Editorial

How apoptosis and epithelial-to-mesenchymal transition are nested in EGFR inhibitors resistance in lung cancer

Simon Garinet[1,2], Audrey Didelot[1], Elena Garelli[3], Karine Pallier[4], Hélène Blons[1,2], Antoine Legras[1,5]

[1]INSERM UMR-S1147, CNRS SNC 5014, Saints-Pères Research Center, 45 rue des Saints-Pères Paris-Descartes University, Sorbonne Paris Cité University, Paris 75006, France; [2]Molecular Biology Department, Georges Pompidou European Hospital, Assistance Publique - Hôpitaux de Paris, Paris 75015, France; [3]Thoracic Surgery Department, Cochin Hospital, Sorbonne Paris Cité University, Assistance Publique des Hôpitaux de Paris, Paris 75014, France; [4]Haute-Vienne Coordination Center in Oncology, Limoges University Hospital Center, Limoges 87000, France; [5]Thoracic Surgery Department, Trousseau Hospital, Tours University Hospital Center, Chambray-lès-Tours 37170, France

Correspondence to: Antoine Legras, MD, PhD. Thoracic Surgery Department, Trousseau Hospital, Tours University Hospital Center, Avenue de la République, Chambray-lès-Tours 37170, France. Email: antoine.legras@chu-tours.fr.

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Non-small cell lung cancer (NSCLC) is today the first cancer worldwide and represents the first cause of cancer-related death. The identification of *EGFR* activating mutations and development of specific EGFR inhibitors (EGFRi) such as tyrosine kinase inhibitor (TKI) has changed the management of metastatic NSCLC. However, TKI resistance progressively appeared and *EGFR* secondary resistance mutation have been identified such as the most frequent T790M (1). Also, the epithelial-to-mesenchymal transition (EMT) was identified as a complex molecular and cellular process involved in tissue remodeling that plays essential roles in cell invasion, migration and drug resistance in many cancer types including NSCLC (2). During EMT, cells undergo conversion from epithelial to mesenchymal state (3). Epithelial differentiated characteristics are lost, including cell-cell adhesion, planar and apical-basal polarity and lack of mobility. On the contrary, mesenchymal features are briefly acquired, such as cell mobility, invasiveness, gain of stem cell properties and a reinforced resistance to apoptosis. After migration, cells may reverse their phenotype through the mesenchymal to epithelial transition (MET) (4).

In their manuscript entitled "Epithelial-to-mesenchymal transition antagonizes response to targeted therapies in lung cancer by suppressing BIM", published at the beginning of 2018, in the well-recognized Clinical Cancer Research journal, Kyung-A Song and co-authors found that mesenchymal *EGFR* mutant lung cancers were resistant to EGFRi-induced apoptosis *via* insufficient expression of BIM, preventing cell death despite potent suppression of oncogenic signaling following EGFRi treatment (5). They initially studied available public datasets, from CCLE and ExpO, to describe an inverse link between Vimentin/ZEB1 and BIM. Their conclusions were drawn after several investigations using real-time quantitative polymerase chain reaction to quantify expression levels of Twist1, ZEB1, Bim and β-actin to characterize EMT, and investigated also different EMT-pathways, based on Twist1 plasmid insertion in NSCLC cell lines, and TGF-β induced EMT. Similarly, BIM was assessed at both the mRNA and protein level. Furthermore, different ways of EGFRi resistance were evaluated, combining different drugs and different models. Mechanistically, they observed that the EMT transcription factor ZEB1 inhibits BIM expression by binding directly to the BIM promoter and repressing transcription. Furthermore, they interestingly found that derepression of BIM expression by depletion of ZEB1 (using shRNA and siRNA methods) or treatment with the BH3 mimetic ABT-263 to enhance "free" cellular BIM levels both led to re-sensitization of mesenchymal EGFR mutant cancers to EGFR inhibitors. This re-

sensitization was observed in cell cultures but also in a xenograft mouse model (SCID mice and subcutaneous tumor cells injection), and confirmed in a recent study, showing that BCL2-inhibitors could restore sensitivity to osimertinib specifically in TWIST1 overexpressed cells (6). Therefore, re-establishing an apoptotic response in EMT-mediated EGFR mutant NSCLC resistant cells is a promising pharmaceutical strategy, in the landscape of a clinical trial (clinical trial number NCT02520778) using ABT-263 (navitoclax) with EGFRi in *EGFR* mutant NSCLC. Finally, they also showed that this relationship between EMT and loss of BIM was not restricted to *EGFR* mutant NSCLC as it was also observed in *KRAS* mutant NSCLC cell lines and in public datasets (CCLE).

This work must be pointed out, considering the excellent scientific tracking and the evidence, in their results, of a significant improvement in the neutralization of EMT. Transposition of these results into reality seems to be the necessary next step, in a clinical trial. The strength also of this work is the correlation between results obtained in cell lines cultures and in *in vivo* tumors. Nevertheless, potential discrepancies might be observed when during the transfer of the strategy, between strictly contained cell lines models, or mouse models, due to tumor microenvironment, constantly not explored in such models.

However, apoptosis escape may also be only an issue of EMT among others, leading to imagine the combination of several therapies, targeting apoptosis, such as navitoclax, and targeting other aspects of EMT. As EMT is a dynamic and complex phenomenon, characterizing and targeting EMT remain difficult. Different models have been proposed to characterize transitions between epithelial, epithelial-mesenchymal state, and mesenchymal phenotypes, using various terms in literature: partial EMT (7), intermediate EMT (8), incomplete EMT (9), hybrid epithelial/mesenchymal (10). MiR-200/ZEB1 seems to behave as a regulator enabling transitions among the constantly evolving EMT phenotypes (11). Finally *in vivo* tumor classification remains a challenge not only because EMT is a dynamic phenomenon but also because EMT markers are heterogeneously expressed with intra-tumor heterogeneity. EMT characterization may probably be restricted to the tumor invasive front (7). Thus, EMT represents a challenging therapeutic target due to their heterogeneous expression and to the complexity of the EMT regulation network.

Inhibition of EMT could restore senescence and apoptosis capacity (2,12). In a recent review, we and others reviewed distinct strategies to target EMT through:

- Extracellular inducers of EMT, like TGF-β blockade (fresolimumab), HIF-1α downregulation (13), MMP inhibitors (14)
- EMT-transcription factors inhibition, targeting TWIST1 (15), PRMT1 (16), glycosylation (17), chromatin (18).
- Downstream effectors of EMT inhibition (19).

MicroRNAs, as EMT regulators, have emerged as a class of therapeutics targets (2). However there are some difficulties because of multiple targets per miRNAs. This can either be harmful due to collateral adverse events but it may also be of interest as miRNA blockage could inhibit a pathway at different levels. Therapeutic strategy could be either direct administration of anti-miRNAs (antisense miRNAs) to block oncomiRs, or restoration of tumor suppressor miRNAs expression (2). In NSCLC, enforced expression of miR-145 inhibited EMT and metastatic ability (20). Sato *et al.* showed that the introduction of miR-200c using pre-miR-200c caused LIN28B suppression in cells with acquired EGFR-TKI resistance that harboured EMT features (HCC4006 after chronic exposure to gefitinib) (21).

Nevertheless, inhibiting or reversing EMT could also lead to a serious adverse event: favouring MET and thus colonisation of metastatic sites by circulating tumor cells (22,23). Furthermore, tumor heterogeneity and the dynamic of EMT encourages the development of multimodal strategy, not focusing only on a single EMT-related target. As examples, combination of anti-miRNA with chemotherapy strategies and also models of miRNA replacement therapy were tested *in vitro* and in mouse models (21,24). Van Roosbroeck *et al.* proposed the use of cisplatin and anti-miR-155 in a mouse model of athymic nude mice (intrapulmonary injections of A549 cells stably infected with lentivirus containing a miR-155-overexpressing lentiviral vector) with significant results in term of primary tumour size and mediastinal lymph nodes (24). Here, by stimulating pro-apoptosis effectors, down-stream to EMT, as investigated by Song and co-authors, might not enhance malignant MET.

Finally, this article should be emphasized based on the rigorous work and on the potential near-future application, in the complex and large field of EMT in cancer.

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Footnote

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