

IN FOCUS

Compensatory Pathways in Oncogenic Kinase Signaling and Resistance to Targeted Therapies: Six Degrees of Separation

Livio Trusolino and Andrea Bertotti

Summary: The efficacy of targeted therapies against mutationally activated kinases is typically limited by the engagement of growth-promoting cues that compensate for inhibition of the targeted kinase. Initial studies have highlighted the contribution of genomic alterations, functional characteristics, and signaling feedback loops—all intrinsic to cancer cells—in sustaining such substitute activities. New evidence now indicates that the relative expression of growth factor ligands produced by the tumor microenvironment can relay redundant survival pathways, which may broadly impair responsiveness to kinase inhibitors. *Cancer Discov*; 2(10); 876–80. ©2012 AACR.

INTRODUCTION

More than 40 years after the signing of the National Cancer Act, researchers, oncologists, and patients are harvesting the fruits of long and silent efforts at laboratory benches and in hospital wards. Mutant genes with causative function in cancer have been identified. Inhibitors that target the faulty products of many such genes—largely those with a kinase activity, which is easily “druggable”—have been developed. Finally, some of these targeted agents have entered the clinic and are now effectively used in patients. The pace and success of scientific discovery in this investigational arena are epitomized by several examples. Mammary tumors with amplification of the *ERBB2* gene, which leads to overproduction and abnormal activation of the encoded tyrosine kinase receptor HER2, are sensitive to monoclonal antibodies and chemical inhibitors that block HER2 function. Non-small cell lung cancers that exhibit mutational activation of the EGF receptor (EGFR) tyrosine kinase respond to EGFR inhibitors, whereas those with an intrachromosomal translocation of *anaplastic lymphoma kinase* (*ALK*) express constitutively active *ALK* fusion proteins that confer hypersensitivity to *ALK* inhibitors. Melanomas displaying a mutationally active *BRAF* kinase undergo massive regression following treatment with specific *BRAF* inhibitors (1).

All these responses in genotype-selected tumors have been met with enthusiasm by the cancer community. “Profile tumors, find the target, inhibit the target” has become such a pervasive mantra that several cancer centers have started establishing institutional programs of personalized cancer medicine. The collective aim is the identification of patient-specific alterations in panels of potentially “actionable” gene

mutations as a means to inform rational therapeutic decisions. Albeit commendable, initiatives like these need to contend with one important notion: The presence of a genetic lesion that is known to be a potential driver of malignant proliferation in a particular, genetically defined cancer subtype does not predict *a priori* response to treatment. For the sake of numbers, only about 30% of patients with *HER2*-amplified breast cancer, 50% of patients with *BRAF*-mutant melanoma, and 60% of patients with *EGFR*-mutant or *ALK*-translocated lung cancer respond to blockade of the corresponding targets (1).

The mechanisms underlying resistance to targeted therapies can be summarized in 5 individual but interconnected principles: the co-existence of DNA alterations additional to those peculiar to the inhibited target, tumor genetic heterogeneity, tumor cellular heterogeneity, negative feedbacks, and tissue-specific modifiers. Here, we discuss the salient aspects of such principles and also elaborate on a sixth, recently proposed scenario: growth factors in the tumor microenvironment as determinants of therapeutic refractoriness (Fig. 1). Importantly, all these mechanisms revolve around the common theme of functional compensation: cancer cells are poorly sensitive to neutralization of the bona fide oncogenic driver whenever independent genetic anomalies, signaling pathways, biologic features, or extracellular ligands provide substitute survival cues that relieve the consequences of target inactivation.

CELL-AUTONOMOUS DETERMINANTS OF RESISTANCE

Whole-scale sequencing of cancer genomes has shown that the mutational load accrued during neoplastic progression is extremely heavy. Although most of these DNA alterations are coincident bystanders with no or limited functional significance, the role of a defined genetic lesion in sustaining the transformed phenotype is inevitably influenced by the overall mutational milieu in which the altered gene resides. This appears to be particularly evident in the case of *HER2*-amplified breast cancer. A critical *HER2* downstream transducer is phosphoinositide 3-kinase (PI3K), a lipid kinase that ultimately impinges on the expression of mitogenic and anti-apoptotic effectors such as cyclins D and E. PI3K activity

Authors' Affiliation: Laboratory of Molecular Pharmacology, Institute for Cancer Research and Treatment, and Department of Oncological Sciences, University of Torino School of Medicine, Candiolo (Torino), Italy

Corresponding Author: Livio Trusolino, Laboratory of Molecular Pharmacology, Institute for Cancer Research and Treatment, Strada Provinciale 142, km 3.95, Candiolo (Torino) 10060, Italy. Phone: 39-011-9933202; Fax: 39-011-9933225; E-mail: livio.trusolino@ircc.it

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is negatively controlled by the PTEN phosphatase. Mammary tumors harboring *HER2* amplification along with mutational activation of PI3K, *PTEN* deletion, or amplification of the gene encoding cyclin E are poorly sensitive to *HER2* neutralization (2). This indicates that relentless activation of pathways downstream from the targeted kinase receptor—due to concurrent genetic alterations—can circumvent inhibition of the receptor itself.

A second level of complexity is provided by the divergent evolutionary trajectory of cancer cells during tumor progression. According to a recent phylogenetic reconstruction of renal cell carcinomas, spatially separated samples from the same cancer lesion display different mutational profiles, allelic imbalances, and chromosomal ploidy (3). Because of this intratumor genetic heterogeneity, only a fraction of cells may possess the mutant gene that predicts response to treatment, and possibly only a subfraction displays the contextual mutational environment that allows this gene to unleash its full malignant potential. In this case, resistance occurs on a cell population basis: when only a minority of cancer cells is genetically susceptible to therapy, tumor growth as a whole is not affected.

Therapeutic resistance may also be inherent in the hierarchical organization of some tumors—for example, breast and colorectal cancer. In such settings, a small subset of “cancer stem cells” capable of long-term replication and tumorigenicity give rise to a larger progeny of more differentiated cells that gradually lose proliferative capacity (4). The committed intermediates derived from the parental stem cells may acquire mutations that are transmitted to the differentiated descendants. However, if only the cancer stem cells contribute to tumor expansion, therapies against targets active in the differentiated tumor bulk but silent in cancer stem cells are likely to be ineffective. Importantly, cancer stem cells have proved to be innately resistant to adverse insults such as hypoxia, ionizing radiation, chemotherapy, and also targeted therapies. The differentiation of cancer stem cells affords a mechanism for producing a certain degree of phenotypic and functional heterogeneity that parallels the diversity produced by clonal evolution. Similar to intratumor genetic heterogeneity, in which resistance is caused by the compensatory activity of nonresponsive subclones, the intratumor cellular heterogeneity embodied in the cancer stem cell model implies that therapeutic resistance is caused by the prevalence of nonresponsive cellular subpopulations.

Negative feedback loops are usually operative within kinase-based oncogenic pathways to mitigate signal overflow and ensure signal adjustment in response to recurring perturbations. When kinase hyperactivation is silenced by pharmacologic treatment, loss of such self-attenuating loops may cause activation of parallel circuits and contribute to the development of resistance. A prototypical example is provided by the mTOR kinase, a regulator of cell growth and metabolism that acts both upstream and downstream of the PI3K pathway. In some cancer cells, an mTOR-dependent negative feedback loop results in the inhibition of PI3K signaling. Thus, when mTOR is inhibited, the ensuing disruption of negative feedback enhances the activity of PI3K and its effector AKT, which blunts the antiproliferative effects of mTOR inhibition (5). Interestingly, these laboratory find-

ings capture the clinical counterpart of the feedback circuit: the modest response of some patients with *PTEN*-deficient glioblastoma treated with mTOR inhibitors correlates with increased activity of AKT as a consequence of therapy (5).

Negative feedbacks also take place along the extracellular signal-regulated kinase (ERK) cascade, a master signaling axis that is triggered by RAS and terminates in the nucleus with the transcriptional modulation of genes involved in cell-cycle progression. RAF, which is present in 3 isoforms (ARAF, BRAF, and CRAF), is a direct effector of RAS and the upstream component of the ERK pathway. Inhibition of the BRAF isoform in cells with active RAS—but not in cells with active BRAF—leads to paradoxical activation of the ERK cascade by favoring the formation of BRAF-CRAF heterodimers (6). This feedback loop may attest to the anecdotal observation that approximately 10% to 15% of patients with *BRAF*-mutant melanoma treated with BRAF inhibitors develop cutaneous squamous cell carcinomas and keratoacanthomas. Indeed, such tumors are caused by BRAF-CRAF-dependent ERK activation, instigated by BRAF-selective drugs in premalignant skin cells harboring existing mutations in RAS (7). More generally, these results argue that BRAF-selective inhibitors should not be administered to patients with RAS-mutant tumors.

Interestingly, some of these feedback pathways appear to be unique to specific cell lineages rather than to specific tumor genotypes. For example, BRAF inhibition relieves a feedback loop that keeps EGFR inactive in *BRAF*-mutant colorectal cancer but not in *BRAF*-mutant melanoma (8, 9). This circuit is active in colorectal cancer and not in melanoma simply because EGFR is abundantly expressed in the former but not in the latter. The inception of EGFR-dependent signals as a consequence of BRAF inhibition leads to downstream stimulation of RAS, which again prompts BRAF-CRAF heterodimer formation. The presence of this feedback explains why, despite harboring identical *BRAF* mutations, melanoma cells are sensitive to BRAF inhibitors whereas colorectal cancer cells tend to be refractory.

Collectively, these findings underscore the inexorable impact, whatever the mechanism, of drug resistance. Of note, although we have tried to provide sufficient context in conceptual terms, we have deliberately presented only a limited number of specific, paradigmatic examples. The ways cancer cells escape targeted therapies are so elusive in essence and multifaceted in appearance that they preclude a comprehensive coverage of the topic. Recent additional information discussed below is a further token of the complexity in the field.

GROWTH FACTORS IN THE TUMOR MICROENVIRONMENT AS A CAUSE OF RESISTANCE

The tumor microenvironment is a reactive stroma replete with growth factors and cytokines that foster tumor cell proliferation, survival, and metastasis, thus exacerbating the intrinsic malignant properties of cancer cells. Using systematic screening approaches, 3 recent articles (10–12) provide strong evidence that this trophic context not only promotes cancer progression but also exerts a causal role in drug resistance.

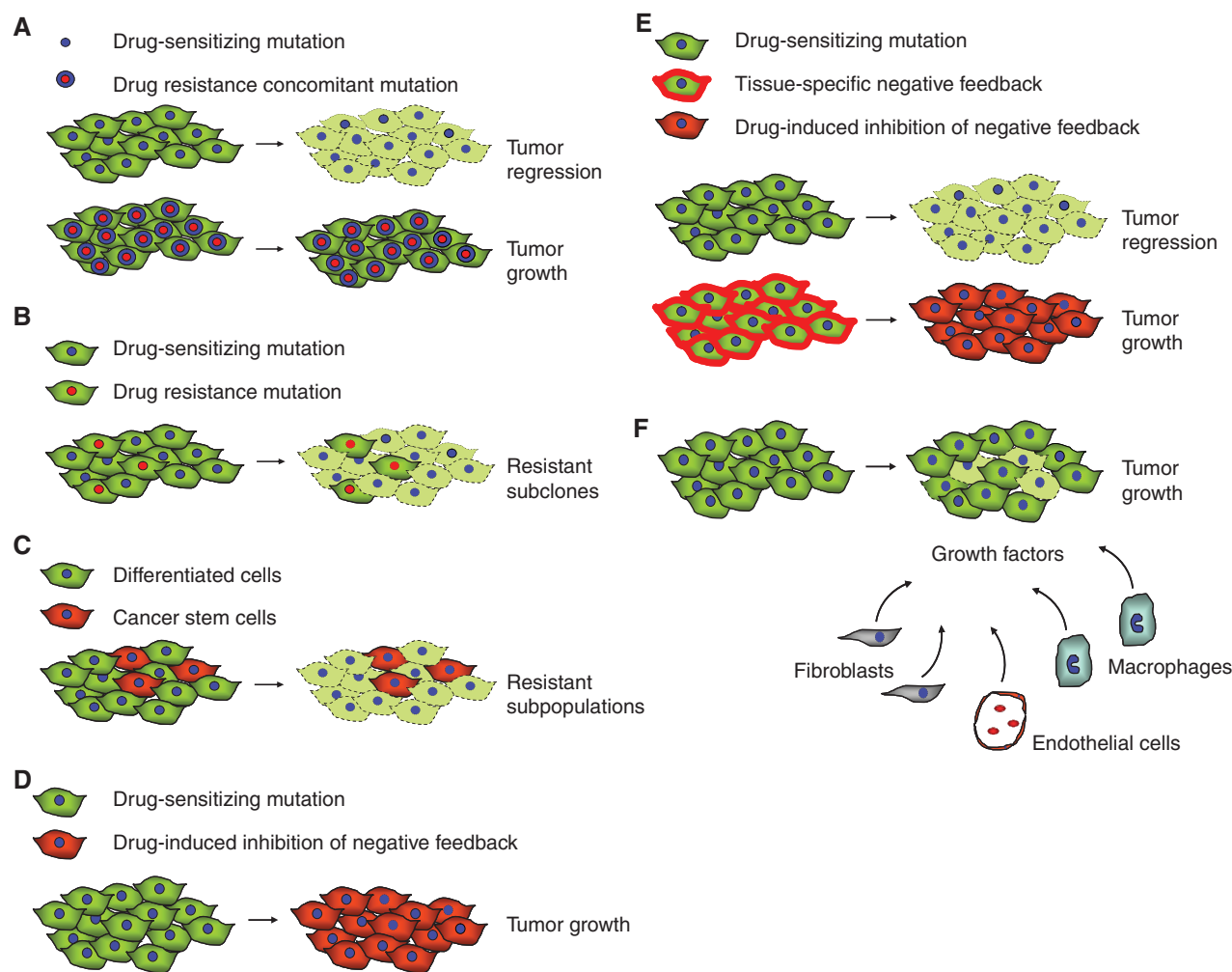


Figure 1. The 6 degrees of separation in drug resistance. Mechanisms involve (A) concurrent genetic lesions that trigger alternative signaling pathways; (B) target activity in only a fraction of tumor cells, due to genetic heterogeneity; (C) target activity in differentiated descendants but not in cancer stem cells; (D) blockade of negative feedback loops by target inhibition, with consequent hyperactivation of compensatory pathways; (E) drug-induced interruption of negative feedback loops in a tissue-specific fashion; and (F) induction of redundant survival signals by stromal growth factors produced by fibroblasts, macrophages, and endothelial cells of blood vessels.

Straussman and colleagues (10) developed a coculture system to analyze the ability of 23 stromal cell types to modulate responsiveness of 45 cancer cell lines to 35 anti-cancer compounds. They found that stromal-derived growth factors have widespread potential to confer drug resistance to targeted agents. The authors then concentrated on *BRAF*-mutant melanoma cell lines and identified stromal hepatocyte growth factor (HGF), the ligand of the MET tyrosine kinase receptor, as the major microenvironmental inducer of resistance to BRAF inhibition.

Similarly, Wilson and colleagues (11) undertook a “matrix analysis” of 41 cancer cell lines dependent on specific oncogenic kinases to analyze the influence of 6 growth factors on drug responsiveness. They observed that several different growth factors can promiscuously rescue cells from treatment sensitivity, which indicates a high degree of redundancy in the ability of growth factors to trigger alternative “salvage”

pathways in drug-inhibited cancer cells. In the specific case of HGF, the protective activity appeared to be more pronounced in HER2-hyperactive cell lines treated with a HER2 inhibitor and, in analogy with the Straussman’s study, in *BRAF*-mutant melanoma cells treated with a BRAF inhibitor. Interestingly, some cell lines in which kinase dependency was not bypassed by HGF in acute experiments (72 hours) were rescued after long-term ligand exposure. This suggests that, under the selective pressure of drug treatment, subsets of MET-expressing cells present before therapy may outcompete a prevalent population of MET-negative, initially sensitive cells whenever HGF levels increase in the tumor microenvironment. A similar mechanism was documented in *EGFR*-mutant non-small cell lung cancers, in which a small fraction of cells harboring *MET* amplification pre-exist before drug administration and undergo massive expansion, driven by stromal HGF, during EGFR inhibitor treatment (13). Intriguingly, HGF was found to stimulate the

clonogenic activity of cancer stem cells in a number of tumor types (14), suggesting that this growth factor can also promote resistance by encouraging the proliferation and maintenance of a drug-insensitive cancer stem cell compartment.

Together with *in vitro* experiments, the 2 studies present some preliminary clinical correlations: The presence of stromal HGF in bioptic specimens (10) or elevated concentrations of HGF in the plasma (11) were associated with reduced response to BRAF inhibitors in patients with *BRAF*-mutant melanoma. However, as Wilson and colleagues rightly point out (11), it is difficult to establish whether the correlation between high HGF levels and poor outcome is due to HGF-induced drug resistance or HGF-induced biologic aggressiveness, regardless of therapy.

The notion that increased levels of growth factor ligands can confer resistance to inhibitors of mutationally active kinases is corroborated by a third study from Harbink and colleagues (12). The authors exploited a high-throughput “secretome” screening platform whereby a cDNA library encoding approximately 3,500 secreted proteins was transfected in a well-by-well high-throughput format, and the resulting supernatants were tested for their ability to abrogate growth inhibition in kinase-dependent cancer cells treated with the appropriate kinase inhibitor. By this approach, a compensatory cross-talk was identified between members of the EGF family, different isoforms of fibroblast growth factor (FGF), HGF, and their respective tyrosine kinase receptors: specifically, EGF members and FGFs could bypass MET inhibition in MET-dependent cell lines and HGF could shield FGF-dependent cell lines from inactivation of the FGF receptor.

All 3 studies show that the rescue pathways activated by exogenous growth factors involve the RAS–ERK cascade and/or the PI3K–AKT axis, with variable predominance of either pathway according to the cellular context and the ligand type. They also present pharmacologic evidence that only concomitant blockade of the kinase that confers dependency and the receptor that is engaged by the protective growth factor re-installs growth inhibition; for example, inhibition of MET (or HGF) in *BRAF*-mutant melanoma resensitized cells to BRAF inactivation.

Besides the specific tumor settings analyzed in these studies, the availability of HGF in the tumor microenvironment and the expression/activity of MET in tumor cells might contribute to drug resistance in a broader perspective. Hypoxia, a hallmark of advanced tumors, induces transcriptional upregulation of *MET* (14). Many inflammatory cytokines and pro-angiogenic growth factors abundant in the tumor-reactive stroma positively modulate the expression of both MET (in the cancer cells) and HGF (in fibroblasts and tumor-associated macrophages; ref. 14). The tumor stroma is also rich in proteases that may activate latent HGF into its mature form. Accordingly, the enhanced reservoir of active ligand in the interstitial compartment and the increased expression of active receptor in cancer cells might be a general mechanism for attenuation of drug sensitivity, which would be prevalent in those neoplasms that feature a hypoxic and/or inflammatory phenotype.

The observation that growth factors induce widespread drug resistance through redundant activation of the RAS

and PI3K pathways raises 2 final implications. First, this rescue activity might also be evoked by therapies against non-kinase targets, namely, anti-endocrine regimens in breast and prostate cancer. The fact that growth factors interface with sexual hormones through RAS- and PI3K-based signaling is well documented (5); in this vein, experimental observations indicate that the antiproliferative effects of estrogen inhibition can be circumvented by hyperactivation of RAF and consequent stimulation of the ERK pathway (15). Second, and from a complementary viewpoint, secreted molecules other than growth factors could influence sensitivity to kinase-directed targeted therapies; cAMP agonists, neurotransmitters, interleukins, and other inflammatory cytokines are all able to stimulate RAS- and PI3K-dependent signaling pathways, suggesting that the response to kinase inactivation could also be affected by systemic cues additional to the “conventional” growth factors present in the tumor-reactive stroma.

The 6 degrees of separation that interconnect the various mechanisms of drug resistance highlight the pervasive nature of this phenomenon and, with it, the difficulties in identifying patients most likely to benefit from therapy. To address these challenges, we first need to address technologic opportunities. Patient stratification based on genomic biomarkers must be optimized with the use of massive DNA sequencing and bioinformatics to understand the logics of multiple concurrent genetic lesions. Phosphoproteomics, transcriptional profiles, and computer-assisted pathway analysis can inform real-time decisional algorithms to identify cancer-causative signals and anticipate compensatory feedback loops. Finally, all this multidimensional knowledge should be gathered dynamically, so as to adjust treatment strategies according to the existing or emerging pathway of resistance. As mentioned, complexity, resilience, flexibility, and adaptation are the conceptual hallmarks of signal compensation. But these peculiarities can also become the operational assets of a new generation of molecular approaches to treat cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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