Commentary

Unraveling epigenetic regulation of epithelial mesenchymal transition

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Epithelial mesenchymal transition (EMT) is a naturally occurred transdifferentiation of epithelial cells that encompasses their plasticity and involves conversion from epithelial to mesenchymal phenotypes (1-3). EMT is a common program in embryonic development, organ fibrogenesis and cancer metastasis. The cells undergone EMT have been evidenced with cancer cell stemness and resistance to chemotherapy (4). Several recent studies on EMT, particularly in cancer entity, have moved forward to better understand molecular mechanisms underlying EMT and its regulation. Since EMT can be modulated by several factors at multiple steps, integrative information of EMT regulation at transcription, post-transcription, post-translation and epigenetic levels is thus required for the new approach on EMT-related cancer therapy (5). Beyond transcriptional control, DNA methylation, histone modification and microRNA (miRNA) have been recognized as epigenetic modifications that can regulate EMT-related genes (5). One of the driving forces for epigenetic modifications in cancer-related EMT is the adaptive mechanism of the cancer cells to alter their metabolism to survive under energy deprivation stage during tumorigenesis (6). Additionally, dysregulated metabolism can be affected by genetic mutations of metabolic enzymes, e.g., succinate dehydrogenase (SDH), fumarate hydratase (FH) and isocitrate dehydrogenase (IDH), that subsequently cause alterations in cellular metabolites and finally initiate cancer development and progression (7). The small metabolites with the potential to trigger cancers are termed "oncometabolites" (8,9). One of the well-known oncometabolites related to cancer progression is fumarate, which has been evidenced to be associated with the development of hereditary leiomyomatosis and renal cell cancer (HLRCC) (8).

HLRCC is an autosomal dominant hereditary cancer syndrome caused by germline mutation of the gene encoding FH, a tricarboxylic acid cycle (TCA) enzyme, which catalyzes hydration of fumarate to malate (10). The consequences of FH inactivation in kidney cancer cells include TCA cycle imbalance, fumarate accumulation, impaired oxidative phosphorylation, and metabolic reprogramming to aerobic glycolysis (also known as Warburg effect), which in turn promote tumor growth (10). Furthermore, stabilization of hypoxia-inducible factor 1 (HIF1), inactivation of AMP-activated protein kinase (AMPK), and dysregulation of Keap1-Nrf2 antioxidant system have been reported in *FH*-deficient kidney cancers (11-13). Although adaptive responses to fumarate accumulation are partially known, there is still a huge gap in understanding epigenetic regulation of tumorigenesis in HLRCC.

The most recent study by Sciacovelli *et al.* (14), has established a connection between an oncometabolite (fumarate) and epigenetic regulation of miR-200 family that plays important roles in EMT program of HLRCC. The authors primarily characterized the EMT phenotypes by proteome and mRNA profilings of $Fh1^{-/-}$ (murine) and UOK262 (human) *FH*-deficient cells (note that the latter

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was derived from HLRCC patient). They demonstrated that a mesenchymal marker, vimentin, was the most increased protein, while a counterpart epithelial marker, E-cadherin, was significantly decreased in the *FH*-deficient cells, suggesting the acquired mesenchymal signature in these cell lines concomitant with the increased cell migratory activity. Interestingly, epithelial features were regained and cell migratory activity was decreased when the *FH*-deficient cells were reintroduced with full-length *FH*. In addition, several EMT transcription factors, including *SNAI1*, *SNAI2*, *TWIST1*, *ZEB1* and *ZEB2*, were elevated in the *FH*-deficient cells (14). It is known that the loss of FH in renal cancer can result in accumulation of fumarate and inhibition of HIF prolyl hydroxylase, thereby stabilizing HIF1 (11). Moreover, silencing of *HIF1α* in UOK262 significantly reduces invasiveness of the cancer cells (12). Unexpectedly, Sciacovelli *et al*. (14) found that silencing of *HIF1β* could not suppress EMT phenotypes, indicating that the involvement of HIF1 in cancer development/progression is (iso)form-specific.

Numerous miRNAs are supposed to be involved in EMT by epigenetic modulation of their mRNA targets especially through DNA methylation at the CpG promoter island or histone acetylation. Among these, ZEB-miR-200 axis has gained wide attention on its role for regulation of transcription factors in cancer EMT (15). miR-200 family is a potent antimetastatic miRNA cluster that suppresses expression of EMT-transcription factors as well as tumor initiation and metastatic cascade (16). Sciacovelli *et al*. (14) hypothesized that epigenetic modification driven by accumulation of fumarate could affect miR200-mediated regulation of EMT-related genes in HLRCC. In concordance with elevated *ZEB1* and *ZEB2* expression, they also showed that miR-200 family was the most down-regulated miRNA in *FH*-deficient cells and *FH* re-expression successfully rescued the expression of miR-200ab and E-cadherin, while suppressed vimentin expression (14). As expected, quantitative PCR (qPCR) revealed hypermethylation of *CpG43* in *FH*-deficient cells that could be returned to unmethylated state by *FH* reconstitution (14).

The same group of investigators also demonstrated that suppression of miR-200 cluster was a consequence of inhibition of ten-eleven translocation (Tet)-mediated demethylation (14). Tet family of dioxygenase comprises three proteins, including Tet1, Tet2 and Tet3, which are responsible for catalyzing the conversion of 5-methylcytosine (5mc) to 5-hydroxymethylcytosine (5hmc). The product 5hmc can be used to implicate the reversible process of DNA methylation/demethylation (17). This group of investigators revealed that combined silencing of *Tet2* and *Tet3* (*Fh1*$^{fl/fl}$ + sh*Tet2-3*) resulted in decreased miR-200abc and E-cadherin expression, suggesting the role of Tet-mediated demethylation in EMT regulation (14). The same results were observed when dioxygenase was reactivated in *Fh1*$^{fl/fl}$ + sh*Tet2-3* cells by using dimethyl alpha-ketoglutarate (DM-a-KG) (14). To decipher whether this molecular scenario occurred in *FH*-deficient cells, the authors performed chromatin immunoprecipitation (ChIP) assay in combination with chromosome conformation capture (3C) analysis to pin-point the region of histone modification, indicating the opened-closed state of chromatin structure. It was clearly shown that the region adjacent to *CpG43* and transcriptional start site of miR-200ba429 was hypermethylated in *FH*-deficient cells, which could be restored to basal level by reintroduction of *FH* (14). Accordingly, the marked decrease in Tet-catalyzed 5hmC was also observed in *FH*-deficient cells (14). Although they did not determine the basal levels of Tet proteins in *FH*-deficient cells, these findings collectively suggest the role of Tet-mediated demethylation in the production of miR-200 family in *FH*-deficient cells. Alternatively, they confirmed the effects of oncometabolite on EMT regulation by using monomethyl fumarate (MMF), a fumarate derivative, in wild-type FH (*Fh1*$^{fl/fl}$) and HK2 cells. As expected, treatment of MMF up-regulated EMT transcription factors and down-regulated miR-200abc and E-cadherin expression in both cell lines, whereas vimentin was increased only in *Fh1*$^{fl/fl}$ cells (14). To avoid the effect of fumarate by-products (i.e., succinic GSH and 2-succinic cysteine), they employed *SDHb*-deficient cell line and demonstrated that only succinate accumulation could raise EMT phenotypes similar to those of FH-deficient cells. However, it was questionable that whether triggering EMT in *SDHb*-deficient cells engaged the same molecular mechanism as revealed in *FH*-deficient cells, since hypermethylation of *CpG43* was not predominant. Finally, the effects of oncometabolite fumarate and epigenetic regulation of EMT were validated in tumor samples collected from HLRCC patients (only two cases included in their own study) (14) and other cancers related to *FH*-mutation, including papillary renal cell carcinoma and clear cell renal cell carcinoma from the published data. By this strategy, it was convincing that the decrease of FH was correlated with down-regulation of miR-200 cluster due to hypermethylation, leading to EMT features. Last but not least, the decreased FH expression

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was related to the worsen prognosis of the patients (14).

In summary, the study by Sciacovelli *et al*. (14) provided a missing piece of the puzzle and demonstrated that oncometabolite fumarate could induce epigenetic regulation of EMT in *FH*-deficient kidney cancers. All these findings raise the possibility that aberrant epigenetics by inhibition of Tet-mediated demethylation may be generalized in other cancers related to miR-200 suppression or loss of FH. Considering epigenetic modifications for anticancer drug design, it is still challenging to figure out the unique set of epigenetic markers specific to EMT (e.g., histone modifications, histone marks, etc.) and chromatin modifying enzymes for the precise targeting of EMT cells. In addition to the discovered linkage, further functional analyses on transcriptome, proteome, and miRNA profiling data provided in this report and by other groups are still necessary for unlocking and conveying the comprehensive message for specific therapeutics of cancers related to *FH*-mutation. However, it should be noted that epigenetic regulation is not limited only to the cancer entity of EMT but also organ fibrogenesis. Several studies have shown a proof-of-concept that aberrant DNA promoter methylation causing gene silencing contributes to many fibrotic diseases, including pulmonary, liver, cardiac, and kidney fibrosis (18). For example, hypermethylation of anti-fibrotic RASAL1 promoter mediated via Tet activity has been recently demonstrated in cardiac and kidney fibrosis (19,20). Nonetheless, this molecular mechanism has been revealed only in differentiated/activated fibroblasts and coronary endothelial cells. Therefore, there is still a plenty of space for investigating the role of epigenetic regulations of EMT in fibrogenesis, including a link between Tet-mediated demethylation and expression of miR-200 cluster or other genes. The study provided by Sciacovelli *et al*. (14) thus sheds light onto the emerging role of epigenetic modifications of EMT process not only in cancers but also for in organ fibrogenesis and may hold a promise for the novel therapeutic strategy in cancers and also in fibrotic diseases in the future.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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