Commentary

Increasing potential of HER3 signaling
in colon cancer progression and therapy

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HER3 protein levels at the cancer cell plasma membrane are directly correlated with reduced survival in colorectal cancer (CRC) patients. In CRC cells, HER3 blockade restricted cellular growth (G2/M arrest), survival, migration, invasion and potentiated the chemotherapeutic effect of 5-FU, supporting strategies targeting HER3 in subsets 3 of CRC patients.
In this issue of *Clinical Cancer Research*, Beji and colleagues (1) report that the HER3 pseudo-kinase is overexpressed in a series of clinical primary colorectal tumors and derived CRC cell lines. Remarkably, CRC patients with high HER3 expression had shorter survival times than cases 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 epidermal growth factor EGFR/HER family comprising HER2 (ErbB-2), HER3 and HER4 has been identified as a critical player in the progression of epithelial neoplasms, including CRC. 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 provide EGF-like ligands targeting HER1 via juxtacrine interactions. Several agonists of G-protein coupled receptors (GPR) were originally described to initiate this GPR-HER1 transactivation loop, e.g. the gastrointestinal regulators bombesin, gastrin-releasing peptide, endothelin-1, lysophosphatic acid and thrombin (2). Additional GPR/HER1 crosstalks have been 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, TGFα, epiregulin and betacellulin), HER3 or HER4 (heregulins/neuregulins, e.g. HRG-β1, HRG-4, 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 are also described for trans-phosphorylation of HERs 1-2-3 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 with HER expression...
levels and activity. These include the activation or invalidation of HER-dependent signalomes and partners via HER activating mutations, down-regulation 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, positive and negative impacts 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, Nrdp1), HER4 (WW domain-containing protein 1, WWP1) and clathrin-dependent endocytosis of HER1, MET and their docking/adaptors (Arkadia, Casitas B-lineage lymphoma, Cbl).

In this context, the study of Beji and colleagues (1) shows a clear localization of HER3 at the plasma membrane of CRC cells, in contrast to the cytoplasmic staining demonstrated in some epithelial cancers. Such a localization of HER3 at the cell surface supports therapeutic interventions using function-blocking antibodies. Addiction of HER3-high expressing CRC cells for HER3 signaling, invasive growth and evasion from apoptosis under 5-FU cytotoxic stress was revealed by HER3 siRNAs and the monoclonal antibody mAb105.5 which selectively antagonize HRG-β1 binding to HER3 (Fig.1). This mAb is characterized by the additional property to down-regulate strongly HER3 expression levels in treated CRC cells, suggesting its ability to induce HER3 internalization/degradation. These data support the notion that the oncogenic potential of HER3 is regulated by a fine balance determining its expression and activation levels in cultured CRC cells. Accordingly, mAb105.5 treatment restricts HER3 phosphorylation and its subsequent association with the PI3K-p85 regulatory subunit. It would be of interest to investigate molecular complexes comprising HER3 and other HER family members in CRC cell lines with high HER3 index and treated by mAb105.5. Notably, the mechanisms driving HER3 upregulation, signaling activation and plasma membrane localization are not fully understood.

The authors further show that down-regulation of HER3 by siRNAs in HER3 high expressing CRC cells attenuates the HER3 signalome using AKT/mTOR and
conversely promotes up-regulation of the CDK inhibitor p27(Kip). Thus, global accumulation of p27 observed in HER3 silenced cells may reflect the down-regulation 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 loss and gain of p27 functions (8,9). Consistently, p27 is described as a double-faceted molecular player acting 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 p27 subcellular localization in clinical colorectal tumors and CRC cells overexpressing HER3 may be essential to establish the role of this fascinating dual protein during treatment by HER3 inhibitors, function-blocking antibodies and siRNAs. Experimental HER3 depletion in CRC cell lines also down-regulates the M-phase promoting factor cyclin B, inducing G2/M phase cell cycle arrest and apoptosis. This was accompanied with 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 demonstrate multiple impacts of HER3 blockade on reversion of critical functions associated with cancer progression, including CRC cell proliferation, migration, invasion and survival (Fig.1). If validated in corresponding tumor xenografts, these results could have a potential implication in the treatment of CRC patients showing high expression levels of HER3 in their primary tumors, circulating cancer cells and metastases. In support with this assumption, recent papers in the field indicate that HER2/HER3 (over)expression is a significant predictor biomarker in 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 CRC as high and low expressing HER3 samples during cancer progression, according to the TNM clinical staging and histological classification. It would be of interest to analyze the HER3 biomarker dosage in correlation with the genetic and molecular background of these HER3 samples in familial and sporadic CRC, i.e. i) genetically instable sporadic CRC linked to chromosomal instability and loss of heterozygosity (LOH) and microsatellite instability-driven (MSI) tumors; ii) non-polyposis forms of the hereditary colon cancer (HNPCC) and familial adenomatous polyposis (FAP) tumors; iii)
frequent mutations and oncogenic signal activations, e.g. Ki-Ras, B-Raf, PTEN, TGFβ. These confrontations will help to better understand the genetic, epigenetic and molecular determinants of HER3 positive and negative tumors, as described by Beji and colleagues (1). This report highlights the possible contribution of HER3 in epithelial-mesenchymal transitions observed in aggressive, chemoresistant, colorectal tumors (12). Further studies are necessary to better understand the adaptor role of HER3 to engage 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 CRC should be considered in relation with combined treatments using multikinase 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 CRC patients with high HER3 index.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
References


**Legend to Figure 1**

**Figure 1.** HER3 overexpression and lower survival outcomes of colorectal cancer patients.

HER3 receptors are engaged with several signaling crosstalks 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 high degree of HER3 protein expression at the plasma membrane. This HER3 biomarker correlates with reduced patient survival. High-HER3 expressing colorectal cancer (CRC) 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 PI3-Kinase/AKT-mTOR axis, induction of cell cycle arrest at the G2/M transition, depletion of the M-phase promoting factor cyclin B1, and accumulation of the cell cycle inhibitors p27 and retinoblastoma protein-1 (pRb1) under 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 in chemotherapy sensitization during the treatment of CRC patients.
HER ligands

- EGF, amphiregulin, TGFα, epiregulin, betacellulin, HB-EGF
- Heregulins HRG-β1, HRG-4
- HB-EGF

Homodimers
Heterotrimers
Lateral crosstalks

HER1  HER2  HER3  HER4  MET

Clinical colorectal tumors
High HER3 human colon cancer cell lines

HER3 overexpression in cancer cells
Plasma membrane-associated HER3
Reduced patient survival

Inhibition of: Invasive growth, cell motility, apoptosis, HER3 phosphorylation and p85-PI3K, AKT-mTOR axis

Induction of: G2/M cell cycle arrest, cell survival p27, pRb1 dephosphorylation, cyclin B1 depletion

mAb105.5 HER3 siRNA others?
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