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Commentary

Targeting CDC34 E2 ubiquitin conjugating enzyme for lung cancer therapy

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Protein ubiquitylation is a common post-translational modification, catalyzed by an E1 ubiquitin activating enzyme, an E2 ubiquitin-conjugating enzyme, and an E3 ubiquitin ligase. CDC34 is a common E2 responsible for assembly of the Lys48-linked polyubiquitylation chains on a substrate for proteasomal degradation [1]. In coupling with unique E3s, CDC34 is well known to promote cell cycle progression by targeting several cyclin-dependent kinase inhibitors for degradation (*e.g.* p21 and p27) [1]. While potential involvement of CDC34 in human cancer has been proposed due to its overexpression in a number of human cancers [2,3], whether CDC34 is an attractive therapeutic target, and in what type(s) of human cancer, remains elusive.

In the March 2020 issue of *EBioMedicine* [4], Zhao and colleagues focused on the involvement of the protein ubiquitylation pathway in controlling the growth of non-small cell lung cancer cells (NSCLCs), and identified CDC34 as a top candidate essential for cell proliferation via the functional screening of a siRNA library consisting of pooled siRNAs targeting 696 ubiquitin pathway genes. Subsequent studies confirmed that CDC34 knockdown inhibited, whereas CDC34 overexpression promoted, the growth and survival of lung cancer cells. Importantly, CDC34 was overexpressed in 66.7% of NSCLC tumor samples, and CDC34 overexpression predicted a worse prognosis of patients, implying an oncogenic role in NSCLCs. Furthermore, CDC34 is stress-responsive and induced by tobacco smoke in normal lung epithelial cells of the A/J mice. Consistently, tumors derived from smokers had higher CDC34 levels than those from nonsmokers. Taken together, these association studies strongly suggest that CDC34 could play a role in lung tumorigenesis. However, whether CDC34 is a valid lung cancer target - and what the underlying mechanism(s) of CDC34 action is - have not been systematically pursued. To this end, Zhao et al., analyzed previously published clinical data of the reverse phase protein array based on 235 lung adenocarcinomas and found a positive correlation between the levels of CDC34 and epidermal growth factor receptor (EGFR), indicating a connection between CDC34 and EGFR.

EGFR is a transmembrane protein and acts as a receptor for members of the epidermal growth factor family (EGF family). EGFR plays an important role in controlling various cellular functions including cell proliferation, differentiation and motility [5,6]. Activation of EGFR by ligand binding, or ligand-independent EGFR signaling, results in its auto-phosphorylation at multiple tyrosine residues and dimerization that trigger numerous signaling cascades within the cell to produce the corresponding biological responses [5,6]. Upon EGFR activation, the phosphotyrosine residue on the cytoplasmic domain recruits a RING finger-type ubiquitin ligase E3, c-Cbl, via interacting with the tyrosine kinase-binding domain of c-Cbl [7]. UBC4/5 as an E2 partner then couples with c-Cbl to mediate EGFR ubiquitylation. The ubiquitylated EGFR is sorted into the endosomes via endocytosis for lysosomal degradation, thus terminating the growth factor signaling as a crucial feedback mechanism to prevent over-proliferation [8]. Here, Zhao et al., made an unexpected finding that CDC34 enhances EGFR signaling by inhibiting EGFR from ubiquitylation and degradation. Mechanistically, CDC34 via its N-terminus binds to the C-terminal intracellular domain of EGFR to directly compete with c-Cbl for EGFR binding, therefore preventing c-Cbl-mediated EGFR polyubiquitylation and subsequent lysosomal degradation. Thus, in contrast to its well-known proteolysis-promoting activity, CDC34 actually plays a previously unknown inhibitory role against EGFR ubiquitylation and degradation.

To determine the biological significance of CDC34-mediated EGFR protection, Zhao et al., used an siRNA-knockdown based approach to validate CDC34 as a promising lung cancer target in an EGFR dependent manner. Indeed, intranasal administration of the lentiviral particles, containing short hairpin RNA targeting CDC34, into the lungs effectively inhibited tumor growth in two mouse transgenic lung tumor models, driven respectively by EGFR mutants $EGFR^{L858R}$ or by $EGFR^{T790M/Del(E746\text{-}A750)}$, commonly found in NSCLC. At the present time, there is no pharmacological approach available to further validate CDC34 as a lung target, although CC0651, a small molecule was reported, that selectively inhibited CDC34 by trapping the weak interaction between ubiquitin and the donor site of CDC34 [9]. However, it is unlikely that this small molecule would be effective to directly disrupt the protein-protein interaction of CDC34-EGFR.

Overall, the finding presented in this study provides a novel mechanism by which CDC34 positively regulates EGFR-mediated oncogenic signaling via competitive inhibition of c-Cbl mediated EGFR ubiquitylation and degradation, leading to EGFR stabilization.

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Using both *in vitro* cell culture and *in vivo* mouse models, the authors clearly showed that CDC34 is indeed an attractive anti-lung cancer target. Thus, the study is highly significant with translational implications. Along the same vein, future studies should be undertaken in two directions. First, to further validate CDC34 as a lung cancer target, the mouse models should be generated with conditional knockout or transgenic expression of CDC34 in the lung alone or in combination with EGFR transgenic mouse models to determine tumor promoting role of CDC34 in a manner dependent of EGFR under "more" physiological conditions. Secondly, when validated, drug discovery efforts should be undertaken to identify small molecules that specifically bind to CDC34. Although it is anticipated that these small molecules would not be able to disrupt the CDC34-EGFR binding—an "undruggable" target, the PROTAC (proteolysis-targeting chimeras) strategy [10] can be employed to hook small molecule to cullin-RING ligase to directly target CDC34 for degradation. Discovery and development of these PROTAC compounds would have unique applications for the treatment of lung cancer with overexpressed CDC34 and EGFR.

Declaration of Competing Interest

The authors declare no conflict of interest.

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