Linking oncogenesis and immune system evasion in acquired resistance to EGFR-targeting antibodies
Lessons from a preclinical model

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Searching for biomarkers that associated with the acquired resistance of malignant cells to epidermal growth factor receptor (EGFR)-targeting monoclonal antibodies is crucial to improve the clinical benefits of these therapeutic agents. We have recently demonstrated that molecular alterations in both oncogenic and immunological pathways may be responsible for such an insensitivity. Our findings suggest that a combination of targeted anticancer agents and immunomodulatory drugs may be useful for overcoming the acquired resistance of cancer cells to EGFR-specific monoclonal antibodies.

The epidermal growth factor receptor (EGFR) arguably constitutes the most comprehensively studied molecular target for anticancer therapy of the past three decades. As a result of this investigational effort, multiple EGFR-targeting monoclonal antibodies (mAbs) such as cetuximab, panitumumab, and nimotuzumab are nowadays commercially available for use in cancer patients. These agents exert profound, but often temporary, antitumor effects in subsets of patients bearing advanced solid neoplasms. Although previous studies have identified somatic mutations in KRAS, BRAF, PIK3CA, and PTEN as biomarkers for the intrinsic resistance of malignant cells to EGFR-targeting mAbs in patients, the molecular basis for acquired resistance remains poorly understood. A few groups have specifically investigated the mechanisms whereby become resistant to EGFR-specific mAbs and undergo disease progression upon treatment. One major barrier to address this question in the clinical setting is the limited access to malignant tissues post-treatment. Using repeated tumor biopsies, Montagut and colleagues identified a mutation in the extracellular domain of EGFR that renders colorectal cancer patients resistant to cetuximab.[1] This said, even when post-treatment neoplastic tissues are available, sampling biases often confound the interpretation of results, as only a small portion of tumors is biopsied, preventing the assessment of both intra- and inter-lesion genetic heterogeneity. Current efforts are being focused on the identification of alternative tissues for the assessment of resistance biomarkers. The DNA released by cancer cells in the blood stream (a form of liquid biopsy) may represent a convenient starting material to carry out these studies. Using this approach, Bardelli and colleagues have recently described secondary KRAS[2,3] or MET[4] mutations as novel sources of acquired resistance to cetuximab or panitumumab. Nonetheless, our knowledge on the potential mechanisms of acquired resistance arises, in large part, from mechanistic insights provided by preclinical studies. Unfortunately, preclinical models of resistance may not reproduce all the aspects of the clinical response to EGFR-targeting mAbs, because resistance tumor variants are established in vitro upon the long-term exposure to EGFR-targeting mAbs, or in vivo upon the administration of these mAbs to immunodeficient mice bearing tumor xenografts. Moreover, studies have largely been focused on the escape of cancer cells from EGFR-targeting mAbs associated with the blockade of EGFR-dependent oncogenic signals, even though accumulating evidence suggests that EGFR promotes tumor progression not only through oncogenic signaling but also by modulating the interactions of cancer cells with the immune system.

In this context, our group generated a surrogate antibody that recognizes murine EGFR named 7A7. Previous studies based on immunocompetent mice (a complete autologous scenario) demonstrated that 7A7 exert antitumor effects via mechanisms that are identical to those ascribed to mAbs targeting human EGFR, namely, (1) EGFR
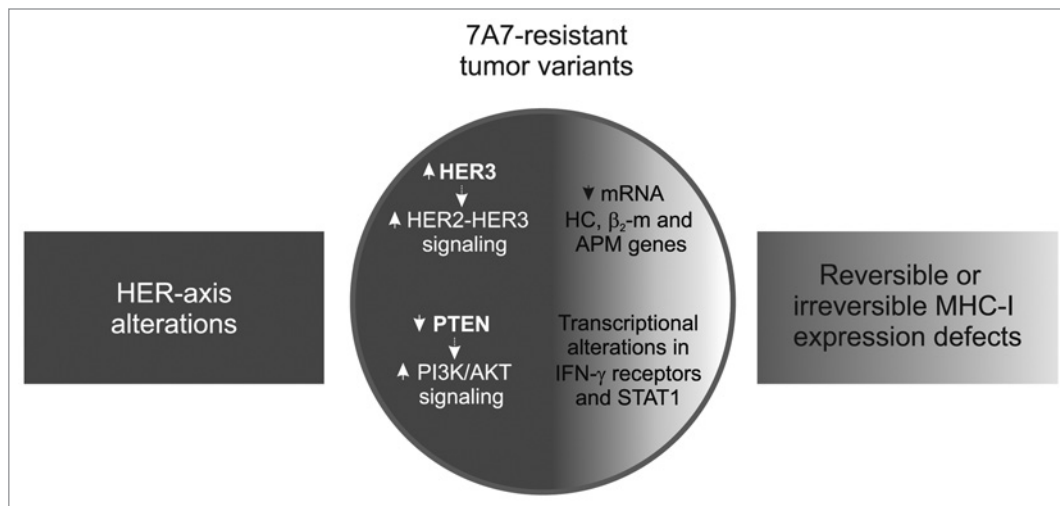
Figure 1. Molecular mechanisms underlying the acquired resistance of malignant cells to the EGFR-targeting antibody 7A7. APM, antigen-processing machinery; β2-m, β2-microglobulin; EGFR, epidermal growth factor receptor; HC, heavy chain; IFNγ, interferon γ; PI3K, phosphoinositide-3-kinase; PTEN, phosphatase and tensin homolog; STAT1, signal transducer and activator of transcription 1.

signaling inhibition, (2) antibody-dependent cell-mediated cytotoxicity, and (3) T cell activation.[5,6] Collectively, these results validated 7A7 as a valuable preclinical tool and prompted us to use it for modeling the acquired resistance of cancer cells to anti-EGFR antibodies in vivo.[7] Our studies demonstrated that, similar to what observed in clinical scenarios, the acquisition of resistance represents an important obstacle against the therapeutic efficacy of 7A7 in mice. Indeed, 26 out of 29 primary cultures generated from 7A7-treated metastases were insensitive to the administration of 7A7 in vivo and in vitro, revealing a high frequency of acquired resistance. Mechanistic studies performed with seven of these 7A7-resistant tumor variants revealed an overlapping pattern of molecular changes. To the best of our knowledge, we were the first to observe convergent alterations in oncogenic and immunological signaling pathways in tumor specimens resistant to EGFR-specific antibodies. In particular, the overexpression of *v-erb-b2* avian erythroblastic leukemia viral oncogene homolog 3 (ERBB3, best known as HER3) and PTEN deficiency occurred concomitantly with reversible and irreversible defects in the expression of MHC class I molecules. Such defects in MHC class I expression stemmed from a significant decrease in the levels of mRNAs coding

for MHC class I heavy chains (HCs), β_2-microglobulin (β_2-m) and various components of the antigen-processing machinery (APM), as well as from transcriptional alterations in the interferon γ (IFNγ) signaling pathway (**Fig. 1**). Our results identify the acquired resistance of cancer cells to EGFR-targeting mAbs as a multifactorial, hence constituting a significant challenge for experimental researchers and oncologists. In addition, our data suggest that the antitumor efficacy of EGFR-specific mAbs may be improved by combination therapies that target the molecular complexity of this phenomenon.

To our knowledge, we were the first to take the immunomodulatory activity of EGFR into consideration for uncovering new mechanisms of resistance to EGFR-specific mAbs. This reflects a novel conceptual paradigm suggesting that both on-target and off-target mechanisms may contribute to the development of acquired resistance. Molecular alterations in the EGFR signaling axis have previously been identified as potential mechanisms whereby cancer cells evade the cytotoxicity of EGFR-targeting mAbs. However, defects in the expression of MHC class I molecules have not been previously associated with this phenomenon. Our earlier findings led us to focus on the ability of 7A7 to induce the immunogenic apoptosis of cancer cells, and hence a tumor-specific

cytotoxic T lymphocyte (CTL) response,[6] as the principal mechanism that would generate defects in MHC class I expression. The presence of IFNγ signaling defects in 7A7-resistant tumor variants (a typical mechanism of escape from T cells) and recent data showing that EGFR-specific CD8+ T cells may contribute to the clinical response of cetuximab-treated cancer patients,[8] reinforced this rationale. Nevertheless, a mechanistic link between changes in oncogenic EGFR signaling and MHC class I alterations cannot be ruled out. This hypothesis is supported by experimental results from several groups. First, HER2 signaling (which in our model is hyperactivated upon HER3 upregulation) results in the downregulation of MHC class I molecules.[9] Second, the blockade of EGFR by cetuximab increased MHC class I expression.[10] Further experiments with our model are required to define the relative contribution of CTL responses vs. the blockade of EGFR signaling to the defects in MHC class I expression linked to 7A7 resistance.

In conclusion, we described a model with strong implications for understanding the acquired resistance of cancer cells to anti-EGFR antibodies. Our findings link this paradigm of resistance not only to cancer cell-intrinsic oncogenic circuits, but also to immunoregulatory components. Moreover, our data support the importance of murine tumor models as a

useful system to identify new biomarkers of acquired resistance to EGFR-specific mAbs. Translational research efforts will guide the next generation of clinical studies to overcome resistance and to increase the efficacy of EGFR-targeting antibodies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Montagut C, Dalmases A, Bellosillo B, Crespo M, Pairet S, Iglesias M, Salido M, Gallen M, Marsters S, Tsai SP, et al. Identification of a mutation in the extracellular domain of the Epidermal Growth Factor Receptor conferring cetuximab resistance in colorectal cancer. Nat Med 2012; 18:221-3; PMID:22270724; http://dx.doi.org/10.1038/nm.2609

2. Misale S, Yaeger R, Hobor S, Scala E, Janakiraman M, Liska D, Valtorta E, Schiavo R, Buscarino M, Siravegna G, et al. Emergence of KRAS mutations and acquired resistance to anti-EGFR therapy in colorectal cancer. Nature 2012; 486:532-6; PMID:22722830

3. Diaz LA Jr., Williams RT, Wu J, Kinde I, Hecht JR, Berlin J, Allen B, Bozic I, Reiter JG, Nowak MA, et al. The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers. Nature 2012; 486:537-40; PMID:22722843

4. Bardelli A, Corso S, Bertotti A, Hobor S, Valtorta E, Siravegna G, Sartore-Bianchi A, Scala E, Cassingena A, Zecchin D, et al. Amplification of the MET receptor drives resistance to anti-EGFR therapies in colorectal cancer. Cancer Discov 2013; 3:658-73; PMID:23729478; http://dx.doi.org/10.1158/2159-8290.CD-12-0558

5. Garrido G, Lorenzano P, Sánchez B, Beausoleil I, Alonso DF, Pérez R, Fernández LE. T cells are crucial for the anti-metastatic effect of anti-epidermal growth factor receptor antibodies. Cancer Immunol Immunother 2007; 56:1701-10; PMID:17415565; http://dx.doi.org/10.1007/s00262-007-0313-4

6. Garrido G, Rabasa A, Sánchez B, López MV, Blanco R, López A, Hernández DR, Pérez R, Fernández LE. Induction of immunogenic apoptosis by blockade of epidermal growth factor receptor activation with a specific antibody. J Immunol 2011; 187:4954-66; PMID:21984704; http://dx.doi.org/10.4049/jimmunol.1003477

7. Garrido G, Rabasa A, Garrido C, López A, Chao L, García-Lora AM, Garrido F, Fernández LE, Sánchez B. Preclinical modeling of EGFR-specific antibody resistance: oncogenic and immune-associated escape mechanisms. Oncogene 2013; Forthcoming; PMID:23975426; http://dx.doi.org/10.1038/onc.2013.288

8. Srivastava RM, Lee SC, Andrade Filho PA, Lord CA, Jie HB, Davidson HC, López-Albaitero A, Gibson SP, Gooding WE, Ferrone S, et al. Cetuximab-activated natural killer and dendritic cells collaborate to trigger tumor antigen-specific T-cell immunity in head and neck cancer patients. Clin Cancer Res 2013; 19:1858-72; PMID:23444227; http://dx.doi.org/10.1158/1078-0432.CCR-12-2426

9. Herrmann F, Lehr HA, Drexler I, Sutter G, Hengstler J, Wollscheid U, Seliger B. HER-2/neu-mediated regulation of components of the MHC class I antigen-processing pathway. Cancer Res 2004; 64:215-20; PMID:14729627; http://dx.doi.org/10.1158/0008-5472.CAN-2522-2

10. Pollack BP, Sapkota B, Cartee TV. Epidermal growth factor receptor inhibition augments the expression of MHC class I and II genes. Clin Cancer Res 2011; 17:4400-13; PMID:21586626; http://dx.doi.org/10.1158/1078-0432.CCR-10-3283