

Paradoxical functions of ZEB1 in *EGFR*-mutant lung cancer: tumor suppressor and driver of therapeutic resistance

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Lung cancer is the leading cause of cancer-related death in the United States and worldwide. While the 5-year survival for lung cancer is a dismal 15%, there have been significant advances in treating patients with non-small cell lung cancer (NSCLC) in the past decade. These recent advances stem from the shift to classifying patients with NSCLC into distinct subtypes based upon the molecular driver, which include *KRAS* mutations (~25%), *EGFR* mutations (~15%), *ALK* translocations (~8%), and *MET* amplification/mutations (~6-7%) (1,2). A subset of patients, such as those patients with *EGFR*- and *ALK*-driven NSCLC, have benefitted significantly from the use of tyrosine-kinase inhibitors (TKIs) that specifically inhibit these oncogenes. While those patients with *EGFR*-mutant NSCLC have benefitted from the use of *EGFR* TKIs, therapeutic resistance to these targeted therapies is inevitable (3). Studies characterizing the resistance mechanisms to *EGFR* inhibitors (*EGFRi*) have identified multiple mechanisms, which include gatekeeper mutations (T790M) within *EGFR* (49%), *MET* amplification (5%), and transformation to small-cell lung cancer (14%) (3). Interestingly, an epithelial-mesenchymal transition (EMT) phenotype has been identified as a driver of resistance to *EGFRi* in potentially as many as 20% of *EGFR*-mutant lung cancer patients (4). Initial studies investigating the role of EMT in *EGFRi* resistance identified the *AXL* kinase and its ligand *GAS6* as drivers of EMT-mediated *EGFRi* resistance (4). However, little is known about other drivers of EMT-mediated resistance. Additional studies are needed to identify both novel drivers of EMT and therapeutic strategies to overcome EMT-mediated *EGFRi* resistance.

Interestingly, Zhang *et al.* recently demonstrated that the EMT transcription factor, *ZEB1* is a potential driver of EMT-mediated resistance to *EGFRi* in *EGFR*-mutant NSCLC (5). In this report, the authors demonstrated that *ZEB1* has opposing functions in *EGFR*-mutant NSCLC, both inhibiting *EGFR*-driven tumorigenesis and promoting resistance to *EGFRi* (Figure 1). The function of *ZEB1* as a tumor suppressor appears to be oncogenic driver specific as *ZEB1* expression inhibited both soft agar and xenograph growth in *EGFR*-mutant NSCLC cell lines, while it promoted soft agar and xenograph growth in *KRAS*-mutant NSCLC. However, this tumor suppressive role of *ZEB1* seems to be independent of its ability to induce EMT, given that *ZEB1* induced EMT in both *KRAS*- and *EGFR*-mutant cell lines. Further investigation of the mechanisms by which *ZEB1* suppressed *EGFR*-driven NSCLC revealed that *ZEB1* suppression of *miR-200c* expression was required for this tumor suppressive phenotype. *ZEB1* has been previously established to inhibit *miR-200c* expression, and suppression of *miR-200c* is required for *ZEB1*-mediated EMT and invasion (5,6). Interestingly, the authors were able to demonstrate that a target of *miR-200c*, *NOTCH1*, directly suppresses *ERRB3* expression, a member of the *EGFR*-family of receptors that has been shown to be required for *EGFR*-driven lung cancer (5). While the authors clearly demonstrated that the *ZEB1-miR-200c-NOTCH1* signaling axis is important for suppression of *ERBB3* (Figure 1), given the lack of phenotypic data presented, it remains an unanswered question if *ZEB1* suppression of *ERBB3* is required for *ZEB1*-suppression of *EGFR*-driven NSCLC. Additionally, the data presented suggests that there are

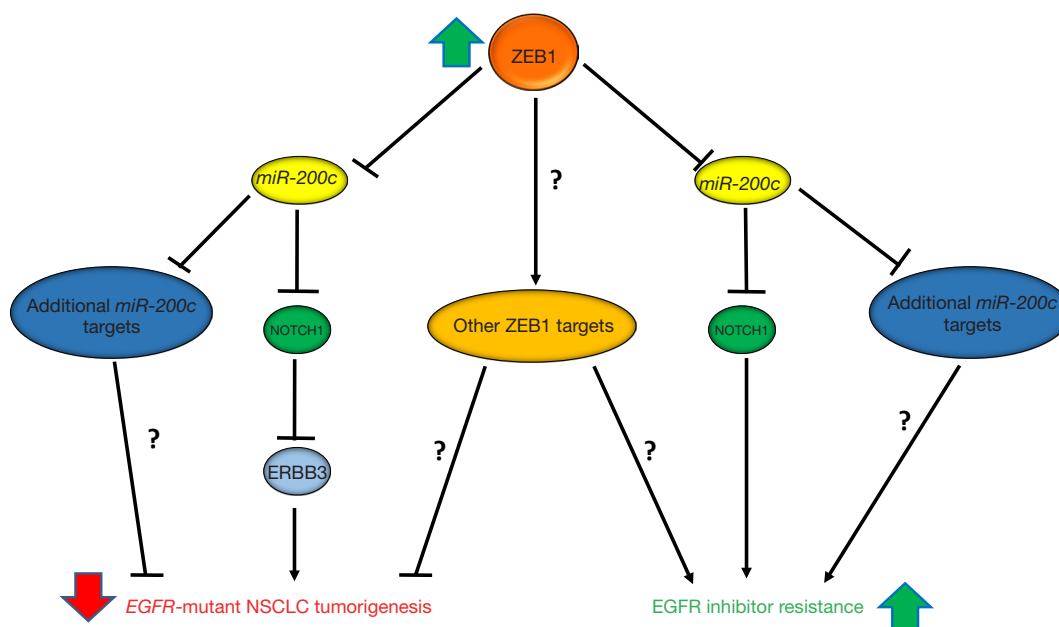


Figure 1 ZEB1 suppresses *EGFR*-mutant non-small cell lung cancer (NSCLC) tumorigenesis and mediates EGFR inhibitor resistance. In the discussed report ZEB1 was shown to inhibit *EGFR*-mutant NSCLC by repressing *miR-200c* expression, which subsequently allows for a target gene of *miR-200c*, NOTCH1, to repress ERBB3 expression. ZEB1 suppression of ERBB3 expression appears to be an important mechanism through which ZEB1 suppresses *EGFR*-mutant tumorigenesis. However, there may be additional *miR-200c* targets and potentially other ZEB1 targets that are important for the tumor suppressive role of ZEB1. Conversely, ZEB1 suppression of *miR-200c* expression and subsequent derepression of NOTCH1 was also shown to be required for ZEB1-mediated EGFR tyrosine kinase inhibitor resistance in NSCLC cell lines. Similar to the tumor suppressive function of ZEB1, ZEB1-mediated resistance most likely involves *miR-200c* targets, beyond NOTCH1, and may require additional ZEB1 target genes. Future studies are required to further delineate the mechanisms of ZEB1-mediated suppression of *EGFR*-mutant NSCLC and EGFR inhibitor resistance.

other *miR-200c* targets and potentially other ZEB1 target genes or signaling pathways that play a role in the tumor suppressive function of ZEB1 (Figure 1).

While a previous report demonstrated that ZEB1 is required for EGFRi resistance in a subset of EGFR-driven NSCLC, Zhang *et al.* provided additional evidence for ZEB1 as a mediator of therapeutic resistance to EGFRi (5,7). The authors demonstrated that ZEB1 overexpression is sufficient to cause gefitinib resistance in a gefitinib sensitive *EGFR*-mutant cell line. Additionally, this resistance is dependent upon the ability of ZEB1 to suppress *miR-200c* and partially dependent upon NOTCH1 expression (Figure 1). The authors demonstrated γ -secretase inhibitors (BMS-708163 and DAPT) are able to partially overcome gefitinib resistance in a gefitinib resistant cell line. However, the *in vitro* evidence provided with γ -secretase inhibitors suggests a relatively minor role for NOTCH1 in the setting of ZEB1-mediated resistance. The data presented suggests that there are other *miR-200c* targets or ZEB1 target

genes that are important for ZEB1-mediated resistance (Figure 1). Additionally, given the non-selectivity and dose-limiting systemic toxicities of γ -secretase inhibitors, there may be a limited role for such therapies in the setting of EMT-mediated resistance (8). However, monoclonal antibody therapies that specifically target NOTCH1 and one of its ligands, Delta-like 4 (DLL4), have been developed and some of which are in early phase trials, may provide a more advantageous therapeutic strategy given their selectivity for the NOTCH1 signaling pathway (8). Further studies, specifically *in vivo*, are needed to explore efficacy and potential toxicities of therapies that combine EGFR and NOTCH1 inhibition. Additionally, in order to determine the clinical relevance of ZEB1-mediated therapeutic resistance, studies are needed to determine how frequently ZEB1 is overexpressed in patient tumors in the EGFRi resistance setting.

While other EMT transcription factors (EMT-TF), such as ZEB2, the TWIST and SNAIL proteins, have

been implicated in therapeutic resistance, it remains unknown if other EMT-TFs play a role in resistance to EGFRi. Interestingly, ZEB2 has been shown to inhibit radiation-induced apoptosis by suppressing the DNA-damage response. The ability of ZEB2 to suppress DNA-damage induced apoptosis seems to be largely independent of its ability to induce EMT (9,10). Additionally, TWIST1 has been shown to lead to chemoresistance in both breast and lung cancer, through direct induction of AKT and modulation of BCL-2 family members, respectively (11,12). SNAIL1 and SNAIL2 have both been linked to chemotherapy and radiation resistance through their ability to inhibit p53-mediated apoptosis, by repressing expression of multiple genes within the p53 pathway (10,13). In addition to the evidence that multiple EMT-TFs mediate therapeutic resistance, there is extensive data that EMT-TFs are often co-expressed in tumors and in many model systems, EMT-TFs can increase expression, often directly, of other EMT-TFs (6,14,15). Given the interconnectedness of the EMT-TFs signaling networks and potential redundancy in their functions, additional studies are needed to determine if other EMT-TFs mediate resistance to EGFRi and if ZEB1-driven resistance occurs independently of other EMT-TFs.

An aspect of EMT-TFs that remains largely unexplored is whether the EMT-TF mediated therapeutic resistance is EMT-dependent or -independent. A previous report on AXL-driven erlotinib-resistance in EGFR-driven NSCLC revealed that AXL-mediated resistance required EMT (4). More specifically, both AXL-mediated EGFRi resistance and AXL-mediated migration and adhesion in this model system required vimentin expression (4). While Zhang *et al.* and others have implicated ZEB1 in EGFRi resistance, it is largely unclear if EMT is simply a consequence of ZEB1 overexpression or if EMT is required for ZEB1-mediated resistance (5,7). Interestingly, TWIST1 has been shown to inhibit oncogene-induce senescence (OIS) and apoptosis in lung cancer, largely independently of EMT (16,17). A previous study demonstrated that ZEB1 can suppress OIS following EGFR overexpression in esophageal cells (18). The ability of ZEB1 to suppress OIS in this model system occurred in the context of EMT. However, ZEB1 was recently shown to mediate radioresistance via increasing CHK1 stabilization and subsequent homologous recombination, a mechanism that seems to be independent of its EMT-function (10,19).

Future studies elucidating the role of EMT in ZEB-

1 mediated EGFRi resistance are needed and may reveal new therapeutic targets and strategies to overcome EGFRi resistance. Given that AXL kinase has been established as an important driver of EMT in lung cancer (4), if ZEB1-mediated resistance proves to require EMT, the relationship between AXL and ZEB1 should be explored. A previous study in hematologic cells demonstrated that activation of AXL can induce expression of TWIST1 (20). A similar relationship may exist for ZEB1. If ZEB1 and AXL signaling pathways prove to be connected, use of small molecule AXL kinase inhibitors may be a therapeutic option to overcome ZEB1-mediated EGFRi resistance. Alternatively, if ZEB1 proves to mediate EGFRi resistance independently of EMT, ZEB1 suppression of apoptosis and modulation of members of the BCL-2 family of proteins should be explored. Given the recent FDA approval of the BCL-2 inhibitor, ABT-199, and additional BCL-2, BCL-XL, and MCL-1 inhibitors currently under investigation, there are potential novel strategies to use these BCL-2 inhibitors to overcome ZEB1-suppression of apoptosis in response to EGFRi. Ultimately, the identification and development of direct inhibitors of ZEB1 and other EMT-TFs may have significant clinical impact; however, this has remained an elusive goal.

In summary, Zhang *et al.* demonstrate unique divergent functions of ZEB1 as both a tumor suppressor and driver of EGFRi resistance in EGFR-driven NSCLC. The authors show that ZEB1 suppression of ERBB3 expression through the ZEB1-*miR-200c*-NOTCH1 signaling axis may be a mechanism by which ZEB1 suppresses EGFR-driven NSCLC tumorigenesis. Contrasting this tumor suppressive function of ZEB1, the authors present evidence that ZEB1 mediates EGFRi resistance in EGFR-mutant NSCLC cell lines. ZEB1-mediated resistance requires ZEB1-suppression of *miR-200c*, partially requires NOTCH1 expression, and is associated with EMT. Furthermore, the authors demonstrate that gefitinib resistance can be partially overcome with γ -secretase inhibitors, providing evidence for use of inhibitors of the NOTCH1 signaling pathway in the setting of EGFRi resistance. Future studies are needed to further elucidate the mechanisms through which ZEB1-mediate therapeutic resistance which may lead to novel combination therapies to combat EGFRi resistance.

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Footnote

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References

1. Cancer Genome Atlas Research Network. Comprehensive molecular profiling of lung adenocarcinoma. *Nature* 2014;511:543-50.
2. Kris MG, Johnson BE, Berry LD, et al. Using multiplexed assays of oncogenic drivers in lung cancers to select targeted drugs. *JAMA* 2014;311:1998-2006.
3. Sequist LV, Waltman BA, Dias-Santagata D, et al. Genotypic and histological evolution of lung cancers acquiring resistance to EGFR inhibitors. *Sci Transl Med* 2011;3:75ra26.
4. Zhang Z, Lee JC, Lin L, et al. Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. *Nat Genet* 2012;44:852-60.
5. Zhang T, Guo L, Creighton CJ, et al. A genetic cell context-dependent role for ZEB1 in lung cancer. *Nat Commun* 2016;7:12231.
6. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* 2014;15:178-96.
7. Yoshida T, Song L, Bai Y, et al. ZEB1 Mediates Acquired Resistance to the Epidermal Growth Factor Receptor-Tyrosine Kinase Inhibitors in Non-Small Cell Lung Cancer. *PLoS One* 2016;11:e0147344.
8. Takebe N, Miele L, Harris PJ, et al. Targeting Notch, Hedgehog, and Wnt pathways in cancer stem cells: clinical update. *Nat Rev Clin Oncol* 2015;12:445-64.
9. Sayan AE, Griffiths TR, Pal R, et al. SIP1 protein protects cells from DNA damage-induced apoptosis and has independent prognostic value in bladder cancer. *Proc Natl Acad Sci U S A* 2009;106:14884-9.
10. Ansieau S, Collin G, Hill L. EMT or EMT-Promoting Transcription Factors, Where to Focus the Light? *Front Oncol* 2014;4:353.
11. Jin HO, Hong SE, Woo SH, et al. Silencing of Twist1 sensitizes NSCLC cells to cisplatin via AMPK-activated mTOR inhibition. *Cell Death Dis* 2012;3:e319.
12. Ansieau S, Morel AP, Hinkal G, et al. TWISTing an embryonic transcription factor into an oncoprotein. *Oncogene* 2010;29:3173-84.
13. Kurrey NK, Jalgaonkar SP, Joglekar AV, et al. Snail and slug mediate radioresistance and chemoresistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells. *Stem Cells* 2009;27:2059-68.
14. Casas E, Kim J, Bendesky A, et al. Snail2 is an essential mediator of Twist1-induced epithelial mesenchymal transition and metastasis. *Cancer Res* 2011;71:245-54.
15. Puisieux A, Brabletz T, Caramel J. Oncogenic roles of EMT-inducing transcription factors. *Nat Cell Biol* 2014;16:488-94.
16. Tran PT, Shroff EH, Burns TF, et al. Twist1 suppresses senescence programs and thereby accelerates and maintains mutant Kras-induced lung tumorigenesis. *PLoS Genet* 2012;8:e1002650.
17. Burns TF, Dobromilskaya I, Murphy SC, et al. Inhibition of TWIST1 leads to activation of oncogene-induced senescence in oncogene-driven non-small cell lung cancer. *Mol Cancer Res* 2013;11:329-38.
18. Ohashi S, Natsuzaka M, Wong GS, et al. Epidermal growth factor receptor and mutant p53 expand an esophageal cellular subpopulation capable of epithelial-to-mesenchymal transition through ZEB transcription factors. *Cancer Res* 2010;70:4174-84.
19. Zhang P, Wei Y, Wang L, et al. ATM-mediated stabilization of ZEB1 promotes DNA damage response and radioresistance through CHK1. *Nat Cell Biol* 2014;16:864-75.
20. Merindol N, Riquet A, Szablewski V, et al. The emerging role of Twist proteins in hematopoietic cells and hematological malignancies. *Blood Cancer J* 2014;4:e206.

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