Adaptive Immune Responses and HER2/neu-Positive Breast Cancer

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Abstract Oncogenic signaling, such as HER2/neu signaling, has been shown to play a major role in tumorigenesis in a subset of breast cancer patients. The use of anti-HER2/neu antibody has not only revealed the mechanisms for HER2/neu signaling, but has also shown a therapeutic advantage of its blockade. The use of trastuzumab has greatly improved the treatment of HER2-positive breast cancer. Although this therapy has been used in the clinic for over 20 years, recent data are still uncovering new mechanisms by which this antibody exerts its antitumor activity. In addition to improved understanding of the molecular mechanisms by which this therapy inhibits growth of tumor cells, it has been discovered that anti-HER2/neu therapy initiates and requires the adaptive immune system. The presence of anti-HER2/neu-initiated adaptive immunity gives credence to efforts targeted at stimulating the immune system in treating HER2-positive breast cancer. This review focuses on the role of the inflammatory response in HER2-positive breast cancer with particular emphasis on trastuzumab therapy.

Keywords Oncogenic signaling · HER2/neu · Adaptive immunity · Breast cancer · Antibody therapies

Introduction

The use of antibody therapies has changed the landscape for treating multiple cancer types. Over the past 12 years, the FDA has approved multiple antibodies for use against human malignancies, establishing this therapy to be effective in treating cancer. In fact, antibody-based therapeutics are now essential components of many cancer treatment regimens [1]. Moreover, the success these therapies are having in the clinic is driving further development of novel immunotherapies [2]. Among the oncoproteins against which antibodies have been generated, human epidermal growth receptor factor 2 (HER2/neu/ErbB2) is among the most targeted, with two molecular targeting agents approved by the FDA [3].

The HER2 oncogene is the human homologue of the rat neu oncogene. First identified in DNA from ethylnitrosourea-induced neuroglioblastomas in rats, HER2/neu is a 185-kDa protein with homology to epidermal growth factor receptor [4–6]. Along with HER3 (ErbB3) and HER4 (ErbB4), these proteins constitute the type 1 growth factor receptor family [7]. These transmembrane proteins are receptor tyrosine kinases that have intrinsic kinase activity and are activated when ligands bind and promote homodimerization or heterodimerization [7, 8]. However, no specific ligand for HER2/neu (ErbB2) has been identified [8, 9]. When normally expressed, heterodimerization of HER2/neu induces downstream signals through the Akt and mitogen-activated protein kinase pathways. Signaling through these receptors supports many physiological processes, including embryogenesis, cell proliferation, differentiation, adhesion, motility, and apoptosis [10, 11].

Studies using monoclonal antibodies specific for the HER2/neu protein revealed that targeting this surface protein could block oncogenic signaling and inhibit tumor

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growth. In vitro treatment of neu-transformed fibroblasts or a human mammary gland adenocarcinoma cell line with an anti-neu antibody (clones 7.16.4 and 4D5, respectively) resulted in downregulation of surface neu protein, and growth inhibition [12, 13]. When used to target xenografts of human tumors in nude mice, anti-neu antibody therapy inhibited tumor growth in vivo [14]. Early studies also demonstrated overexpression of HER2/neu provided transformed cells with resistance to TNF α -mediated tumor inhibition in vitro [15]. However, treatment of these cells with anti-HER2/neu antibody countered the resistance to TNF α and induced tumor cell death [13].

Owing to the accessibility of HER2/neu at the cell membrane, and its low expression in normal tissues and overexpression in a high percentage of tumors, this protein was an ideal candidate for immunotherapeutic intervention [16]. Trastuzumab is a humanized monoclonal antibody that was engineered by inserting the complementary regions of the murine antibody (clone 4D5) into a human IgG1 framework [17, 18]. Since its development, trastuzumab has been tested in several clinical trials and has proved to be an effective adjuvant therapy for HER2/neu-positive metastatic breast cancers [19]. In 1998, the FDA approved trastuzumab (Herceptin) for use in treating human breast cancer patients. It was the first monoclonal antibody to be approved for treating solid tumors. The therapeutic activity of trastuzumab has been evaluated in women with metastatic breast cancer as a single agent given before or after traditional chemotherapy, and in combination with a variety of chemotherapy agents [11, 20–22]. The first phase II trials demonstrated objective response rates from 12 to 15 % [22, 23]. These studies established that trastuzumab therapy can be effective in patients [24]. On the basis of the convincing preclinical studies, clinical trials were conducted and demonstrated the benefits of combining chemotherapy with trastuzumab therapy [24–26]; one such study enrolled women who had not received previous adjuvant therapy to examine the combination of trastuzumab therapy and chemotherapy [20, 24]. The addition of trastuzumab therapy to chemotherapy was associated with a longer time to disease progression, a higher objective response rate, and longer survival [20]. This study was instrumental, and on the basis of these results, the FDA approved trastuzumab, given either alone or in combination with chemotherapy, for treating patients with metastatic breast cancer overexpressing HER2/neu [1, 9].

Immune Responses to Breast Cancer

Multiple studies have been published regarding the mechanisms by which anti-HER2/neu therapy inhibits tumor

growth. Anti-HER2/neu therapy has both direct and indirect effects that ultimately lead to tumor cell death. The direct effects include diminishing cell signaling, induction of cell cycle arrest, inhibition of receptor shedding, and when combined with chemotherapy, inhibition of DNA repair. The indirect effects involve inhibition of angiogenesis and inflammatory cell engagement. These multiple effects are due to the ability of anti-HER2/neu to prevent HER2/neu dimerization while the effector Fc arm of the antibody engages Fc receptor (FcR)-positive inflammatory cells, such as natural killer (NK) cells and macrophages. In fact, FcR-positive cells were shown to be essential for mediating the therapeutic effects of the anti-HER2/neu antibody [27]. A study by Clynes et al. [27] demonstrated that the therapeutic effect of the anti-HER2/neu antibody was significantly reduced in *Fc γ R*-knockout mice. In addition, when a mutation was made in the CH2 domain of the mouse IgG2 heavy chain to inhibit FcR binding, anti-HER2/neu maintained an ability to inhibit tumor growth in vitro, but did not induce antibody-dependent cellular cytotoxicity (ADCC) by NK effector cells. Although Fc-mediated mechanisms do not exclude other mechanisms of action by anti-HER2/neu, these data promoted ADCC as the major mechanism for the in vivo effects of anti-HER2/neu therapy. Over the past few years, the direct effect anti-HER2/neu therapy has on HER2-positive tumors has been reviewed extensively [18, 28]. Therefore, this review will focus on new evidence for how anti-HER2/neu therapy induces an adaptive immune response.

Understanding the role of adaptive immunity in HER2-positive breast cancer has been an important area of study for many years. For example, in the early 1990s, many studies demonstrated that both humoral and adaptive immune responses against HER2/neu are present in HER2/neu-positive breast cancer patients [29]. However, how anti-HER2/neu therapy induces this response and if adaptive immunity was even necessary was largely underappreciated. This was due in part to the overwhelming evidence that anti-HER2/neu therapy exerts multiple direct effects on the tumor, and these mechanisms were observed and supported by in vivo evidence [24]. One caveat to many of these studies, however, was the use of xenografting of human tumors into nude mice. As a result, the role of adaptive immunity in anti-HER2/neu therapy was underexplored.

Using an immunocompetent mouse model, we recently demonstrated that adaptive immunity and T cells are necessary for tumor reduction by anti-HER2/neu therapy alone [30•]. Wild-type BALB/c mice were inoculated with a syngeneic HER2/neu-positive breast cancer cell line and treated with a mouse anti-HER2/neu antibody. The critical role of adaptive immunity was supported by the observation that the efficacy of anti-HER2/neu therapy was greatly reduced in *Rag1*-knockout mice that lack adaptive

immunity. Anti-HER2/neu therapy had some effect in *Rag1*-knockout mice, suggesting that the direct effects of anti-HER2/neu therapy were intact, but the overall antitumor effect was significantly reduced in these mice. To explore the cell types involved, wild-type BALB/c mice depleted of CD8⁺ T cells were unable to completely respond to anti-neu therapy, and this CD8 dependency was recapitulated in tolerized neu transgenic mice. Moreover, wild-type BALB/c mice with undetectable tumors for greater than 30 days after anti-HER2/neu therapy were subsequently resistant to a high-dose tumor rechallenge, suggesting the presence of immune memory. Together these data suggest that, in addition to the direct effects anti-HER2/neu therapy has on inhibiting tumor growth, this treatment also induces and requires an adaptive immune response.

We also investigated the inflammatory events leading to the induction of adaptive immunity after anti-HER2/neu therapy. Stimulation of Toll-like receptors (TLRs) via the MyD88 pathway is an important means by which antigen-presenting cells are activated and respond to foreign pathogens [31]. Accordingly, the antitumor effect of anti-HER2/neu therapy was abolished in MyD88-deficient mice. Typically, TLRs are activated via pathogen-associated molecular patterns from bacteria and viruses. However, some self-ligands associated with tissue damage, such as high mobility group box 1 (HMGB1), are also capable of activating TLRs to increase cross-priming and activation of dendritic cells in both mice and humans [32, 33]. Neutralization of HMGB1 alone greatly reduced the efficacy of anti-HER2/neu therapy. These data indicate that HMGB1, an endogenous danger signal, is essential for antibody-mediated tumor regression, and it is conceivable that anti-HER2/neu antibody induces HMGB1 release in the tumor microenvironment, enhancing innate responses via the MyD88 pathway.

These observations were extended and confirmed by work from Smyth's group [34•], who—through the use of another HER2/neu-dependent tumor model and adoptive cell transfer experiments with CD8 and interferon- γ knockout mice—demonstrated that not only were CD8⁺ cells essential, but interferon- γ from CD8⁺ cells was required for the antitumor effect of anti-neu therapy. Although this study also demonstrated that anti-HER2/neu therapy was dependent on TLR signaling through MyD88, it established the necessity of NK cells and the additional role of type I interferons. Taken together, these two studies suggest that NK cells induce ADCC after engaging the anti-HER2/neu antibody to promote the release of HMGB-1, stimulate MyD88-dependent TLRs, and thereby cause the release of type I interferons to prime the adaptive immune system (Fig. 1).

Pertuzumab is a humanized monoclonal antibody that binds extracellular domain II of the HER2 receptor, and has been effective in treating trastuzumab-resistant tumors when given in combination with trastuzumab [35]. The

binding site of pertuzumab differs from that of trastuzumab, which binds extracellular domain IV, but it nonetheless shares many of the same mechanisms of action [35]. Because pertuzumab can induce ADCC, it follows that induction of an adaptive immune response would be possible. It remains to be determined, however, if adaptive immunity is necessary for pertuzumab therapy.

Although there is still much to learn about how anti-HER2/neu therapy inhibits tumorigenesis and how to combine it with standard care—be it radiation, chemotherapy, or surgery—these studies identified a new mechanism of action for anti-HER2/neu therapy and support continued efforts aimed at harnessing this response for enhanced tumor reduction.

Immunotherapy for Breast Cancer

Prior to our study, other researchers had been attempting vaccination, regulatory T cell depletion, and adoptive cell transfer; but these immunotherapies have had limited and various effects. Despite a measurable immune response, increased numbers of tumor-specific CD8⁺ T cells do not always lead to tumor reduction. Furthermore, recognition of the tumor antigen alone is not sufficient for the host to eradicate established solid tumors [36–38]. Thus, natural immune responses fail to clear tumor cells and immune tolerance may occur throughout tumor progression. Several factors contribute to this dampened immune response. First, poor direct or indirect antigen presentation in lymphoid tissues reduces early T cell priming. This may be due to inadequate numbers of tumor cells migrating to the lymph node, low expression of MHC class I on tumor cells, or low expression of costimulatory molecules on antigen-presenting cells. Second, physical (e.g., hydrostatic pressure) and biological (poor adhesion molecule activation) barriers around tumor tissues can cause an inadequate number of immune cells to migrate into and though the tumor [39, 40]. Third, suppressive cell types residing within the tumor microenvironment can induce anergy or deletion of infiltrating T cells to promote tumor growth [41]. Fourth, release of soluble factors such as IL-10, transforming growth factor β , IL-17, and vascular endothelial growth factor by the tumor or the surrounding stroma can inhibit T cell function or promote angiogenesis [38, 42]. Finally, immune tolerance and/or suppression can be induced by the tumor itself though the expression of the inhibitory molecule PD-L1.

Combination Therapies

Owing to the multitude of factors limiting the immune response, the need for therapies that reactivate the immune

Fig. 1 Anti-HER2/neu therapy induces an adaptive immune response. Tumor cell death induced by oncogenic signal blockade and antibody-dependent cellular cytotoxicity upon binding of anti-Her2/neu to the Her2 receptor induces release of danger signals, such as high mobility group box 1 (HMGB1), from apoptotic or stressed tumor cells (A). HMGB1 is recognized alone or in complex with other molecules, such as single-stranded DNA, to activate dendritic cells and promote cytokine production via activation of the MyD88

pathway (B). MyD88 signaling enhances cross-presentation and activates dendritic cells and also induces the release of type I interferons (IFN) (C). These dendritic cells can either remain in the tumor or travel to the lymph node and prime CD4⁺ and CD8⁺ T cells of the adaptive immune system (D). Primed T cells leave the lymph node and travel back to the tumor. FcR Fc receptor. NK natural killer cell

system is imperative. Because targeted therapies such as anti-HER2/neu therapy can hasten tumor reduction and reduce tumor immune suppression, they may also reset the microenvironment and open a window of opportunity for immunotherapies [43•]. Indeed, immunotherapy can lead to the reduction or even eradication of some established human tumors, but these responses are only achieved in a minority of patients [37, 44, 45]. However, monotherapies are unlikely to overcome the multitude of suppressive mechanisms that inhibit antitumor immunity in all patients [43•]. Thus, new strategies and protocols are critically needed for treating most cancer patients.

Among the recent strategies has been the combination of anti-HER2/neu therapy with immunomodulatory therapies. Typically this involves combining monoclonal antibody therapy with tyrosine kinase inhibitors. However, some studies are focused on altering the adaptive immune system. One such strategy has been the combination of anti-HER2/neu therapy with an agonistic anti-CD137 antibody or with blocking PD-1. CD137 mediates lymphocyte survival, and can enhance the function of CD8⁺ T cells, NK cells, and dendritic cells, whereas PD-1 is an inhibitory receptor expressed on activated and anergic T cells. Thus, combining either of these therapies with anti-HER2/neu therapy may have synergistic effects involving both adaptive and innate immunity. Indeed, Stagg et al. [34••] demonstrated that addition of an agonistic anti-CD137 or anti-PD-1 blocking antibody significantly enhanced the efficacy of anti-HER2/

neu therapy when administered to both tumor-bearing wild-type mice and neu transgenic mice. In a similar fashion, Kohrt et al. [46] recently reported that CD137 expression is increased on human NK cells after anti-HER2/neu therapy, and selectively activating this protein with an agonistic anti-CD137 antibody enhanced the efficacy of anti-HER2/neu therapy in a xenograft model. Transplanting human tumors into athymic mice allowed these researchers to identify how additional anti-CD137 treatment enhances ADCC; however, how activating signals through anti-CD137 therapy enhances adaptive immune responses still requires further elucidation.

In our laboratory, we have focused on the immunostimulatory properties of LIGHT. Expression of LIGHT, a TNF family member, within the tumor microenvironment can attract various immune cells, including substantial numbers of FcR⁺ cells, dendritic cells, and T cells [47]. In the context of HER2/neu-positive breast cancer, targeting tumors with an adenovirus expressing LIGHT enhanced the efficacy of anti-HER2/neu therapy [30••]. Moreover, tumor-free mice after combination therapy were resistant to a lethal rechallenge of HER2-positive tumor cells but not HER2-negative cells. These data suggest that expression of LIGHT works by enhancing the adaptive immune response and promoting antigen-specific memory. Together, these preclinical studies support further effort aimed at enhancing the adaptive immune response initiated by anti-HER2/neu therapy.

Antibody–Drug Conjugates

Given the harsh side effects of chemotherapy, approaches that selectively target cytotoxic agents to cancer cells are an attractive strategy. This approach is very promising in the treatment of HER2/neu-positive cancer. In particular, trastuzumab–emtansine (T-DM1), an antibody–drug conjugate which has a chemotherapeutic agent linked to trastuzumab, has proven to be effective in treating tumors that had progressed on previous therapies, including trastuzumab therapy [48]. We have demonstrated that timing of chemotherapeutic administration can have different effects on the immune response initiated by anti-HER2/neu therapy [30••]. It will be important, therefore, to determine whether and to what extent trastuzumab–emtansine induces an immune response.

Conclusion

The discovery of the association between HER2/neu overexpression and breast cancer prognosis has promoted a succession of therapies aimed at exploiting this tumor oncoantigen, and the introduction of anti-HER2/neu antibody therapy has revolutionized the treatment of HER2-positive breast cancer. Since approval of trastuzumab by the FDA, many studies have sought to understand the mechanisms by which this therapy reduces tumor burden. The recent identification that the adaptive immune response is necessary for mediating the effects of this antibody treatment has furthered the understanding of anti-HER2/neu therapy and given credence to studies testing immunotherapies to fight HER2/neu-positive breast cancer. Further understanding the mechanisms behind current therapies, such as anti-HER2/neu antibody therapy, will benefit the development of these strategies.

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Conflict of interest No potential conflicts of interest relevant to this article were reported.

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- Of importance
- Of major importance

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