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MET-Mediated Resistance to EGFR Inhibitors: An Old Liaison Rooted in Colorectal Cancer Stem Cells

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Abstract

Inhibitors of EGFR are currently approved for the therapy of metastatic colorectal cancer (as well as other tumors), but their benefits are limited by inherent and acquired resistance, whose mechanisms are the subject of intense investigation. It is known that such resistance relies on a handful of genetic lesions and/or extracellular signals bypassing the requirement of EGF for cell proliferation and survival. As recently shown, these mechanisms may imply oncogenic activation of MET or its stimulation by the ligand hepatocyte growth factor. However, it is still largely obscure if sensitivity or resistance to EGFR inhibitors operates in cancer stem cells. Convincing evidence indicates that this elusive cell subpopulation is present at the roots of colorectal cancer. Conceivably, cancer stem cells accumulate the genetic lesions driving tumor onset and progression, as well as the genetic determinants of sensitivity or resistance to conventional and targeted therapies. Recent studies enlighten the expression of functional EGFR and MET in colorectal cancer stem cells and the outcome of their inhibition. Evidence is provided that, in patients sensitive to EGFR therapy, association of MET inhibitors fosters cancer stem cell eradication and durable tumor regression. *Cancer Res*; 74(14); 3647–51.

EGFR Is a Therapeutic Target in Colorectal Cancer

Targeted therapies are usually effective when they hit the product of an activated oncogene that plays an indispensable role in cell proliferation and survival, thereby sustaining the so-called “oncogene addiction” (1). However, this rule has apparent exceptions. Colorectal cancer is an example, where antibodies targeting EGFR offer clinical benefits even in the presence of a wild-type receptor (2, 3). This benefit is explained, at least in part, by the observation that EGFR signaling is crucial for homeostasis of intestinal stem cells, the putative origin of colorectal cancer (4, 5). However, colorectal cancer is frequently resistant to EGFR therapy, often as a result of genetic lesions that constitutively activate signal transduction pathways downstream EGFR, namely the RAS–ERK and PI3K–AKT pathways, controlling cell proliferation and survival. Direct stimulation of these pathways by activated oncogenes affords a “bypass track”—or compensatory signaling mechanism—that subtracts cell proliferation and survival from EGFR control, making EGFR inhibition ineffective (2). Such a resistance can manifest *ab initio* (“intrinsic” or “primary” resistance) or after the selective pressure imposed on tumor subclones by EGFR therapy itself (“acquired” or “secondary” resistance). It is not surprising that the same oncogene can be the protagonist of both intrinsic and acquired resistance to EGFR therapy, as it was shown in the case of RAS (6–8) or ERBB2 (9, 10). The same could occur for other genes implied in resistance such as PIK3CA (11), BRAF, PTEN (12), and MET (13).

***MET* Activation Is a Mechanism of Resistance to EGFR Inhibition**

The *MET* oncogene, encoding the tyrosine kinase receptor for hepatocyte growth factor (HGF; ref. 14), was first recognized as a factor of resistance to EGFR inhibitors in lung cancers driven by EGFR mutations. Amplification of this oncogene was found in 20% of patients that developed acquired resistance to the selective EGFR kinase inhibitor gefitinib (15, 16). In experimental settings, *MET* amplification emerged concomitantly with resistance in an “EGFR-addicted” cell line treated with increasing concentration of gefitinib. In these cells, *MET* was shown to reactivate the proliferative/antiapoptotic MEK–ERK and PI3K–AKT pathways, quenched by the inhibitor, thereby conferring bypass track resistance (15). These studies also provided the proof of principle that the combination of EGFR and *MET* inhibitors could be beneficial in lung cancers driven by EGFR mutations (15, 16). Interestingly, later, it was shown that rare subclones harboring *MET* amplification may pre-exist in EGFR-mutated lung cancers. These subclones are not driven by EGFR mutations and thus are positively selected by therapy with EGFR inhibitors (17).

The finding that *MET* amplification (or another genetic alteration, ref. 3) confers acquired resistance to EGFR therapy links the concept of resistance to the presence of a cell-autonomous, selectable genetic lesion, mirroring the mechanism of oncogene addiction. On the other hand, sensitivity to targeted therapy may rely on the normal activity of an essential pathway—such as the one downstream EGFR in colorectal cancer—and refractoriness may be sustained by physiologic, inherent signaling circuits. Indeed, it has recently emerged that resistance to targeted therapies may be sustained by growth-promoting cues coming from the tumor microenvironment. As an example, in cancer cell lines addicted to mutationally active kinases, the response to specific inhibitors was efficiently counteracted by a variety of growth factors (18–20). In this context, among the many factors screened, HGF displayed a prominent role in protecting BRAF-mutant melanomas or ERBB2-driven carcinomas from their respective inhibitors (18, 19). Consistently, an association between high levels of HGF expression and resistance to kinase-targeting agents was found also in bioptical samples of BRAF-mutant melanomas (18,19). Again, in an experimental setting, HGF, EGF, and FGF were shown to reciprocally rescue cells from inhibition of their respective receptors (20), a compensation mechanism that may be critical for resistance of colorectal cancer stem cells to targeting agents (see below).

The specific role of HGF in sustaining resistance to EGFR inhibition was first shown in lung adenocarcinomas driven by EGFR mutations (17, 21). Lately, it was shown that HGF protects colorectal cancer cells from inhibition of wild-type EGFR (13, 22, 23). Recently, by analyzing microarrays of colorectal tumors treated with an anti-EGFR antibody as monotherapy, a correlation between increased expression of HGF and poor therapeutic response was highlighted (24, 25).

As *MET* is widely expressed in human tumors (14), and HGF is abundantly produced by cancer-associated fibroblasts (26, 27), activation of the wild-type receptor by its ligand may have a widespread role, sustaining both biologic aggressiveness and protection from antibodies or drugs targeting kinases, EGFR in particular. So far, however, the contribution of HGF may have been underestimated in the experimental setting, for a limitation inherent in cancer cell xenografting: murine HGF does not efficiently cross-react with human *MET* (28, 29).

Colorectal Cancer Stem Cells: The Root of the Tumor Resistance to Therapy

Colorectal tumors contain cells endowed with heterogeneous tumorigenic potential (30, 31). Indeed, different cell subpopulations were isolated from whole tumors by cell surface markers and cell sorting: only a minor cell subset displayed the distinctive properties of “cancer stem cells” such as (i) to generate a xenograft that phenocopies the original tumor, (ii) to self-renew *in vivo*, that is to sustain serial passages of xenografting, and (iii) to give rise to a progeny that, although proliferating, cannot establish or propagate a xenografted tumor (30).

From colorectal tumors, it was also possible to isolate cells—retaining the above properties—which were not prospectively isolated through cell surface markers, but were selected in a stem cell culture medium (25, 32). This methodology generated the so-called “colospheres”: spheroid colonies growing in suspension, each virtually clonal, and originated by cells with stem properties. As they were not prospectively isolated by cell surface markers, such cells should be more appropriately referred to as “cancer-initiating cells” (C-IC; refs. 30, 32).

It is debated whether cancer stem cells (or C-IC) are fixed entities, or represent a transient functional status, and if there is interconversion between the nonstem and the stem status; if so, it is unclear which extracellular cues could mediate such interconversion (30). Interestingly, among a few hints, it has been shown that induction of the so-called “epithelial–mesenchymal transition” by exogenous signals reprograms cells to reactivate latent stem properties (33). HGF, also known as “scatter factor,” is a well-known inducer of cell dissemination and invasive growth, a complex process starting with epithelial–mesenchymal transition (14, 34). Moreover, HGF induces Wnt signaling in colorectal C-IC (35). These features whisper that HGF sustains the cancer stem cell phenotype, as envisioned by considering the role of its receptor MET during development and tissue regeneration (36).

At present, the dilemma about the “stem status” (fixed vs. transitory and interchangeable) is largely unresolved, and the “cancer stem cell model” is burdened with controversy on the relative extent of the stem cell subpopulation in individual tumors (30). However, whatever the outcome of the arguments, the relevance of stem cells in cancer therapy is increasingly evident. It is quite established that cancer cells obeying to the operational definition of “stem cells” are highly resistant to conventional therapies, and thus are the most likely cause of tumor recurrence (30). In colorectal C-IC, chemoresistance has been associated with the activity of autocrine IL4 (37), or the paracrine stimulation by IL17A, a factor that promotes also self-renewal (38), or the activity of transcription factors ID1 and ID3, critical for sustaining the stem phenotype (32). Altogether, these data support the conclusion that “determinants of stemness” deeply contribute to therapeutic resistance (30) and enhance the interest in the stemness promoting activity of HGF as a cause of failure of conventional and targeted therapy.

EGFR: A Master Regulator of Colorectal Cancer Stem Cells

In cancer stem cells, the role of EGFR and MET signaling is largely obscure, as well as the outcome of their targeted inhibition. To shed light on these issues, we systematically isolated C-IC from a previously established ample cohort of patient-derived xenografts of metastatic colorectal cancer (“xenopatients”). These xenografts were validated as faithful representatives of the original tumors, able to retain their genetic and phenotypic features across multiple serial passages, and displaying a therapeutic response to EGFR inhibitors comparable with that of matched patients, and correlated with the genotype (9). As expected, mutations in genes encoding signal transducers of the RAS pathway conferred primary resistance. Moreover, this xenopatient cohort was used to perform population-based studies, resulting in the identification of two new mechanisms of resistance to therapy with the anti-EGFR antibody cetuximab: *ERBB2* amplification (for primary resistance; ref. 9) and *MET* amplification (for acquired resistance; ref. 13). Colospheres were generated from xenopatient tumors by culture in stem cell–selective medium. These cells, for brevity called “xenospheres,” displayed the operational properties of C-IC (see above). Indeed, xenospheres long-term self-propagated *in vitro* and generated phenocopies of the original tumors after transplantation in mice (“spheropatients”; Fig. 1). Notably, xenospheres retained the most essential property of C-IC, as they could be rederived from the spheropatient and sustain serial transplantation. Finally, the matched human patient, xenopatient, xenosphere, and spheropatient retained the genetic determinants of response to anti-EGFR therapy. Altogether, these

findings provide a proof of concept that therapeutic sensitivity/resistance of colorectal cancer to EGFR inhibitors resides in C-IC (25).

In the majority of colorectal C-IC, isolated as xenospheres, EGFR was highly expressed. However, those harboring *RAS* mutations (*RAS*^{mut}) were completely autonomous in their growth, insensitive to EGF, and refractory to EGFR inhibitors (even when cultured in the presence of EGF). This confirmed that, in colorectal C-IC, *RAS* constitutive activation sustains a bypass track compensatory mechanism of cell proliferation and survival. On the contrary, xenospheres harboring wild-type *RAS* pathway genes and normal *ERBB2* gene copy number (for brevity referred to as *RAS*^{wt}) displayed strong dependence on EGF for their growth and survival, and, in some cases, expressed autocrine loops of EGFR ligands. As expected, when *RAS*^{wt} xenospheres were grown in the presence of exogenous or autocrine EGF as the sole growth factor, cetuximab was sufficient to fully prevent proliferation and survival. However, when the same cells were cultured in a medium produced by cancer-associated fibroblasts, secreting a plethora of cytokines and growth factors, and mimicking the tumor microenvironment (27), a strong inherent resistance to EGFR inhibition became evident (25).

MET: A Dangerous Coregulator of Colorectal Cancer Stem Cells

Among factors produced by cancer-associated fibroblasts, HGF is prominent (25, 27). Its receptor MET was widely expressed, at high levels, in all xenospheres, and, unlike EGFR, was mostly downregulated when cells were cultured in prodifferentiating conditions. This observation strongly associates MET functions with the stem/progenitor status. When *RAS*^{wt} xenospheres were cultured in the medium produced by cancer-associated fibroblasts, in spite of the presence of multiple growth factors, MET inhibition was sufficient to abolish proliferation, survival, and the underlying *RAS*/*ERK* and *PI3K*/*AKT* signaling. On the contrary, in the same setting, EGFR inhibition was ineffective (25). These results prompted us to reconsider the importance of MET inhibitors for colorectal cancer therapy. Indeed, previous preclinical models, based on xenografts in regular *NOD/SCID* mice, failed to show a response to MET inhibitors (39). However, as mentioned (see above), dependence of HGF is difficult to assess in the mouse, because murine HGF does not efficiently cross-react with human MET expressed by colorectal C-IC. Thus, a preclinical model was set up to ensure the presence of human HGF, either by inducing an HGF autocrine loop in xenospheres or by transplanting xenospheres in a genetically engineered *NOD/SCID* mouse, where the endogenous gene was replaced by the human *HGF* gene. In this setting, the superior efficacy of the combination of EGFR and MET inhibitors for the therapy of *RAS*^{wt} tumors was striking. Interestingly, this combination treatment was accompanied by signs of stem cell marker loss and increased tumor differentiation (25).

Studying the Therapeutic Response at Cancer Stem Cell Level: Opportunities and Pitfalls

To date, a major limitation to study the therapeutic response of cancer stem cells has been the lack of integration between genomic and functional traits (30). We can now move ahead and bridge the gap by isolating cancer stem cells (or C-IC) from individual tumors, by validating the correspondence between the genetic profile of the original tumor and the stem cell derivatives, and by studying in this setting the mechanism of sensitivity or resistance to targeted therapies.

In the past, cell lines have been invaluable for identifying and validating targets for cancer therapy, but C-IC growing in cultures may open a new era of discovery, as they more faithfully retain the genetic make-up of the original tumor (40). This is essential when assessing the mechanisms of intrinsic and acquired resistance, which often depend on cell-autonomous-specific genetic lesions. Besides the conceptual implications, the practical value of C-IC *in vitro* cultures resides in their property to indefinitely propagate while retaining a remarkable genetic stability. This value is independent of the definition of “cancer stem cells,” a concept still

controversial and suffering from prejudicial interpretation that leaves uncomfortable many researchers working at the interface between bench and bed. Of course, drawbacks exist, first of all the cumbersome technology for isolating stem cells from human tumors. However, the improvement of platforms of patient-derived xenopatients—feasible for the majority of tumors—may significantly increase the efficiency of cell derivation. By characterizing cancer stem cells *in vitro*, the interplay between the genetic make-up and the signaling circuits inherently operating in these cells can be recognized. Many of these circuits are expected to depend on extracellular cues emanated from the tumor microenvironment, which often plays the role of a “cancer stem cell niche.” The impact of these cues on the response to targeted therapies may be underestimated in classical xenopatients, if, as in the case of HGF, murine environmental factors weakly cross-react with human receptors. This limitation can be now circumvented by genetic modification of either cancer stem cells or the murine host, the spheropatient (Fig. 1). In sum, the population-based studies in xenopatients can be integrated by a reductionist approach in isolated cancer stem cells, and a new hypothesis emerging *in vitro* can be assessed by a further xenografting step in a more sophisticated mouse recipient. The hope is that such a xenopatient–xenosphere–spheropatient system, in colorectal as well as in other tumors, may offer a more rapid and robust flux of information reverberating to the patient. This information should contribute to rigorously define the sensitivity of the critical cellular target (the cancer stem cell) to the targeting agent, to recognize the factors supporting primary and secondary resistance, and to identify biomarkers for monitoring target inhibition. Sad to notice, but these obvious actions, required to predict and control the therapeutic response, are often slackly implemented in the translational setting, and fail to guide the design of clinical trials. As a result, the clinical and molecular interpretation of the therapeutic outcomes may remain inconclusive. The case of combined inhibition of EGFR and MET in lung cancers (41), recently discontinued for lack of clinical benefit, is one of the last, paradigmatic examples.

Disclosure of Potential Conflicts of Interest

P.M Comoglio is a consultant/advisory board member for Metheresis Translational Research SA. No potential conflicts of interest were disclosed by the other authors.

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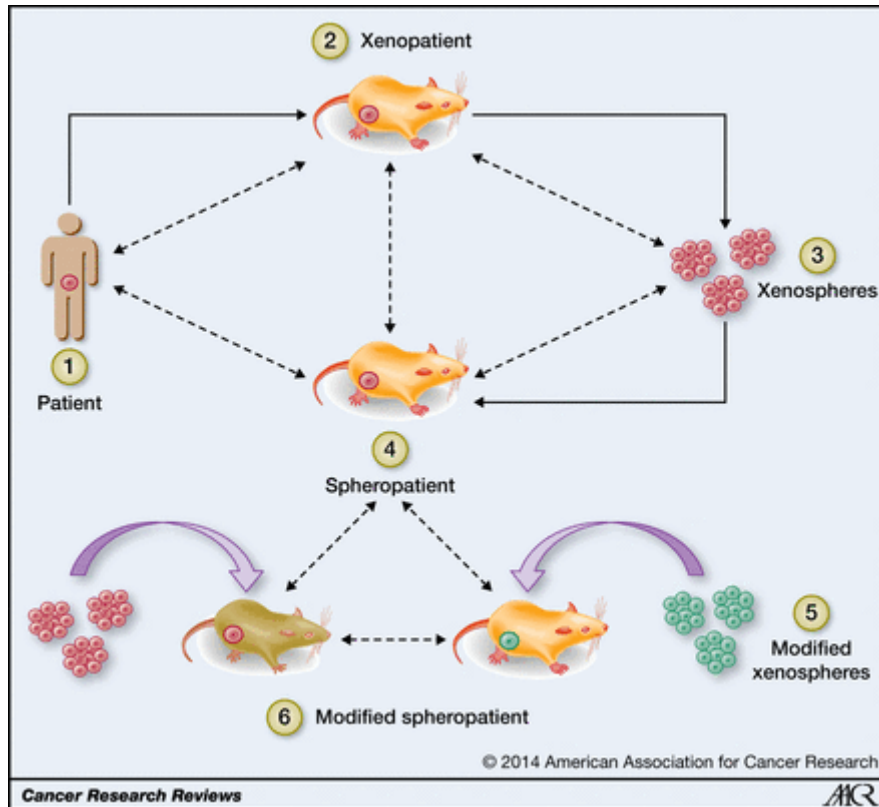


Figure 1.

The xenopatient–xenosphere–spheropatient platform. The patient's surgical sample (1) is xenografted in NOD/SCID mice and propagated (xenopatient, 2). From these xenografts, cancer-initiating cells (cancer stem cells) are selected and expanded in stem cell medium (xenospheres, 3). After transplantation in mice (spheropatient, 4), these cells generate tumors that faithfully retain the genotypic and phenotypic features of the original tissues. Xenospheres provide an in vitro model to identify the cell-autonomous and non-cell-autonomous determinants of sensitivity and resistance to therapies that cannot be fully elucidated in vivo. Both xenospheres (5) and the recipient mice (6) are amenable to genetic manipulation so as to assess experimental hypothesis. Integrated information (dotted arrows) among the patient and the different experimental settings fosters discovery and validation of new therapeutic targets.