EDITORIAL

Microenvironment and endocrine resistance in breast cancer: Friend or foe?

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Abstract

Breast cancer affects one in eight women around the world. Seventy five percent of these patients have tumors that are estrogen receptor positive and as a consequence receive endocrine therapy. However, about one third eventually develop resistance and cancer reappears. In the last decade our vision of cancer has evolved to consider it more of a tissue-related disease than a cell-centered one. This editorial argues that we are only starting to understand the role the tumor microenvironment plays in therapy resistance in breast cancer. The development of new therapeutic strategies that target the microenvironment will come when we clearly understand this extremely complicated scenario. As such, and as a scientific community, we have extremely challenging work ahead. We share our views regarding these matters.

Key words: Breast cancer; Tumor microenvironment; Endocrine resistance; Tamoxifen; Stroma; Estrogen receptor; Aromatase inhibitors; Cancer stem cells

Core tip: Resistance to endocrine therapy in breast cancer is an important clinical problem that requires further insight to develop a solution. We here discuss a paradigm shift, where the interplay of the tumor cells with the microenvironment, and the role of cancer stem cells are discussed as key targets in the development of novel therapeutic strategies.

TEXT

Breast cancer is the most frequent cancer in women in the Western world and one of the main causes of death. Seventy five percent of breast cancer patients have estrogen receptor-alpha (ERα) positive tumors and endocrine therapy is the adjuvant treatment of choice in this scenario. However, a high percentage of patients develop resistance and cancer reappears; up to one third of patients recur within 15 years of the initial diagnosis[1]. Resistance to endocrine therapy is considered as *de novo* when there is no primary response to treatment. However, when therapy is initially successful but cancer eventually recurs, endocrine resistance is considered as acquired[2].

The most widely used endocrine therapy for breast cancer patients has been tamoxifen, a selective ER modulator. Tamoxifen was developed in the 70's and is considered the first targeted therapy for cancer, as it specifically targets the ER[3]. Other endocrine treatments include selective ER down modulators such as Fulvestrant and aromatase inhibitors like Letrozole and Anastrozole[4].

WHY DOES ENDOCRINE RESISTANCE DEVELOP?

A number of mechanisms have been proposed as responsible for inducing acquired tamoxifen resistance. In particular a great number of papers have historically dealt with alterations in growth factor receptor pathways, in particular the HER family of growth factor receptors, as the responsible for this phenomenon[5]. A quick search in PubMed while we are writing this editorial shows that when we use the key words "breast cancer and tamoxifen resistance" we find 1869 publications; if the word "growth factor" is added, the number is reduced to 503 and "Her-2" leads to 236. However, if we look into other plausible mechanisms very few papers are found: For example adding the word "microenvironment" leads to 17 citations, "inflammation" accounts for 4, "integrins" 4, "stroma" 15, "fibroblasts" 40 and "stem cells" leads to 40. However, when we look at the first publications related to these topics we find that the first paper listed in PubMed related to growth factors and tamoxifen resistance was published in 1988[6], whereas "stem cells", for example, dates to 1985[7]. So evidently researchers have been thinking about other mechanisms but probably the means to carry out these investigations were not available, or very few researchers thought that mechanisms other than autocrine loops within the tumor cell population could be responsible for the progression of the disease. We know today, however, that tumors are not only composed of neoplastic cells themselves, but that other cell types and extracellular components are critical both to tumor progression and response to treatment[8]. Thus, considering these as key players in the development of endocrine resistance is critical. The following paragraphs aim at highlighting some of the main findings related to endocrine resistance through mechanisms that need extensive research to lead us to the development of novel strategies for the treatment of breast cancer.

INFLAMMATION AND ENDOCRINE RESISTANCE

A growing body of evidence supports the role of the immune system as a regulator of tumor development and dissemination. Infiltrating immune cells produce cytokines, proteinases, chemokines and growth factors that promote extracellular matrix remodeling and angiogenesis. In particular gene-profiling studies have linked inflammation related gene clusters to resistance in patients treated with tamoxifen[9] and the aromatase inhibitor Anastrozole[10]. Moreover, a number of cytokines have been associated to suppression of ERα in breast cancer cells such as TNF, IL1β, IL6 and amphiregulin[11]; ER negative tumors are associated to more aggressive and invasive phenotypes. Epithelial to mesenchymal transition can be induced by factors such as IL6 with the upregulation of stem-related transcription factors[12,13]. Moreover, increased IL6 serum levels are correlated with decreased response to endocrine therapy in breast cancer and poorer survival[14,15]. In the ERα negative scenario IL1β is correlated with increased invasiveness and poor prognosis[16]. In ERα expressing breast tumors, IL1β has been shown to activate ER's transcriptional activity[17,18] and to modulate the response to 4-OH-tamoxifen; in particular in the presence of IL1β tamoxifen acts as an agonist instead of an antagonist[19]. The SDF-1-CXCR4 axis has also been implicated in breast tumor progression[20,21]. CXCR4 overexpression is correlated with worse prognosis and decreased survival in both the ER positive and negative scenario[21,22]. Moreover, using MCF-7 cells, treatment with SDF-1 induced tamoxifen and fulvestrant resistance in cells overexpressing CXCR4[21].

Tumor associated macrophages (TAMs) are associated to increased angiogenesis and survival[23]. Results from experimental models in mice suggest that TAMs are key players in the progression to metastasis[24]. Moreover, experiments suggest that TAMs produce estrogens that directly stimulate the proliferation of ERα positive breast cancer cells[25]. CD68, a macrophage marker, has been associated to increased recurrence suggesting that in ER positive breast cancers the presence of macrophages may lead to endocrine resistance[26].

STEM CELLS AND ENDOCRINE RESISTANCE

Cancer stem cells have gained attention in the last years as responsible for tumor progression and resistance to therapy[27]. Experiments carried out using human samples have clearly shown that both chemo

and radiotherapy increase the percentage of breast cancer stem cells in a neo-adjuvant setting[28]. In the context of endocrine therapy our results together with those of other groups strongly suggest that endocrine treatment leads to enrichment in breast cancer stem cells. Our working hypothesis, based on the literature and our results, is that breast cancer stem cells express reduced levels or no ER-α, and would thus not be efficiently targeted by endocrine treatment[29]. A study testing the effect of neo-adjuvant treatment in patients with Letrozole shows that it leads to enrichment in cells with mammosphere forming capacity[30]. Simões *et al*[31] analyzed the impact of estrogen signaling on MCF-7 mammosphere forming capacity. They plated MCF-7 cells straight onto nonadherent plates and treated the suspension cultures with hormones finding that estradiol decreased, and 4-OH-tamoxifen increased mammosphere forming capacity. The same results were true for suspension cultures of primary human normal and tumor breast cell suspensions, where treatment with 4-OH-tamoxifen led to an increase in Nanog and Sox-2. Ao *et al*[32] on the other hand treated suspension cultures with 4-OH-tamoxifen and then passaged the cells to media without antiestrogen (still in suspension) and found that under these conditions a greater amount of mammospheres were formed. We showed that tamoxifen selects for cells with stem cell properties in the human MCF-7 cells line, as well as in mouse LM05-E cells and the M05 tumor from which they derive[33]. Mammosphere assays revealed that pretreatment of either cell line with 4-OH-tamoxifen leads to an increase in cells with increased clonogenicity in suspension. Additionally, we analyzed the gene expression of transcription factors associated to pluripotency and found that they were increased both in the mammospheres and in cells growing on 2D treated with 4-OH-tamoxifen for 5 d. *In vivo* studies using the M05 tumor showed similar results with an increase in the amount of cells with mammosphere forming capacity in tumors derived from mice treated with tamoxifen containing pellets. These tumors were enriched in CD29^h/CD24^l cells, in comparison to the parental tumor. Additionally, when passaged to untreated mice, those tumors that derived from mice that had been previously exposed to tamoxifen generated "secondary tumors" that grew at a faster rate compared to controls, and had a higher capacity of giving rise to mammospheres as well as maintaining an increased CD29^h/CD24^l cell population. Finally, M05 tumor passages that had progressed to hormone independence had a higher amount of cells with mammosphere forming capacity supporting the notion that increased aggressiveness and endocrine independence are correlated with an increase in cells with stem cell properties[33]. These results, in conjunction, strongly suggest that breast cancer stem cells are involved in endocrine resistance.

THE EXTRACELLULAR MATRIX, CANCER ASSOCIATED FIBROBLASTS AND ENDOCRINE RESISTANCE

Cancer associated fibroblasts have long been believed to play a key role in cancer progression[34]. In breast cancer in particular, several lines of evidence strongly suggest that they are vital in determining tumor progression and the outcome of therapy[35]. A seminal paper by Finak *et al*[36] identified distinct stromal signatures that corresponded to good and poor-outcome breast cancers. They were able to identify a 26 gene predictor that forecasted disease outcome with higher precision than predictors or signatures derived from whole tissues. In this line of evidence, but in the context of endocrine resistance, an extracellular matrix gene cluster has been associated to prognosis and response to treatment[37]. In particular they found that fibronectin, lysyl oxidase, SPARC and TIMP3 expression levels were associated to the prognosis of patients with breast cancer whereas levels of tenascin C were associated to resistance to treatment with tamoxifen. A recent paper by Holton *et al*[38] using 3D cultures and Fourier transform infrared spectroscopic imaging shows that fibroblasts induce epithelial to mesenchymal transition in cancer cells together with a downregulation in ERα levels. Our work also suggests that stromal factors modulate response to endocrine resistance. In particular we showed that conditioned media derived from carcinoma associated fibroblasts induced tamoxifen resistance in otherwise sensitive cells, using a mouse model of estrogen dependent breast cancer[39,40]. Moreover, we found that fibronectin, which is mostly produced by fibroblasts in breast tumors, induces resistance in both LM05-E and MCF-7 cells. This effect is accompanied by an induction in ERα phosphorylation at serine-118. Interestingly, high levels of phospho-serine-118 has been previously associated to endocrine resistance in breast cancer[41].

BACK TO THE BEGINNING

The examples above are just a snapshot of the recent findings regarding endocrine resistance and microenvironment. So is the microenvironment a friend or a foe in this context? Clearly I believe that we are just beginning to unravel the complexity of cancer and that evidently there is no single culprit to failure when it comes to treatment. However, we definitely need a lot of work to be carried out to understand exactly what the role of each player is in this complicated challenge. Moreover, the work that needs to be done is extremely delicate given that it implies analyzing and understanding how tumor and tumor "associated" cells behave in different contexts. This type of work is time consuming, needs to be carried out in different model systems and is very expensive. The challenge I believe

is patience. The other key point here is that researchers that study different types of cancer need to interact more and companies must be willing to openly share new chemicals when requested even though they are not being developed for the purpose the scientist asking is wanting to explore.

The road ahead is exciting and may result frustrating at times. We are just beginning to understand that the microenvironment plays a key role in endocrine resistance in breast cancer. The numbers in PubMed are clear evidence that we are in the dawn of our understanding in this matter. As the poet Robert Frost would say, we are taking the road less travelled, and that should make the difference.

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