ephrins (ephrin-B1 to ephrin-B3), respectively. Significant inter-class interactions between Ephs and their ligands exist too: EphA4 can bind to class B ephrins and EphB2 to ephrin-A5 [3]. Similar to Ephs, ephrins are also cell-bound proteins; this is a key difference to the soluble nature of ligands (e.g. growth factors) that activate other RTK families [4–7].

Eph/ephrin juxtacrine signaling impacts the actin cytoskeleton organization and the expression of various adhesion molecules that regulate cell shape, adhesion and movement [6,8]. This Eph/ephrin binding typically generates a ligand-dependent and bidirectional (forward; reverse) signaling, followed by the activation of downstream molecular pathways in both attached cells through the Eph and its associated ephrin, respectively. Forward signaling into the receptor expressing cell triggers its kinase domain [9], and causes cell-cell de-adhesion [10–12]; the latter is a typical mechanism of tissue patterning during CNS development [13]. Reverse signaling into the ligand expressing cell activates Src kinases [14]. In contrast to the rest of the Ephs, two members (EphA10 and EphB6) have no kinase activity but can regulate signaling by forming heterodimers with Ephs that have competent kinase activity [15–17]. Of interest, cis Eph/ephrin interactions on the same cell can weaken the ones between opposing cells; for instance, ephrin-A3 can silence ligand-dependent bidirectional signaling [5]. On the top of that, Ephs may also be involved in ligand-independent signaling through their crosstalk with other molecules, such as the HER2 [3,18–22]. Ligand-independent signaling causes cell-cell adhesion [10–12]. In health, the Eph/ephrin system is implicated in processes such as cardiovascular and nervous system embryogenesis, as well as in cardiovascular, nervous, bone, immune, metabolic and stem cell homeostasis [19]. In disease, the Eph/eph system is implicated in

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viral, neurodegenerative, and neoplastic pathologic processes [19].

Accumulating evidence suggests that the Eph/ephrin system could be a promising target for cancer therapeutics [3,11,20,23,24]. Aberrant Eph or ephrin expression levels – caused by chromosome gains/losses [25], mutations [26–30], and deregulations in the transcriptomic [7], or epigenetic levels [31,32], – have been reported in diverse cancer sites including the lungs, breasts, colon, pancreas, ovary, esophagus, thyroid, tongue, liver, brain, skin and the lymphoreticular system [3,7,23,24,33–40]. The Eph/ephrin system is implicated in neoplastic cell proliferation, evasion of apoptosis, invasion, metastasis and angiogenesis and can be deregulated not only in tumor cells, but also the tumor microenvironment [3,8,20,41–45]. Both upregulation and downregulation of Ephs have been associated with cancer progression [3], and both can be seen during evolution from premalignant to malignant state [31]. Their dichotomous behavior is based on the oncogenic context, tumor stage and microenvironment; therefore, even the same family member can act as tumor promoter or suppressor in different scenarios [3,11,20]. In most cellular contexts, ligand-dependent forward signaling seems to suppress growth and migration/invasiveness through the inhibition pathways such as the Ras/Erk and Akt/mToR [6,46–49], or the activation of pathways such as the Abl/Crk [50]. In contrast, ligand-independent signaling promotes tumor proliferation, invasion and metastasis through crosstalk with other surface receptors or signal transduction pathways [3,11,20].

2. Breast cancer heterogeneity

The biggest challenge towards assessing prognosis and designing effective treatments in the era of personalized medicine is that breast cancer appears heterogeneous either among distinct individuals (intertumor heterogeneity) or even within each single tumor (intratumor heterogeneity) thus representing a collection of different cancers rather than a single disease [51–54]. Both intertumor and intratumor heterogeneity can be studied at morphologic, molecular and clinical behavior levels [51–54].

Intertumor heterogeneity at the morphologic level is best shown in the latest WHO Classification: breast neoplasms include various histologic types such as the invasive carcinoma of no special type (the most common of all) and the invasive lobular carcinoma (second most common), as well as diverse precursor lesions, non-epithelial neoplasms and metastases [55]. Invasive breast cancers are typically graded, and the most common grading system used worldwide is the “Nottingham Histologic Index” that groups breast cancers in three grades (I, II, III) based on the extent of gland formation, nuclear atypia and mitotic activity [56]. It is now obvious that, even in breast cancers of the same histologic type (e.g. invasive carcinoma of no special type), morphologic heterogeneity could still be present and “uglier” cases of higher grade are associated with more aggressive prognosis [55,56].

At the molecular level, intertumor heterogeneity is highlighted by the existence of four main intrinsic breast cancer subtypes that result in different prognostic outcomes: luminal A, luminal B, human epidermal growth factor receptor 2 (HER2)-enriched, and basal-like breast cancers (BLBCs) [57–59]. BLBCs generally come with a worse prognosis compared to the most common luminal subtypes [57,58,60,61]. Intrasubtype intertumor heterogeneity also exists and BLBCs are the most typical example [59,61–65]. Of interest, several research groups have lately described another intrinsic molecular subtype, the claudin-low; cancers of this subtype are characterized by enriched stem cell and epithelial-mesenchymal transition (EMT) features, high residual disease rates after treatment and ominous prognosis [64,66,67]. Multigene-based biomarker assays (e.g. Prosigna/PAM50, BluePrint and MammaTyper) have been on the market and can allocate breast cancer patients to each of the four aforementioned subtypes, albeit with limitations and moderate agreement with one another [68,69]. However, as such assays, despite their higher accuracy, are not available to most breast cancer patients, the latest two St. Gallen Consensus Meetings propose the simplified use of four surrogate immunohistochemical biomarkers (Estrogen Receptor (ER), Progesterone Receptor (PR), HER2, and the proliferative marker Ki-67) to form immunohistochemistry (IHC)-based clinical groups that attempt to recapitulate the four intrinsic subtypes [61,70,71]. These groups guide oncologists to provide evidence-based care to their patients, stratify them into risk categories, assess their risk of recurrence/metastasis and predict their response to targeted therapies: hormone positive patients can be treated by anti-estrogens such as the tamoxifen (with the addition of chemotherapy in case of high Ki-67 or high histologic grade), HER2 positive patients with the antibody trastuzumab plus chemotherapy, and triple negative breast cancer patients (TNBC; ER, PR and HER2 negative) with chemotherapy alone [61,70,71]. TNBCs comprise around 15% of these IHC-based groups and is associated with young age, African American women, germline *BRCA1* and *BRCA2* mutations and dismal prognosis [60,72,73]. Around 80% of TNBCs are classified as BLBCs or claudin-low while the remaining 20% as either luminal or HER2-enriched breast cancers [64]. In a similar mode, while most BLBCs are classified as TNBCs, some of them could be hormone or HER2 positive [61,63,64].

Breast cancer intratumor heterogeneity at the morphologic and molecular levels can be highlighted by mixed tumor histology (e.g. invasive carcinomas of no special type and mucinous or lobular features; metaplastic carcinomas), also by ER, PR and/or HER2 discordant IHC distribution among the cells of a primary tumor or between a primary tumor and its metastatic lesion(s) [51,74–76]. Spatial intratumor heterogeneity refers to the distinct features of various cell populations (tumor cells; microenvironment) found in an individual tumor or its synchronous metastatic site. Temporal intratumor heterogeneity assesses cancer evolution over time or due to therapeutic response and includes differences between primary tumor and metachronous metastatic sites, also evolution from *in situ* to invasive lesions [51,53]. Intratumor heterogeneity areas of interest include the study of cancer stem cells, alterations of immune cells, endothelial cells and fibroblasts along with their interaction with tumor cells, the process of angiogenesis and the significance of circulating tumor cells (CTCs) [51,52,77–83].

Regarding heterogeneity at the clinical level, variations between different tumors or within an individual tumor can lead to different prognostic outcomes and treatment strategies [52,70,71]. Notably, the extent of each cancer – directly associated with prognostic and therapeutic implications – is traditionally assessed with the classic TNM staging system in a population-based approach. This evaluates tumor size (T), lymph node involvement (N) and the presence of distant metastases (M) [84]. Particularly for breast cancer, the latest American Joint Committee on Cancer (AJCC)/TNM system focused on incorporating recent evidence-based research towards a more personalized approach for each breast cancer. The classic TNM system was enriched with the histologic grading and “precision” molecular data discussed above – proliferation rate, immunohistochemistry, multigene-based assays – to formulate five prognostic groups: 0 (reserved for *in situ* breast cancers), I, II, III and IV [85].

3. The Eph/ephrin system: focus on breast cancer heterogeneity

Based on the information from the previous chapter, we conclude that any heterogeneity at each of the aforementioned levels leads to different breast cancers accompanied by diverse prognosis and management strategies. Consequently, doctors are in constant search for new biomarkers to assess prognosis and guide targeted treatment for this highly heterogeneous disease.

Several groups have described deregulations of the Eph/ephrin system in breast cancer using cell lines, xenograft and mutant animal models besides human tissue samples. They have also correlated aberrant expression levels of various family members with poor prognosis; EphA2, EphB4 and EphB6 are the members most extensively studied.