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# Oncogenic growth factor signaling mediating tumor escape from cellular immunity

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Unrestrained growth factor signals can promote carcinogenesis, as well as other hallmarks of cancer such as immune evasion. Our understanding of the function and complex regulation of HER family of receptors has led to the development of targeted therapeutic agents that suppress tumor growth. However, these receptors also mediate escape from recognition by the host immune system. We discuss how HER family of oncogenic receptors downregulate tumor antigen presentation and upregulate suppressive membrane-bound or soluble secreted inhibitory molecules that ultimately lead to impaired cellular immunity mediated by cytotoxic T lymphocyte (CTL) recognition. Implementing this knowledge into new therapeutic strategies to enhance tumor immunogenicity may restore effector cell mediated immune clearance of tumors and clinical efficacy of tumor-targeted immunotherapy against HER receptor overexpression.

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## Introduction

Growth factor receptors transduce extracellular signals through the activation of intracellular messengers or directly through receptor translocation to the nucleus. Of the receptor tyrosine kinases (RTKs), the HER family, also called ErbB, is one of the most extensively studied for its role in development, physiology, and human cancer [1]. The HER family is considered a prototypical oncogenic growth factor receptor, since it activates multiple intracellular signal transduction cascades including the mitogen activated protein kinase (MAPK), phosphatidylinositol-3 kinase (PI3K/AKT), Janus kinase/signal transduced and activator of transcription (JAK/STAT) and protein kinase C (PKC) pathways [2,3]. In turn, these signals induce malignant transformation of tumor cells through increased cell proliferation and survival, resistance to growth inhibition or apoptosis and increased invasion and metastasis, capabilities that are common to the majority of tumors and were recognized as initial 'Hallmarks of Cancer' [4].

In addition, recent work has provided evidence for enlarging this list of tumor transforming competences, where cellular metabolism re-conditioning, inflammation-promoting tumor development and evasion of immune destruction have been noted as important additional 'Hallmarks of Cancer' [5]. In this context, the concept of 'tumor immunogenicity' arises, where tumor cells could be more or less immunogenic in regard to expression of molecules that activate or inhibit the host immune system. Three major signals determine a successful immune response, intact antigen processing machinery (APM) and HLA class I mediated antigen presentation (Signal 1), proper co-stimulation (Signal 2) and proinflammatory cytokine stimulation (Signal 3). Tumor cells evade immune recognition by downregulating signal 1 and providing aberrant signals 2 and 3, which in the setting of tumors are represented by increased surface expression of checkpoint receptor ligands, particularly programmed death ligand-1 (PD-L1) and increased secretion of immunosuppressive cytokines and chemokines. In this review, we focus on the molecular mechanisms of how HER family of receptors regulate tumor cell-induced immune escape, not only by downregulating HLA class I antigen presentation but also by upregulating expression of PD-L1 and suppressive cytokines. Ultimately, these oncogenic signals lead to evasion of cellular immunity mediated by cytotoxic T lymphocyte (CTL) and natural killer (NK) cell cytotoxicity, which may be reversed by specific anti-HER monoclonal antibody (mAb) or tyrosine kinase inhibitor (TKI) therapy.

## HER family of growth factor receptors: overview and targeted immunotherapy

The HER family of receptors comprises four members: EGFR (ErbB1, HER1), ErbB2 (HER2, *neu* in rodents), ErbB3 (HER3) and ErbB4 (HER4) [1]. Soluble ligand binding to the ectodomain of the receptor promotes homodimerization and heterodimerization which in turn induces activation of the intracellular tyrosine kinase domain and phosphorylation of the C-terminal tail, intracellular phosphoproteins then bind and activate adaptors that transduce signals that activate MAPK, PI3K, PKC and JAK/STAT pathways [2]. Importantly, two members

of this growth factor receptor family, the epidermal growth factor receptor (EGFR) and HER2 are the most strongly associated with tumor progression of various human neoplasms, including breast, lung, head and neck squamous cell carcinoma (HNSCC) and glioblastoma [6–8]. Furthermore, in the case of the EGFR, in addition to overexpression of the wild type receptor some tumors also exhibit activating mutant forms such as glioblastoma, where a variant called EGFRvIII has been reported [9] or non-small cell lung cancer (NSCLC) where mutations in the EGFR kinase domain (L858R *human*/T790M *murine*) have been associated with tumor resistance [10]. Given that the mechanism of action of HER receptors depends heavily on the interaction of their extracellular domains with activating ligands, their function can be readily disrupted by targeted receptor-blocking-specific mAbs, which prevent ligand binding and possibly induce endocytosis and degradation of the receptor [11]. Three humanized mAbs targeting this family of receptors have been approved for clinical use, trastuzumab which targets HER2 and cetuximab or panitumumab which target the EGFR. Moreover, a second strategy to block HER receptor signaling works by inhibiting their tyrosine kinase function with specific small molecule inhibitors. In the setting of cancer treatment, three TKI have been approved for clinical use, gefitinib and erlotinib that are specific for the EGFR and lapatinib that is equally specific for the EGFR and HER2 [12,13]. EGFR blocking mAbs have an additional therapeutic benefit over TKIs, that is the activation of the innate immune system NK cells through interaction of the Fc portion with the FcγRIIIa (CD16) receptor, triggering antibody-dependent cellular cytotoxicity (ADCC), activation of dendritic cells (DC) via NK-DC IFNγ-mediated crosstalk and ultimately, inducing upregulation of tumor antigen-specific CTL [14].

## EGFR and HER2 mediated downregulation of APM components and HLA class I mediated antigen presentation

Tumor antigen presentation is a major pre-requisite for appropriate T cell responses, especially because of the crucial role of this process in the generation of tumor antigen (TA)-specific adaptive immune responses [15,16]. TA-antibody-based immunotherapies may rely on this process, where mAbs such as cetuximab or trastuzumab enhance adaptive T cell responses by promoting NK cell activation, Th1 cytokine secretion and DC cross presentation [17,18]. Importantly, impaired HLA class I antigen presentation is associated with reduced CTL recognition, disease progression and metastasis [19,20]. Interestingly, recent studies showed that EGFR signaling regulates the expression of APM components and HLA class I [17,21]. In this regard, EGFR-induced activation of protein phosphatase type 11 (PTNP11, best known as SHP2) dephosphorylates signal transducer and activator of transcription 1 (STAT1), a known transcription factor that mediates expression of HLA class I and APM molecules (Figure 1). Interestingly, treatment with IFNγ and inhibition or depletion of SHP2 in tumor cell lines induced upregulation of phosphorylated STAT1 and restored expression of HLA class I and APM components [17], which ultimately enhanced HLA class I restricted antigen presentation and expansion of EGFR-specific CTL. Furthermore, EGFR-mediated SHP2 activation may not only downregulate HLA class I-peptide presentation (signal 1) but also induce secretion of immunostimulatory cytokines and chemokines (signal 3), since its inhibition upregulated tumor cell secretion of IL-12p70, RANTES (CCL5) and IP-10 (CXCL10). Importantly, a second mechanism of downregulation of HLA class I and APM components downstream the EGFR has been reported, where activated SHP2 dephosphorylates GDP, inducing RAS/MAPK pathway activation [19]. Notably, restoration of signal 1 via EGFR inhibition is clinically relevant, since patients from a novel phase II clinical trial who responded to cetuximab single agent therapy showed significant upregulation of tumor HLA class I expression [22•], and induction of EGFR-specific CTL correlated with better clinical response.

In the setting of HER2, a recent report demonstrated that its oncogenic transformation in murine models was associated with low levels of MHC class I and reduced CTL recognition [23,24], which could be reversed by IFNγ treatment [25]. In humans, HER2 overexpressing tumors showed poor CTL recognition even after IFNγ treatment [26]. Furthermore, siRNA mediated silencing of HER2 induced upregulation of HLA class I molecules in several ErbB2+ breast cancer cell lines [27]. Likewise, HER2 overexpression in melanoma cells resulted in poor recognition by tyrosinase-specific CTL [28], the mechanism by which HLA class I antigen presentation was affected may be related to HER2-induced mutations within the promoter binding sites of the transcription factor E2F1 in the tapasin locus [29]. Moreover, the mechanism by which HER2 downmodulates HLA class I expression may also be associated with MAPK pathway activation since MAPK inhibition with PD98059 resulted in a dose-dependent upregulation of surface HLA class I expression in breast, esophageal and gastric carcinoma cells. Further evidence of the role of the MAPK pathway in the regulation of antigen presentation is the finding of Sapkota *et al.* which demonstrated that vemurafenib-mediated BRAF inhibition enhanced IFNγ-mediated upregulation of HLA class I expression in melanoma [20]. In addition, the view that HER2 orchestrates tumor immune escape is clinically relevant since patients who had high HER2 expression had low HLA class I levels, as determined by immunohistochemistry staining of breast cancer specimens [30]. In summary, these findings demonstrate that HER family receptors play an active role mediating immune evasion by downregulating antigen