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REVIEW

Adaptive Immune Responses Associated with Breast Cancer Relapse

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Abstract The generation, survival, and differentiation of breast cancer stem cells (BCSC) in immunocompetent hosts remain elusive. Some investigators have shown that BCSC can be induced from epithelial tumor cells by the pathologic epithelial to mesenchymal transition (EMT). Emerging evidence suggests that the induction of EMT among epithelial tumor cells originates from signals produced by the non-tumor cells that constitute the tumor microenvironment, including the immune effectors that infiltrate the tumors. Thus, this suggests that the immune system not only has anti-tumor function, but also paradoxically immunoedits tumors, facilitating tumor escape and progression. Indeed, many studies in human breast cancers show both positive and negative associations between the infiltration of various immune effectors (e.g., CD4 and CD8 T cells) and the propensity to relapse with metastatic disease. These observations suggest that distinct types of immune effector cells may induce or inhibit tumor relapse. This review focuses on recent advances in identifying components of the immune system that may directly induce tumor escape and relapse. We propose that levels of interferon (IFN)-γ production or levels of the expression of IFN-γ receptor α on tumor cells may determine whether tumor inhibitory or relapse-promoting effect of IFN-γ may prevail.

Keywords Breast cancer stem cells · Relapse · Her-2/neu · IFN-γ · Immunoediting · Tumor escape

Abbreviations

EMT	Epithelial to mesenchymal transition
BCSC	Breast cancer stem cells
CTL	Cytotoxic T cells
MMC	Mouse mammary carcinoma
DCIS	Ductal carcinoma in situ
IRF	IFN-γ regulatory factor

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Cancer Immunotherapy and a Double-Edged Function of IFN-γ

A body of experimental and clinical evidence has emerged over the last decade showing that the immune system can prevent the development of cancers (Bui and Schreiber 2007; Dunn et al. 2002; Ljunggren 2008; Sauer et al. 2008). This one-sided view has recently been challenged. Two relevant publications are worth mentioning from the early papers on this topic. Daniel et al. (2003) reported that HPV16 transgenic mice deficient in $CD4^+$ cells had a delay in tumor progression, albeit small, associated with decreased inflammation. These findings suggest a role for $CD4^+$ T cells in tumor progression, an observation that makes sense from the perspective of $CD4^+$ T cell or NKT cell contribution in inflammatory responses. In a second relevant study, tumor-specific $CD8^+$ T cells were shown to cause mutation in cytotoxic T cell (CTL) epitopes within the tumor antigen that led to tumor progression (Singh and Paterson 2007). In recent years, there has been more compelling evidence in both pre-clinical and clinical studies that challenge the one-sided view on the role of interferon (IFN)-γ producing T cells in protecting the host against cancers.

Pre-Clinical Studies

CTL adoptive immunotherapy has been reported to mediate tumor regression and tumor escape concurrently (Liu

et al. 2006). For example, IFN-γ was shown to promote immunoediting and subsequent tumor escape in the CT26 colon carcinoma by down-regulation of the expression of gp70 immunogenic tumor antigen (Beatty and Paterson 2000). Berner et al. (2007) showed that tumor-specific IFN-γ was essential for successful initial immunotherapy, but it also impaired subsequent secondary or durable anti-tumor immune responses. Farrar et al. (1999) showed that IFN-γ-producing T cells can establish and maintain cancer dormancy. Along this line of evidence, Wu et al. (2011) showed that a highly aggressive variant of the UV-induced 4102 tumor cells was resistant to IFN-γ in spite of the expression of MHC class I molecule and an intact STAT-1 phosphorylation. In fact, progression of cancer from indolent to aggressive tumors occurred in the presence of IFN-γ-inducible genes (Wu et al. 2011). Romieu-Mourez et al. (2010) also showed that treatment of HER-2/neu positive cells with IFN-γ resulted in the suppression of protective anti-tumor immune responses. We reported that immunotherapy of rat neu overexpressing mouse mammary carcinoma (MMC) elicited neu-specific IFN-γ-producing $CD8^+$ T cells that in turn facilitated breast cancer recurrence of neu antigen negative variant tumors following initial rejection of MMC tumor cells in immunocompetent mice (Kmieciak et al. 2007; Worschech et al. 2008). The tumor antigen loss was due to hypermethylation of the neu promoter and loss of neu both at mRNA and protein levels (Kmieciak et al. 2007; Santisteban et al. 2009), resulting in escape of the tumor from further neu-specific immune responses. It is likely that DNA methylation may also impact HER-2/neu gene amplification in humans, as suggested by others (Terada et al. 2009). Subsequent studies showed that such tumor-editing function of $CD8^+$ T cells induced epithelial to mesenchymal transition (EMT) of MMC, and as a result induced neu antigen loss and generated breast cancer stem cells (BCSC)-like tumor clones (Santisteban et al. 2009). Recently, it was demonstrated that lack of expression of IFN-γ receptor by forcing the expression of dominant negative IFN-γ receptor reduced the ability of renal carcinoma cells to metastasize (Hall et al. 2007). This may suggest the involvement of IFN-γ/IFN-γ receptor α axis in tumor invasion.

Clinical Studies

HER-2/neu-targeted immunotherapies have been employed against breast cancers where IFN-γ has been used as key marker for the efficacy of anti-tumor T cell responses. However, tumor progression occurs despite the presence of tumor-specific IFN-γ-secreting T cells (Rosenberg et al. 2005). Matkowski and Sheu both showed in cohorts of 88 and 24 patients with operable breast cancer, respectively, that relapse or disease progression was associated with strong $CD8^+$ T cell infiltration (Matkowski et al. 2009; Sheu et al. 2008). In uveal melanoma patients, elevated serum levels of IFN-γ correlate with the spread of metastasis and represent a negative prognostic marker (Hallermalm et al. 2008). A prospective randomized trial showed that tumor relapse in melanoma patients who were immunized with NY-ESO-1 vaccine was associated with down-regulation of NY-ESO-1 and HLA class I in their tumor. This suggests immunoediting and escape of the tumor under pressure from NY-ESO-1-specific immune responses (Nicholaou et al. (2011). In patients with ductal carcinoma in situ (DCIS), HER-2/neu-targeted vaccination induced HER-2/neu-specific IFN-γ-producing T cell responses and resulted in HER-2/neu antigen loss (Czerniecki et al. 2007). Although the authors considered this HER-2/neu loss a positive outcome of the immune response, no follow-up studies have been performed to determine whether patients with HER-2/neu loss in their tumors might end up with recurrence of more invasive tumors. Very recently, we reported that patients with HER-2/neu negative breast cancer had HER-2/neu-specific IFN-γ-producing T cell responses which were associated with nuclear translocation of IFN-γ receptor α in the tumor site (Kmieciak et al. 2011). This suggests that patients with HER-2/neu negative breast cancer might have had undetectable HER-2/neu positive premalignant tumors in the past that had lost HER-2/neu expression and progressed to invasive carcinoma under immune pressure. The fact that 55–75 % of patients with premalignant DCIS overexpress HER-2/neu in their tumor lesions and 75 % of breast cancers are HER-2/neu negative may suggest the progression of HER-2/neu positive DCIS to HER-2/neu negative breast cancer only in the tumor clones that express variable levels IFN-γ receptor α. This possibility is also supported by the observation that overexpression of HER-2/neu in DCIS lesions are usually accompanied by invasive foci (Roses et al. 2009). In fact, the low frequency of HER-2/neu expression (20–25 %) in invasive breast cancer implies that HER-2/neu loss is an epiphenomenon of disease progression (Hoque et al. 2002). Evaluation of HER-2/neu expression in tumor lesions of patients with breast cancer revealed overexpression of HER-2/neu in primary tumors and its loss in synchronous metastasis (Santinelli et al. 2008) as well as gain of HER-2/neu overexpression in metastatic tumors (Houssami et al. 2011). Such discordance between primary and metastatic tumors in the expression of HER-2/neu suggests that HER-2/neu loss in vivo may be associated with distant recurrence or metastasis only in certain tumor clones. In prostate cancer, HER-2/neu positive tumors, DU145 and PC-3, which responded to IFN-γ (probably because of the expression of IFN-γ receptor α), showed down-regulation of HER-2/neu expression, whereas another prostate tumor line, LNCaP, which failed

to respond to IFN-γ did not show any change in the expression of HER-2/neu (Kominsky et al. 2000). Such failure of LNCaP was later shown to be due to the lack of JAK1 gene expression (Dunn et al. 2005).

IFN-γ and Epigenetic Modulation of Cancer

Despite the presence of pre-existing tumor-specific immune responses, breast cancer patients often do not benefit from immunotherapy (e.g., vaccines, antibodies). For example, although trastuzumab (in combination with chemotherapy) prolongs the survival of women with advanced HER-2/neu^{+} breast cancer, the vast majority of women will develop resistance within 1 year of treatment initiation and 15 % of patients are de novo resistant (Pohlmann et al. 2009). Similarly, in the phase II E75 HER-2/neu peptide vaccine studies conducted by Peoples and colleagues, the benefit (i.e., prevention of recurrence) was minimal and nonsignificant despite the generation of high level of HER-2/neu-specific CD8^{+} T cell responses (Holmes et al. 2008). Although the reason for the resistance or lack of benefit is unclear, it could be related to the recent findings of Reim et al. (2009), who showed that trastuzumab associated with IFN-γ-producing NK cells expanded tumorigenic CD44^{high}CD24^{low}HER-2/neu^{low} BCSC in vitro. IFN-γ signal transduction in the target cells, including breast tumors, involves early response genes such as IFN-γ regulatory factor 1 (IRF1) and late IFN-γ negative feedback regulatory responses such as IRF2 (Blanco et al. 2000; Connett et al. 2003; Doherty et al. 2001; Yim et al. 2003). IRF1 induces apoptosis (Harada et al. 1990) and IRF2 is an IFN-γ negative regulatory feedback response that has been shown to render the tumor cells refractory to apoptosis (Taniguchi et al. 2001). IRF2 can repress IRF1-induced transcriptional activation (Harada et al. 1990; Hida et al. 2000). The IRF1 protein is very unstable (half-life, $\sim$30 min), whereas IRF2 protein is apparently stable (half-life, $\sim$8 h) (Watanabe et al. 1991). In addition, evidence has also been provided that IRF2 functions as a transcriptional activator for vascular cell adhesion molecule-1 (VCAM-1) (Jesse et al. 1998). Elevated VCAM-1 is associated with poor prognosis and breast cancer recurrence (Silva et al. 2006) as well as cancer stem cell (CSC) characteristics (Levina et al. 2008). Very recently, IRF2 was shown to support the self-renewal and multilineage differentiation capacity of stem cells (Sato et al. 2009). Oncogenic activity of IRF2 that represses the tumor suppressor activity of IRF1 may also result in the repression of other tumor suppressor genes. For example, it was shown that IFN-γ can interfere with the p53-mediated transactivation of p21 (Koeppel et al. 2009), which may lead to alterations in p53 function. In fact, IFN-γ may contribute to the induction of methylation of several genes that are altered by hypermethylation of their promoter region during tumor relapse. For example, loss of E-cadherin during EMT and silencing of p53 during tumorigenesis have been shown to be associated with DNA methylation (Dumont et al. 2008; Kang et al. 2001). The p53 alteration in turn induces symmetrical division in BCSCs that accelerates their renewal (Cicalese et al. 2009), leading to breast cancer recurrence. We have observed such epigenetic effects of IFN-γ during neu antigen loss and tumor relapse in our mouse model of mammary carcinoma (Kmieciak et al. 2007). Therefore, levels of IFN-γ production or of the expression of IFN-γ receptor α on tumor cells may determine whether tumor inhibitory or relapse-promoting effect of IFN-γ may prevail. High levels of IFN-γ and/or IFN-γ receptor α expression or lack of the expression can induce a robust tumor rejection via IFN-γ-dependent or -independent mechanisms, whereas intermediate levels of IFN-γ and/or IFN-γ receptor α expression may facilitate tumor escape and relapse (Table 1). This hypothetical model which is suggested by our recent observations (unpublished data) may also predict the direction of discordance between primary and metastatic breast cancers in the expression of HER-2/neu such that tumor clones with low levels of IFN-γ receptor α that escaped anti-tumor immune responses may retain HER-2/neu expression during metastasis. However, gain of HER-2/neu expression in metastatic tumor cells remain unknown. The role of microenvironment in inducing epigenetic changes in tumor cells which may drive HER-2/neu expression is yet to be determined. Advances in our understanding of the role of anti-tumor immune responses in epigenetic modulation of cancer may improve our ability to develop highly tailored immunotherapeutic strategies that can overcome tumor escape and relapse.

Table 1 Levels of IFN-γ and/or IFN-γ receptor α expression determine T cell-mediated tumor inhibition or tumor escape

	Levels of IFN-γ production and/or IFN-γ receptor α expression		
	High	Intermediate	Low
Tumor inhibition	Yes	No	Yes
Tumor escape and relapse	No	Yes	No

Tumor Relapse and BCSC

Tumor dormancy remains a major hurdle in the treatment of cancers. The exact identity of cells that contribute to tumor dormancy and subsequent relapse of cancers remain poorly understood. Despite conventional therapies of breast cancer, most of the US deaths are attributed to relapse (Benson et al. 2009), which is likely due to the failure of current treatment regimens to eliminate BCSC, a subset of

cells with potent tumorigenicity and a profound resistance to apoptosis. In fact, an association between CSC and tumor recurrence has been observed (Joung et al. 2010; Kusumbe and Bapat 2009; Vesuna et al. 2009), thus implicating CSCs as facilitators in relapse. What remain unknown about BCSC are the generation, survival, and heterogeneity of these transformed tumor-initiating cells in the immunologically hostile microenvironment. Currently, the major hypothesis describing the origin of BCSC is that transformed resident tissue stem cells undergo asymmetrical division, occasionally renewing a copy of themselves, but most often generating proliferative epithelial daughter progeny with limited tumorigenicity (Santisteban et al. 2009). An alternative hypothesis is that BCSC are derived from transformed, differentiated epithelial cells by acquisition of stem cell properties through EMT (Mani et al. 2008). These two hypotheses were derived from observations implying that heterogeneous BCSC phenotypes may exist in breast cancer patients.

Inflammatory cytokines, IL-1, IL-6 and IL-8 in particular, have been suggested to support CSC through activation of STAT3 and NF-κB pathways (Korkaya et al. 2011). These cytokine pathways mimic those involved in wound healing. Two key publications that highlight the role of these inflammatory cytokines in supporting BCSC include: (i) Wicha's group, which showed that blockade of the IL-8 receptor CXCR1 can selectively target BCSC and reduce tumor growth and metastasis (Ginestier et al. 2010); (ii) Struhl's group, which showed that IL-6 was sufficient to convert human breast and prostate tumor cells to CSC (Iliopoulos et al. 2011). IL-6 secreted by breast tumor cells has also been reported to be involved in the activation and recruitment of mesenchymal stem cells to the tumor site (Rattigan et al. 2010). IL-6-mediated induction of EMT has also been reported (Xie et al. 2012).

It was reported that expression of the HER-2/neu oncogene regulates BCSC in driving tumor invasion and metastasis (Korkaya et al. 2008). Along this line of evidence, Magnifico et al. (2009) reported that, in breast cell lines, $CD44^{+}CD24^{-/low}$ BCSC clones that were capable of mammosphere formation also had increased HER-2/neu expression. Expression of HER-2/neu increased sensitivity of BCSC to trastuzumab and lapatinib (Korkaya and Wicha 2009; Magnifico et al. 2009). On the other hand, Reim et al. (2009) showed that treatment with trastuzumab and polyclonal NK cells resulted in the preferential survival of individual sphere-forming cells that displayed a $CD44^{+}CD24^{-/low}$ "cancer stem cell-like" phenotype and expressed significantly less HER-2/neu compared with non-stem cells. This suggested that the induction of antibody-dependent cell cytotoxicity by trastuzumab and NK cells may spare the actual tumor-initiating cells, leading to tumor relapse. The $CD44^{+}CD24^{-/low}$ population was also found to be more immunoresistant in SK-BR3, MDA-MB231, and BT474 breast cancer cell lines. Such contradictory reports may be because some groups focused merely on trastuzumab as an antibody therapy that relays signals downstream of HER-2/neu receptor, whereas others included NK cells, which are associated with IFN-γ production and its interaction with IFN-γ receptor α.

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References

Beatty GL, Paterson Y (2000) IFN-gamma can promote tumor evasion of the immune system in vivo by down-regulating cellular levels of an endogenous tumor antigen. J Immunol 165:5502–5508

Benson JR, Jatoi I, Keisch M et al (2009) Early breast cancer. Lancet 373:1463–1479

Berner V, Liu H, Zhou Q et al (2007) IFN-gamma mediates CD4+ T-cell loss and impairs secondary antitumor responses after successful initial immunotherapy. Nat Med 13:354–360

Blanco JC, Contursi C, Salkowski CA et al (2000) Interferon regulatory factor (IRF)-1 and IRF-2 regulate interferon gamma-dependent cyclooxygenase 2 expression. J Exp Med 191:2131–2144

Bui JD, Schreiber RD (2007) Cancer immunosurveillance, immunoediting and inflammation: independent or interdependent processes? Curr Opin Immunol 19:203–208

Cicalese A, Bonizzi G, Pasi CE et al (2009) The tumor suppressor p53 regulates polarity of self-renewing divisions in mammary stem cells. Cell 138:1083–1095

Connett JM, Hunt SR, Hickerson SM et al (2003) Localization of IFN-gamma-activated Stat1 and IFN regulatory factors 1 and 2 in breast cancer cells. J Interf Cytokine Res 23:621–630

Czerniecki BJ, Koski GK, Koldovsky U et al (2007) Targeting HER-2/neu in early breast cancer development using dendritic cells with staged interleukin-12 burst secretion. Cancer Res 67:1842–1852

Daniel N, Meyer-Morse EK, Bergsland K et al (2003) Immune enhancement of skin carcinogenesis by CD4+ T cells. J Exp Med 197:1017–1028

Doherty GM, Boucher L, Sorenson K et al (2001) Interferon regulatory factor expression in human breast cancer. Ann Surg 233:623–629

Dumont N, Wilson MB, Crawford YG et al (2008) Sustained induction of epithelial to mesenchymal transition activates DNA methylation of genes silenced in basal-like breast cancers. Proc Natl Acad Sci USA 105:14867–14872

Dunn GP, Bruce AT, Ikeda H et al (2002) Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol 3:991–998

Dunn GP, Sheehan KC, Old LJ et al (2005) IFN unresponsiveness in LNCaP cells due to the lack of JAK1 gene expression. Cancer Res 65:3447–3453

Farrar JD, Katz KH, Windsor J et al (1999) Cancer dormancy. VII. A regulatory role for CD8+ T cells and IFN-gamma in establishing and maintaining the tumor-dormant state. J Immunol 162:2842–2849

Ginestier C, Liu S, Diebel ME et al (2010) CXCR1 blockade selectively targets human breast cancer stem cells in vitro and in xenografts. J Clin Invest 120:485–497

Hall VL, Subleski J, Back TC et al (2007) Friend or foe? IFNgamma mediates pro-metastatic gene expression in the tumor microenvironment. J Immunol 178(48):34

Hallermalm K, Seki K, De Geer A et al (2008) Modulation of the tumor cell phenotype by IFN-gamma results in resistance of uveal melanoma cells to granule-mediated lysis by cytotoxic lymphocytes. J Immunol 180:3766–3774

Harada H, Willison K, Sakakibara J et al (1990) Absence of the type I IFN system in EC cells: transcriptional activator (IRF-1) and repressor (IRF-2) genes are developmentally regulated. Cell 63:303–312

Hida S, Ogasawara K, Sato K et al (2000) CD8+ T cell mediated skin disease in mice lacking IRF-2, the transcriptional attenuator of interferon-α/β signalling. Immunity 13:643–655

Holmes JP, Gates JD, Benavides LC et al (2008) Optimal dose and schedule of an HER-2/neu (E75) peptide vaccine to prevent breast cancer recurrence: from US Military Cancer Institute Clinical Trials Group Study I-01 and I-02. Cancer 113:1666–1675

Hoque A, Sneige N, Sahin AA et al (2002) Her-2/neu gene amplification in ductal carcinoma in situ of the breast. Cancer Epidemiol Biomarkers Prev 11:587–590

Houssami N, Macaskill P, Balleine RL et al (2011) HER2 discordance between primary breast cancer and its paired metastasis: tumor biology or test artefact? Insights through meta-analysis. Breast Cancer Res Treat 129:659–674

Iliopoulos D, Hirsch HA, Wang G et al (2011) Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. Proc Natl Acad Sci USA 108:1397–1402

Jesse TL, LaChance R, Iademarco MF et al (1998) Interferon regulatory factor-2 is a transcriptional activator in muscle where it regulates expression of vascular cell adhesion molecule-1. J Cell Biol 140:1265–1276

Joung JY, Cho KS, Kim JE et al (2010) Prostate stem cell antigen mRNA in peripheral blood as a potential predictor of biochemical recurrence in high-risk prostate cancer. J Surg Oncol 101:145–148

Kang JH, Kim SJ, Noh DY et al (2001) Methylation in the p53 promoter is a supplementary route to breast carcinogenesis: correlation between CpG methylation in the p53 promoter and the mutation of the p53 gene in the progression from ductal carcinoma in situ to invasive ductal carcinoma. Lab Invest 81:573–579

Kmieciak M, Knutson KL, Dumur CI et al (2007) HER-2/neu antigen loss and relapse of mammary carcinoma are actively induced by T cell-mediated anti-tumor immune responses. Eur J Immunol 37:675–685

Kmieciak M, Payne KK, Idowu MO et al (2011) Tumor escape and progression of HER-2/neu negative breast cancer under immune pressure. J Transl Med 9:35

Koeppel M, van Heeringen SJ, Smeenk L et al (2009) The novel p53 target gene IRF2BP2 participates in cell survival during the p53 stress response. Nucleic Acids Res 37:322–335

Kominsky SL, Hobeika AC, Lake FA et al (2000) Down-regulation of neu/HER-2 by interferon-gamma in prostate cancer cells. Cancer Res 60:3904–3908

Korkaya H, Wicha MS (2009) HER-2, Notch, and breast cancer stem cells: targeting an axis of evil. Clin Cancer Res 15:1845–1847

Korkaya H, Paulson A, Iovino F et al (2008) HER2 regulates the mammary stem/progenitor cell population driving tumorigenesis and invasion. Oncogene 27:6120–6130

Korkaya H, Liu S, Wicha MS (2011) Regulation of cancer stem cells by cytokine networks: attacking cancer's inflammatory roots. Clin Cancer Res 17:6125–6129

Kusumbe AP, Bapat SA (2009) Cancer stem cells and aneuploid populations within developing tumors are the major determinants of tumor dormancy. Cancer Res 69:9245–9253

Levina V, Marrangoni AM, De Marco R et al (2008) Drug-selected human lung cancer stem cells: cytokine network, tumorigenic and metastatic properties. PLoS One 3:e3077

Liu K, Caldwell SA, Greeneltch KM et al (2006) CTL adoptive immunotherapy concurrently mediates tumor regression and tumor escape. J Immunol 176:3374–3382

Ljunggren HG (2008) Cancer immunosurveillance: NKG2D breaks cover. Immunity 28:492–494

Magnifico A, Albano L, Campaner S et al (2009) Tumour-initiating cells of HER2-positive carcinoma cell lines express the highest oncoprotein levels and are trastuzumab sensitive. Clin Cancer Res 15:2010–2021

Mani SA, Guo W, Liao MJ et al (2008) The epithelial–mesenchymal transition generates cells with properties of stem cells. Cell 133:704–715

Matkowski R, Gisterek I, Halon A et al (2009) The prognostic role of tumor-infiltrating CD4 and CD8 T lymphocytes in breast cancer. Anticancer Res 29:2445–2451

Nicholaou T, Chen W, Davis ID et al (2011) Immunoediting and persistence of antigen-specific immunity in patients who have previously been vaccinated with NY-ESO-1 protein formulated in ISCOMATRIX^{TM}. Cancer Immunol Immunother 60:1625–1637

Pohlmann PR, Mayer IA, Mernaugh R (2009) Resistance to trastuzumab in breast cancer. Clin Cancer Res 15:7479–7491

Rattigan Y, Hsu JM, Mishra PJ et al (2010) Interleukin 6 mediated recruitment of mesenchymal stem cells to the hypoxic tumor milieu. Exp Cell Res 316:3417–3424

Reim F, Dombrowski Y, Ritter C et al (2009) Immunoselection of breast and ovarian cancer cells with trastuzumab and natural killer cells: selective escape of CD44high/CD24low/HER2low breast cancer stem cells. Cancer Res 69:8058–8066

Romieu-Mourez R, François M, Abate A et al (2010) Mesenchymal stromal cells expressing ErbB-2/neu elicit protective antibreast tumor immunity in vivo, which is paradoxically suppressed by IFN-gamma and tumor necrosis factor-alpha priming. Cancer Res 70:7742–7747

Rosenberg SA, Sherry RM, Morton KE et al (2005) Tumor progression can occur despite the induction of very high levels of self/tumor antigen-specific CD8+ T cells in patients with melanoma. J Immunol 175:6169–6176

Roses RE, Paulson EC, Sharma A et al (2009) HER-2/neu overexpression as a predictor for the transition from in situ to invasive breast cancer. Cancer Epidemiol Biomarkers Prev 18:1386–1389

Santinelli A, Pisa E, Stramazzotti D et al (2008) HER-2 status discrepancy between primary breast cancer and metastatic sites. Impact on target therapy. Int J Cancer 122:999–1004

Santisteban M, Reiman JM, Asiedu MK et al (2009) Immune-induced epithelial to mesenchymal transition in vivo generates breast cancer stem cells. Cancer Res 69:2887–2895

Sato T, Onai N, Yoshihara H et al (2009) Interferon regulatory factor-2 protects quiescent hematopoietic stem cells from type I interferon-dependent exhaustion. Nat Med 15:696–700

Sauer KA, Maxeiner JH, Karwot R et al (2008) Immunosurveillance of lung melanoma metastasis in EBI-3-deficient mice mediated by CD8+ T cells. J Immunol 181:6148–6157

Sheu BC, Kuo WH, Chen RJ et al (2008) Clinical significance of tumor-infiltrating lymphocytes in neoplastic progression and lymph node metastasis of human breast cancer. Breast 17:604–610

Silva HC, Garcao F, Coutinho EC et al (2006) Soluble VCAM-1 and E-selectin in breast cancer: relationship with staging and with the detection of circulating cancer cells. Neoplasma 53:538–543

Singh R, Paterson Y (2007) Immunoediting sculpts tumor epitopes during immunotherapy. Cancer Res 67:1887–1892

Taniguchi T, Ogasawara K, Takaoka A et al (2001) IRF family of transcription factors as regulators of host defense. Annu Rev Immunol 19:623–655

Terada K, Okochi-Takada E, Akashi-Tanaka S et al (2009) Association between frequent CpG island methylation and HER2 amplification in human breast cancers. Carcinogenesis 30:466–471

Vesuna F, Lisok A, Kimble B et al (2009) Twist modulates breast cancer stem cells by transcriptional regulation of CD24 expression. Neoplasia 11:1318–1328

Watanabe N, Sakakibara J, Hovanessian A et al (1991) Activation of IFN-β promoter element by IRF-1 requires a post-translational event in addition to IRF-1 synthesis. Nucleic Acids Res 19:4421–4428

Worschech A, Kmieciak M, Knutson KL et al (2008) Signatures associated with rejection or recurrence in HER-2/neu positive mammary tumors. Cancer Res 68:2436–2446

Wu TH, Schreiber K, Arina A et al (2011) Progression of cancer from indolent to aggressive despite antigen retention and increased expression of interferon-gamma inducible genes. Cancer Immun 11:2

Xie G, Yao Q, Liu Y et al (2012) IL-6-induced epithelial–mesenchymal transition promotes the generation of breast cancer stem-like cells analogous to mammosphere cultures. Int J Oncol 40:1171–1179

Yim JH, Ro SH, Lowney JK et al (2003) The role of interferon regulatory factor-1 and interferon regulatory factor-2 in IFN-gamma growth inhibition of human breast carcinoma cell lines. J Interf Cytokine Res 23:501–511