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## Increasing Potential of HER3 Signaling in Colon Cancer Progression and Therapy

Christian Gerspach

HER3 protein levels at the cancer cell plasma membrane are directly correlated with reduced survival in patients with colorectal cancer. In colorectal cancer cells, HER3 blockade restricted cellular growth ( $G_2$ -M arrest), survival, migration, and invasion, and potentiated the chemotherapeutic effect of 5-FU, supporting strategies that target HER3 in subsets of patients with colorectal cancer. *Clin Cancer Res*; 18(4); 917-9. ©2011 AACR.

In this issue of *Clinical Cancer Research*, Beji and colleagues (1) report that the HER3 pseudokinase is overexpressed in a series of clinical primary colorectal tumors and derived colorectal cancer cell lines. Remarkably, colorectal cancer patients with high HER3 expression had shorter survival times compared with patients with lower expression. Moderate and high HER3 expression was identified as an independent prognostic marker for low survival associated with a relative risk of 3.29.

The HER1 prototype of the EGFR/HER family comprising HER2 (ErbB-2), HER3, and HER4 has been identified as a critical player in the progression of epithelial neoplasms, including colorectal cancer. HER agonists initiate receptor homo- or heterodimerization and connections with a vast array of intracellular signaling pathways through paracrine and autocrine loops (Fig. 1). In addition, ectodomain shedding of heparin-binding (HB)-EGF from the transmembrane-anchored pro-HB-EGF by matrix metalloproteases provides EGF-like ligands that target HER1 via juxtacrine interactions. Several agonists of G-protein coupled receptors (GPR), such as the gastrointestinal regulators bombesin, gastrin-releasing peptide, endothelin-1, lysophosphatidic acid, and thrombin, were originally described to initiate this GPR-HER1 transactivation loop (2). Additional GPR-HER1 cross-talks were subsequently described for diverse GPR and pathophysiological processes, including fMLP-receptor-dependent chemotaxis in inflammatory cells and the alternative estrogen receptor GPR30 in breast cancer (3).

Another level of complexity is determined by the ability of HER receptors to form heterodimers with HER family members (i.e., HER1/HER2 or HER3; HER2/HER3 or HER4

modules) upon ligand binding to HER1 (EGF, amphiregulin, TGF- $\alpha$ , epiregulin, and betacellulin), HER3, or HER4 (heregulins/neuregulins, e.g., HRG- $\beta$ 1, HRG-4, and HB-EGF). HER2 ligand has not been identified, but overexpressed HER2 is constitutively active according to the generation of its cognate heterodimers with HER partners. HER heterodimerization and synergies associated with MET amplification have also been described for *trans*-phosphorylation of HER1, HER2, HER3, and RET, in a MET kinase-dependent manner (4). These molecular alliances support the indirect activation of a given HER member by agonists targeting other HER partners, as well as the contribution of HERs in multifactorial chemoresistances to DNA-damaging agents and other anticancer drugs (5). Several other genetic, epigenetic, and post-transcriptional mechanisms should be considered in relation to HER expression levels and activity. These include the activation or invalidation of HER-dependent signalomes and partners via HER activating mutations, downregulation by ubiquitin-dependent and -independent degradation, receptor endocytosis, lysosomal degradation or recycling from endosomes, receptor subcellular localization, and regulation by noncoding microRNAs (6, 7). In recent years, both direct and indirect, and positive and negative effects of ubiquitin and deubiquitin ligases on HER family members and elements of their molecular scaffolds and downstream signaling machinery have been described. Numerous examples concern E3 ubiquitin ligases targeting the degradation of HER3 (neuregulin receptor degrading protein 1) and HER4 (WW domain-containing protein 1), and clathrin-dependent endocytosis of HER1, MET, and their docking/adaptors (Arkadia and Casitas B-lineage lymphoma).

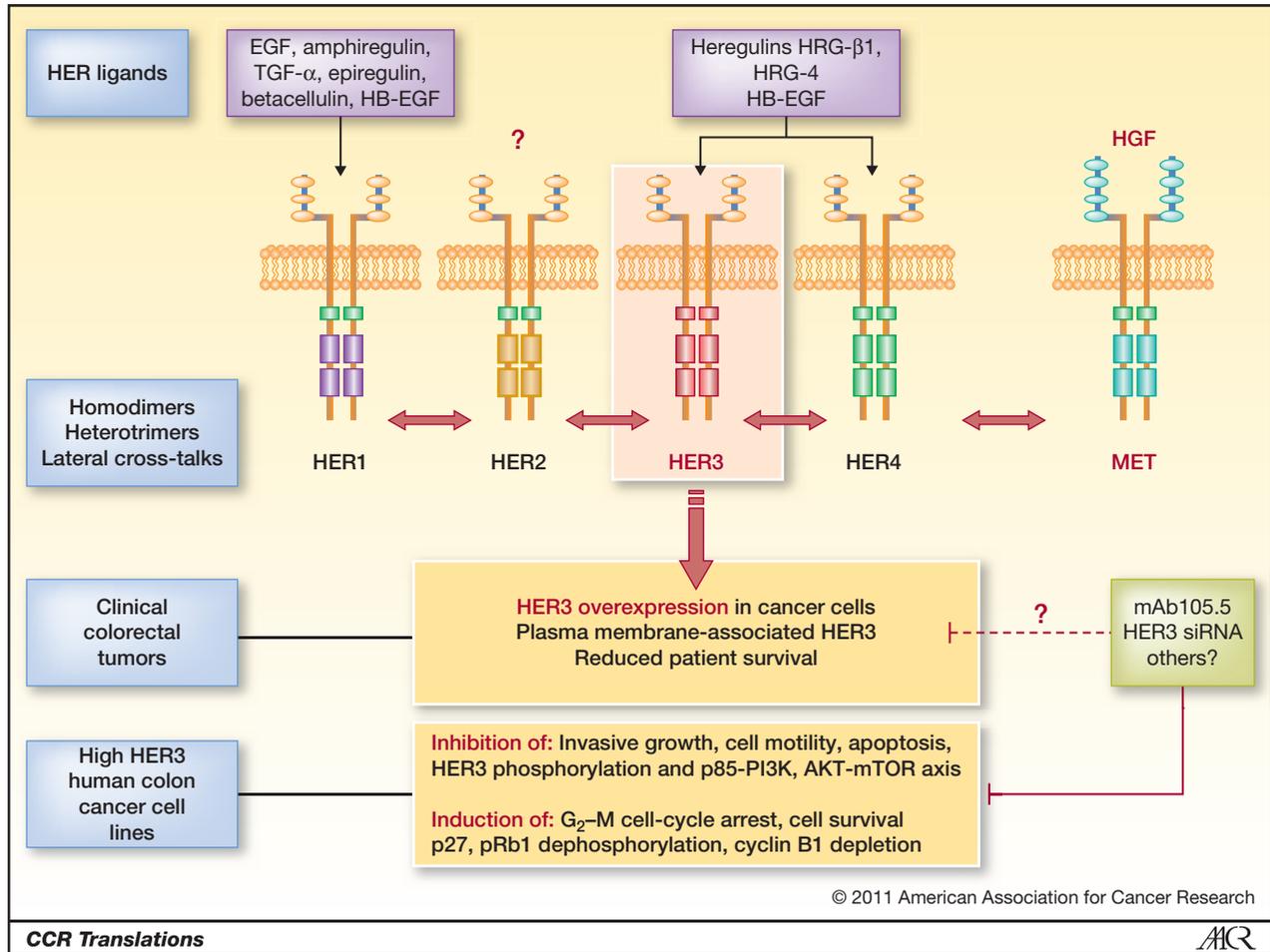
In this context, the study of Beji and colleagues (1) shows a clear localization of HER3 at the plasma membrane of colorectal cancer cells, in contrast with the cytoplasmic staining shown in some epithelial cancers. Such a localization of HER3 at the cell surface supports the use of therapeutic interventions with function-blocking antibodies. Addition of high-HER3-expressing colorectal cancer cells for HER3 signaling, invasive growth, and evasion from apoptosis under 5-fluorouracil

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**Figure 1.** HER3 overexpression and lower survival outcomes of patients with colorectal cancer. HER3 receptors are engaged with several signaling cross-talks and molecular complexes with other HER family members, directly or indirectly activated by their cognate ligands. Other tyrosine kinase receptors, such as MET, may participate in the formation of HER-based heterodimers and execution of *trans*-phosphorylations activating HER members. Cancer cells in clinical colorectal tumors exhibit a high degree of HER3 protein expression at the plasma membrane. This HER3 biomarker correlates with reduced patient survival. High-HER3-expressing colorectal cancer cells in culture display several hallmarks of aggressive epithelial tumors, including invasive growth, migratory potential, and evasion from apoptosis. These phenotypes were reversed by treatment with the monoclonal antibody mAb105.5 or HER3 siRNAs. These cellular responses were determined through invalidation of the phosphoinositide 3-kinase (PI3K)/AKT-mTOR axis, induction of cell-cycle arrest at the G<sub>2</sub>-M transition, depletion of the M-phase promoting factor cyclin B1, and accumulation of the cell-cycle inhibitors p27 and pRb1 under the dephosphorylated, active form. It is anticipated that therapeutic strategies targeting HER3 signalomes in combination with DNA-damaging agents, cytotoxic and antimetabolic drugs, or multikinase inhibitors may prove to be beneficial for chemotherapy sensitization during the treatment of colorectal cancer patients.

cytotoxic stress was revealed by HER3 siRNAs and the monoclonal antibody mAb105.5, which selectively antagonize HRG- $\beta$ 1 binding to HER3 (Fig. 1). This mAb is additionally characterized by its ability to strongly downregulate HER3 expression levels in treated colorectal cancer cells, which suggests that it may also be able to induce HER3 internalization/degradation. These data support the notion that the oncogenic potential of HER3 is regulated by a fine balance that determines its expression and activation levels in cultured colorectal cancer cells. Accordingly, mAb105.5 treatment restricts HER3 phosphorylation and its subsequent association with the phosphoinositide 3-kinase (PI3K)-p85 regulatory subunit. It would be of interest to investigate molecular complexes comprising HER3 and other HER family mem-

bers in colorectal cancer cell lines that have a high HER3 index and have been treated with mAb105.5. Of note, the mechanisms that drive HER3 upregulation, signaling activation, and plasma membrane localization are not fully understood.

The authors further show that downregulation of HER3 by siRNAs in high-HER3-expressing colorectal cancer cells attenuates the HER3 signalome using AKT/mTOR, and conversely promotes upregulation of the cyclin-dependent kinase inhibitor p27(Kip). Thus, the global accumulation of p27 observed in HER3 silenced cells may reflect the downregulation of PI3K associated with p27 stability (8). Moreover, the PI3K/AKT/mTOR axis has been shown to regulate the accumulation, translocation, and activity of p27 in nuclear and cytoplasmic compartments according to the

loss and gain of p27 functions (8, 9). p27 is consistently described as a double-faceted molecular player that acts as tumor suppressor and negative regulator of cancer cell growth versus its invasion/metastasis promoter activity, pending its nuclear/cytoplasmic localization (8–11). Eventually, the subcellular localization of p27 in clinical colorectal tumors and colorectal cancer cells overexpressing HER3 may be essential to establish the role of this fascinating dual protein during treatment with HER3 inhibitors, function-blocking antibodies, and siRNAs. Experimental HER3 depletion in colorectal cancer cell lines also down-regulates the M-phase, promoting factor cyclin B, inducing G<sub>2</sub>-M-phase cell-cycle arrest, and apoptosis. This is accompanied by a remarkable conversion of multiphosphorylated forms of the cell-cycle inhibitor retinoblastoma protein-1 (pRb1/p105) into their dephosphorylated pRb1 active counterparts, promoting cell-cycle arrest. Beji and colleagues showed multiple effects of HER3 blockade on reversal of critical functions associated with cancer progression, including colorectal cancer cell proliferation, migration, invasion, and survival (Fig. 1). If these results are validated in corresponding tumor xenografts, they could have potential implications for the treatment of patients with colorectal cancer who have high expression levels of HER3 in their primary tumors, circulating cancer cells, and metastases. In support of this assumption, recent studies in the field indicate that HER2/HER3 (over)expression is a significant predictor biomarker for anticancer therapy and patient survival in epithelial tumors of the stomach, pancreas, and breast.

This interesting study is opening new avenues for the stratification of colorectal cancer as high- or low-expressing HER3 samples during cancer progression, according to the tumor–node–metastasis clinical staging and histologic clas-

sification. It would be of interest to analyze the HER3 biomarker dosage in correlation with the genetic and molecular backgrounds of these HER3 samples in familial and sporadic colorectal cancer, that is, genetically unstable sporadic colorectal cancer linked to chromosomal instability and LOH and microsatellite instability-driven tumors, nonpolyposis forms of hereditary colon cancer and familial adenomatous polyposis tumors, and frequent mutations and oncogenic signal activations (e.g., Ki-Ras, B-Raf, PTEN, and TGF- $\beta$ ). Such findings would help elucidate the genetic, epigenetic, and molecular determinants of HER3-positive and -negative tumors, as described by Beji and colleagues (1). Their report highlights the possible contribution of HER3 to the epithelial–mesenchymal transitions observed in aggressive, chemoresistant colorectal tumors (12). Further studies are necessary to better understand the adaptor role of HER3 in engaging the PI3K pathway predominantly activated by a given tyrosine kinase receptor or oncogene, as well as the impact of HER3 signaling inhibitors acting at the HER3-agonist interface. Thus, therapeutic targeting of HER3 signalomes in colorectal cancer should be considered in relation with combined treatments using multi-kinase inhibitors or neutralizing antibodies acting at these HER3 connections. Such a strategy may potentiate tumor responses and chemosensitization to DNA-damaging agents such as the FOLFOX and FOLFIRI regimens in colorectal cancer patients with a high HER3 index.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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