Colorectal cancer (CRC) patients with distant metastasis suffer from poorer prognosis as compared with those without, necessitating identification of novel preventive or therapeutic targets for metastasis. Epithelial-mesenchymal transition (EMT) is considered to play a major role in invasion and metastasis of solid cancers including CRC, while mesenchymal-epithelial transition (MET), the reverse of EMT, is associated with their reduced invasion ability (1,2). EMT is regulated primarily by so-called EMT transcription factors (EMT-TFs) including SNAI1/2, ZEB1, and TWIST1, but its complex regulatory networks include microRNAs and splicing factors that affect EMT-TFs and/or their targets (1,2). For example, miR-34a and miR-200c target SNAI1 and ZEB1, respectively, and thereby regulate EMT of CRC cells (3-5). miR-34a encodes a p53-inducible microRNA (11) and intriguingly, p53-mediated induction of miR-34a appeared to be dominant over HIF1A-mediated repression of miR-34a.

The authors first showed that culturing p53-deficient CRC cells under hypoxic condition (0.5% O_2) for 30 hours repressed mature miR-34a expression in a HIF1A-dependent manner, while the same treatment on p53-proficient CRC cells induced the expression of mature miR-34a. Ectopic expression of miR-34a in p53-deficient DLD-1 cells suppressed the levels of EMT markers and the invasion activity induced by hypoxia, indicating that HIF1A-mediated downregulation of miR-34a is required for hypoxia-induced EMT in p53-deficient CRC cells.

The authors next identified the mRNA encoding protein phosphatase 1 regulatory inhibitor subunit 11 (PPP1R11), also called INH3, as a direct and conserved target of miR-34a. As its name indicates, PPP1R11 can inhibit PP1, the phosphatase that can dephosphorylate STAT3 at serine 727 (13,14), a phosphorylation site known to enhance homodimerization and transcriptional activity of STAT3 (15). They then showed that the PPP1R11 gene
The promoter contains 5 HIF1A binding sites and its expression can be induced directly by HIF1A, suggesting a coherent feed-forward regulation of PPP1R11 by HIF1A and miR-34a under hypoxia. The authors subsequently showed that PPP1R11 is necessary for EMT, migration, and invasion of DLD-1 and HT-29 cells, and that overexpression of PPP1R11 is sufficient for inducing EMT, migration, and invasion in DLD-1 cells. In addition to these in vitro results, they showed that knocking down PPP1R11 in DLD-1 cells pretreated with a hypoxic culture condition inhibits their lung metastasis upon tail-vein injection, demonstrating that PPP1R11 mediates hypoxia-induced metastasis of p53-deficient CRC cells. Negative regulation of PPP1R11 and the EMT phenotypes by miR-34a in vivo was further demonstrated using genetically-engineered mouse models of early-stage colorectal carcinogenesis. Namely, intestinal tumors from miR-34a^-/-;miR-34b^-/-;Apc^Min/+ compound mutant mice showed elevated levels of PPP1R11, phospho-Stat3 (S727), and the EMT marker Vimentin. In addition, culture at 0.5% O₂ enhanced the levels of PPP1R11 and phospho-Stat3 (S727) in the organoids derived from the intestinal tumors of the compound mutant mice, but not in those from Apc^Min/+ tumors.

To address whether the above-mentioned difference in the response to hypoxia between p53-deficient and p53-proficient CRC cells is determined by p53 status, the authors next tested the effect of p53 overexpression in p53-deficient SW480 cells. Ectopic expression of p53 abolished the enhancement of their invasion and migration activity induced by CoCl₂, a chemical inducer of HIF1A, accompanied by reduced levels of PPP1R11 protein and phospho-STAT3 (S727). This repression of PPP1R11 by p53 was shown to be mediated by miR-34a. The authors further employed 3 isogenic CRC cell lines, namely HCT116, RKO, and SW48 cells, that differ only in TP53 status. In all three cell lines, TP53^-/- cells under hypoxia did not adopt a mesenchymal morphology and showed upregulation of miR-34a and downregulation of PPP1R11. These results clearly indicate the essential roles of the TP53 status in determining the hypoxia responses of CRC cells.

Li et al. moved on to address the clinical relevance of their findings. They showed that while 5-FU treatment significantly reduced colony formation by HCT116 TP53^-/- cells under hypoxia (0.5% O₂), 5-FU barely affected the colony forming activity of HCT116 TP53^+/+ cells at the same condition. Furthermore, knockdown of PPP1R11 abrogated the 5-FU resistance of HCT116 TP53^-/- cells under hypoxia, connecting the p53/HIF1A/miR-34a/PPP1R11/STAT3 regulatory pathway to drug resistance of CRC. The authors finally evaluated their findings in clinical samples of CRC. Using the Cancer Genome Atlas (TCGA) database, they first showed that CRC samples with mutant TP53 displayed significantly higher expression of PPP1R11 mRNA compared with TP53-wild-type CRC samples. They then showed that the elevated PPP1R11 protein expression at invasion front of CRCs was significantly associated with liver metastasis. Indeed, the immunostaining pattern of

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**Figure 1** Proposed model for the response of p53-proficient and p53-deficient CRC cells to hypoxia. In TP53^+/+ CRC cells, hypoxia-induced p53 upregulates miR-34a expression and reduces HIF1A induction. The enhanced level of miR-34a and reduced level of HIF1A cause repression of PPP1R11 (INH3), resulting in suppression of STAT3 phosphorylation at serine 727 and induction of MET (upper panel). In contrast, hypoxia strongly induces HIF1A in TP53^-/- CRC cells. HIFA1 represses miR-34a expression and enhances PPP1R11 (INH3) expression. PPP1R11 then inhibits PP1, resulting in enhancement of STAT3 phosphorylation and induction of EMT (lower panel). CRC, colorectal cancer; MET, mesenchymal-epithelial transition; EMT, epithelial-mesenchymal transition.
PPP1R11 at invasion front overlapped with that of the EMT marker Laminin 52 and the hypoxia marker GLUT1, and the elevated expression of INH at the infiltrative tumor edge of CRCs was negatively correlated with the expression of \( \text{miR-34a} \). They further demonstrated significant association between elevated expression of PPP1R11 and that of GLUT1 and Laminin 52 at the invasion front of primary tumors and metastases of CRCs.

As mentioned above, hypoxia has been implicated in invasion and metastasis of solid cancers through promotion of EMT, but hypoxia also induces p53. The meticulous and convincing study by Li et al. has unveiled the molecular mechanism by which the loss of p53 contributes to EMT and thereby invasion and metastasis of CRC through downregulation of \( \text{miR-34a} \) (Figure 1). Identification of PPP1R11 (INH3) as the key downstream effector that induces EMT via STAT3 phosphorylation provides a promising candidate target for therapy of invasive CRC containing hypoxic regions, in addition to the miR-34 mimics that are being developed (16,17). Like all excellent studies, the findings by Li et al. raise many questions. Is the p53/HIF1A/miR-34a/PPP1R11/STAT3 pathway involved in oncogenic signaling initiated by hypoxia-independent activation of HIF1A, such as the loss of VHL in kidney cancer? Is the axis also involved in the biology of cancer stem cells, which share many molecular characteristics with cancer cells undergoing EMT? CRC cells that have acquired EMT phenotypes in hypoxic environment will eventually invade into normoxic area to undergo intravasation. By citing their previous work (18), the authors state that it is conceivable that the transient repression of \( \text{miR-34a} \) by hypoxia observed here is fixed over time by DNA hypermethylation in its promoter region. Is such a fixation really taking place in CRC cells \textit{in vivo}? Further studies are awaited to answer these and other remaining questions, and to develop effective means for preventing metastasis and/or circumventing drug resistance of CRCs.

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**Footnote**

**Conflicts of Interest:** The author has no conflicts of interest to declare.

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