

Regulation of autophagy and EMT by the interplay between p53 and RAS during cancer progression (Review)

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Abstract. Cellular autophagy and epithelial-mesenchymal transition (EMT) are key events mostly resulted from the interplay of tumor suppressors and oncogenes during cancer progression. The master tumor suppressor p53 may control tumor cell autophagy and EMT through the transcriptional induction of multiple target genes, while the activated oncogene RAS may also play a critical role in regulating mitogenic signaling to tumor cell autophagy and EMT. Although the fundamental functions of p53 and RAS are well understood, the interactive effects of p53 and RAS on autophagy and EMT are still unclear. In this review, we highlight the recent advances in the regulation of autophagy and EMT by p53 and RAS, aiming to explore novel therapeutic targets and biomarkers in cancer treatment and prevention.

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1. Introduction

In response to various stresses such as DNA damage or hypoxia, the tumor suppressor p53 can be activated to

regulate cell cycle, differentiation, apoptosis, senescence and autophagy (1,2). Mutations of p53 in single allele may lead to loss of the tumor suppressor functions, gain of oncogenic functions, or exert dominant-negative effects which may disrupt the normal functions of the wild-type allelic p53 (3). Under normal circumstances, p53 is rapidly turned over by ubiquitination through binding to MDM2. Mutant p53 is usually much more stable than the wild-type p53 due to the loss of the binding activity to MDM2, and is often accumulated in tumor cells (4,5). While the wild-type p53 predominantly functions as a transcription factor, the mutant p53 also has the ability to transactivate multiple genes involved in cell proliferation, apoptosis inhibition, chemoresistance and matrix degradation (4).

On the other hand, oncogenic mutations of RAS are detected in many cancer types including pancreatic, lung, ovarian and colon cancers, which usually lead to chemo- and/or radio-resistance of cancer cells (6,7). RAS activates several downstream cascade branches including the RAF/MEK/ERK, PI3K/AKT and RalGDS/Ral signal molecules critical for cancer progression (8,9). Although p53 and RAS are individually reported to contribute to cellular autophagy and EMT, how they functionally interact with each other to cooperatively regulate the downstream signaling cancer progression is unclear. In this mini review, we will summarize the recent findings regarding the functional interaction of mutant p53 and RAS in modulating cancer progression through some key events of cell autophagy and EMT (10-13).

2. Mutations of p53 and RAS synergistically promote cell autophagy

Autophagy, an intracellular catabolic process in response to stress and nutrient deprivation, plays multiple roles during tumorigenesis and cancer therapy (14). To maintain metabolic homeostasis, autophagy occurs to deliver excessive or unnecessary cytoplasmic components as well as injured or aged organelles to the lysosomes for degradation (15,16). As a homeostatic process, autophagy has both tumor-promoting and tumor-suppressing properties depending on cancer cell type and the tumorigenic context (17). The main regulators of autophagy include the PI3K-Akt-mTOR pathway associated

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molecules, RAS and p53 (14). Several studies have shown that the nuclear p53 stimulates cellular autophagy via the transactivation of multiple target genes, while the cytoplasmic p53 inhibits autophagy in a transcription-independent manner, therefore the subcellular localization of p53 may determine the outcome of autophagy (18,19). On the other hand, RAS can modulate autophagy via various signaling cascades in cancer cells, conversely, autophagy also mediates and promotes the RAS-driven cancer progression and invasion (20,21). RAS renders mitochondrial health particularly reliance on autophagy to the extent that RAS-driven cancer cells seem more autophagy-dependent for survival to nutrient starvation than normal cells. Thus, that RAS-driven cancers are susceptible to autophagy inhibition therapy (22).

Both Ras and p53 are reported to interact with several identical binding partners and signaling cascades during the autophagy process, suggesting the possible interplay between their corresponding pathways. In the nucleus, p53 activates Sestrin1 (also known as p53-activated gene 26, PA26) and Sestrin2 (also known as hypoxia-inducible gene 95, Hi95), to induce autophagy through the activation of adenosine monophosphate-activated protein kinase (AMPK) (23). The activated AMPK inhibits mTOR1 activity by phosphorylating the mTORC1 binding factor Raptor or the tumor suppressor tuberous sclerosis protein 1/2 (TSC1/2) complex (24,25). Studies on metastatic pancreatic ductal adenocarcinomas showed that two K-RAS activation pathways RAF/MEK/ERK and PI3K/AKT also converge to the TSC1/2 (26). In addition, inhibition of mTORC1 or activation of AMPK can activate the unc-51-like autophagy activating kinase 1/2 (ULK1/2) to eventually initiate autophagy (27,28).

The autophagy related genes (ATG) have been recognized to execute autophagy directly and the ATG proteins play pivotal roles in the formation of the autophagosomes (29). p53 and RAS regulate autophagy mostly relying on these ATG proteins. *In vitro* studies on various cancer cell lines showed that overexpression of the p53 target gene Isg20L1 promotes autophagy that can be partially rescued by ATG5 depletion (30). The nucleus p53 induces autophagy through direct activation of serial genes such as ULK1, ULK2 and ATG7 in multiple cell lines such as MEFs, lung cancer cells and HCT116 cells (31,32), indicating that the nucleus p53 induces autophagy at least partially relying on ATG5/7. A recent study on ATG7-deletion genetically engineered mouse models of K-RAS^{G12D}-driven on small-cell lung cancer (NSCLC) showed that the functional status of p53 determined the metabolic requirement for autophagy. During tumor development, intact p53 with ATG7 deletion leads to the premature p53 induction and blocks tumor proliferation, while p53 loss of function restored the proliferation and growth during ATG7 deletion (33). Finally, both H-RAS^{V12} and K-RAS^{V12} can initiate autophagy by upregulating ATG5 and ATG7 through the Rac1/mitogen-activated kinase kinase 7 (MKK7)/c-Jun N-terminal kinase (JNK) signaling pathways in normal fibroblasts and human breast epithelial cell line MCF10A (34,35).

Activated RAS and mutant p53 may synergistically regulate autophagy. In human pancreatic cancer cell lines CAPAN-2, PANC-1 and Panc10.05, activated K-RAS and p53 loss of function collaboratively upregulate Plac8 to facilitate autophagosome-lysosome fusion (36). Both RAS and p53

signaling pathways can regulate the heat shock transcription factor 1 (HSF1) that stimulates autophagy through direct binding to the ATG7 promoter and activating its expression during breast cancer progression (37,38). The RAS/RAF/MEK/ERK signaling pathway activates HSF1 through its phosphorylation at Ser326 in human neurofibrosarcoma cell line MpNST while HSF1 and p53 interfere with each other during cancer development (39). The HSF1 signaling usually depends on p53 mutation status and HSF1 is also required for the nuclear localization of p53 in multiple cell lines (38,40).

Studies have also shown that autophagy plays a cardinal role in response to hypoxia microenvironment of tumors. For example, the hypoxia-inducible factor-1 α (HIF-1 α) can activate autophagy and alter cancer metabolism (41). During anti-angiogenic therapy, some cancer cells activated both AMPK and HIF-1 α pathways to initiate autophagy and thus survive under the hypoxic insult (42). A study revealed that H-RAS can transform Rat1 fibroblasts through upregulation of HIF-1 α expression, but treatment with either MAPK or PI3K inhibitors suppresses the HIF-1 α level (43). HIF-1 α also stabilizes p53 through direct interaction with and inhibition of MDM2 (44,45). Although it is known that HIF-1 α interacts with both RAS and p53 in hypoxia conditions, how these interactions induce autophagy is still unclear. The detailed signaling pathways involving p53 and RAS in autophagy are presented in Fig. 1.

3. p53 and RAS participate in regulation of cancer cell EMT

Epithelial-mesenchymal transition (EMT) is a process of certain cells switching from an epithelial to a mesenchymal status (46). During EMT, epithelial cells lose their characteristics as apical-basal polarity and tight junction but gain the mesenchymal properties such as reduced intercellular adhesion and increased motility (47). EMT may play an important role in the initiation and development of cancers and chemoresistance of metastatic cancers (47-50).

It is well established that oncogenic RAS promotes EMT in collaboration with other pathways including p53 (51,52). p53 inhibits the RAS-mediated EMT and EMT-associated stemness of human mammary epithelial cells via the RAS/RAF/MEK/ERK and the RAS/PI3K/AKT pathways. Moreover, inhibition of the RAS/RAF/MEK/ERK pathway upregulates E-cadherin and β -catenin expression (53). Both RAS/PI3K/AKT and RAS/RAF/MEK/ERK pathways stimulate EMT through the activation of Snail2 (also known as Slug) expression and the reduction of E-cadherin in multiple cell lines including colorectal carcinoma cells HCT-116, HKe-3 and HKh-2, rat parotid gland epithelial cell Pa4, and endometrial cancer cell lines Ishikawa and Hec251 (51,54,55). In non-small cell lung cancer, mutation of p53 is associated with high expression of Slug and low expression of E-cadherin, leading to poor prognosis of patients (56). The study suggested that wt p53 can bind to MDM2 and Slug simultaneously to form a p53-MDM2-Slug complex, which then facilitates MDM2-mediated Slug degradation (56). The H-RAS^{V12}-induced EMT can be inhibited by ASPP2 without p53 binding (57). In mouse primary kidney epithelial cells, ASPP2 represses ZEB1 expression by forming ASPP2- β -catenin-E-cadherin ternary

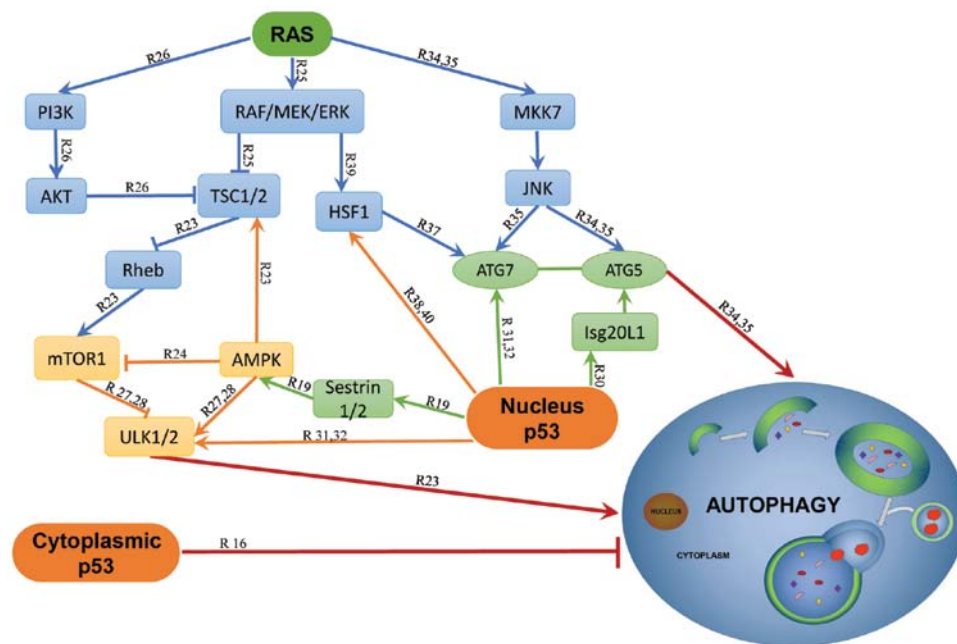


Figure 1. Mutant p53 and RAS synergistically promote cell autophagy. R, reference

complex at cell-cell junctions to negatively regulate the WNT signaling (57). Although ASPP2 suppresses ZEB1 without regard to the p53 mutation status, in hepatocellular carcinoma cell lines and immortal normal mammary epithelial cells, p53 represses ZEB1 and ZEB2 expression through the transcriptional activation of the miRNA-200 family members (58,59). In murine and human cancer cells, Twist1 and Twist2 may also cooperate with H-RAS^{V12} to overcome premature senescence of mouse embryonic fibroblasts through inhibition of the p53 pathway and promotion of EMT by suppressing E-cadherin and stimulating vimentin expression (60).

Concurrent mutations of RAS and p53 have been found to play a critical role in EMT and tumor metastasis via multiple pathways. The Raf kinase trapping to Golgi (RKTG), the negative regulator of the RAS/RAF/MEK/ERK pathway, may also collaborate with p53 to regulate EMT (61). Concomitant knockdown of p53 and RKTG in mice contribute to skin cancer development and epidermal EMT. Studies of A431 and HepG2 cells suggested that loss of p53 and PKTG at the same time reduced E-cadherin but increased vimentin to promote EMT (61). Furthermore, the AKT activator IGF-1 induces EMT with p53 silencing while the AKT inhibitor VIII blocks the E-cadherin/ β -catenin complex formation induced by p53 and RKTG, implicating that the RAS/PI3K/AKT cascades enhance EMT function likely through inhibition of the p53 function (61). On the other hand, miR-200 blocks EMT and metastasis in syngeneic mice with metastatic lung adenocarcinoma carrying both K-RAS^{G12D} and p53R172HAG mutations (62). Several studies have shown that loss of p53 can enhance the RAS signaling induced EMT. p53 may act as a checkpoint controller to inhibit EMT while loss of p53 allows other signal cascades such as RAS activation to induce EMT (61,63-65). Activation of K-RAS^{V12} and loss of p53 may cooperate to induce EMT and cell motility by triggering the RhoA activity (10). In metastatic mouse models, depletion of the Rho-GTPase Rnd1 inhibits the RAS/RAF/MEK/ERK

pathway to promote EMT in collaboration with the loss of p53 (66).

Hypoxia-induced EMT, in particular, is well-known in several cancers such as breast, ovarian, hepatocellular carcinomas and oesophageal squamous cell cancer (67-70). HIF-1 α targets several EMT transcriptional factors including Snail, Slug, Twist and ZEB in hypoxia conditions (71). In response to hypoxia stress, HIF-1 α can activate PI3K/AKT to promote EMT and to enhance the tumor cell metastatic potential (67). As mentioned above, p53 and RAS may have an intimate crosstalk with HIF-1 α , indicating the interactive potentials among the three molecules during EMT or MET.

Since the first step of tumor metastasis is characterized by the increased motility and invasiveness, it has been implicated that EMT plays a cordial role in promoting metastasis, although the role of EMT for invasion and metastasis remains contested (72). Mutant p53 and oncogenic RAS promote EMT while the upregulation of wt p53 suppresses RAS-induced EMT phenotypes. p53 may interact with the RAS signaling to inhibit or promote EMT process via multiple pathways depending on the p53 status and RAS activation level. The detailed signaling pathways involving p53 and RAS in EMT are depicted in Fig. 2.

4. The relationship between autophagy and EMT in cancer

Autophagy and EMT are two key processes during cancer progression and linked in a close relationship with each other according to recent studies. The interactions between autophagy and EMT is complicated. Just like its dual role in cancer, autophagy also has two-tier functions on EMT according to the cellular type and the stage of tumor progression (73). Several studies showed the controversial effect of autophagy on EMT. Autophagy inhibition promotes EMT while autophagy activation reverses EMT mainly by regulating several mesenchymal markers. A recent study on gastric cancer cells

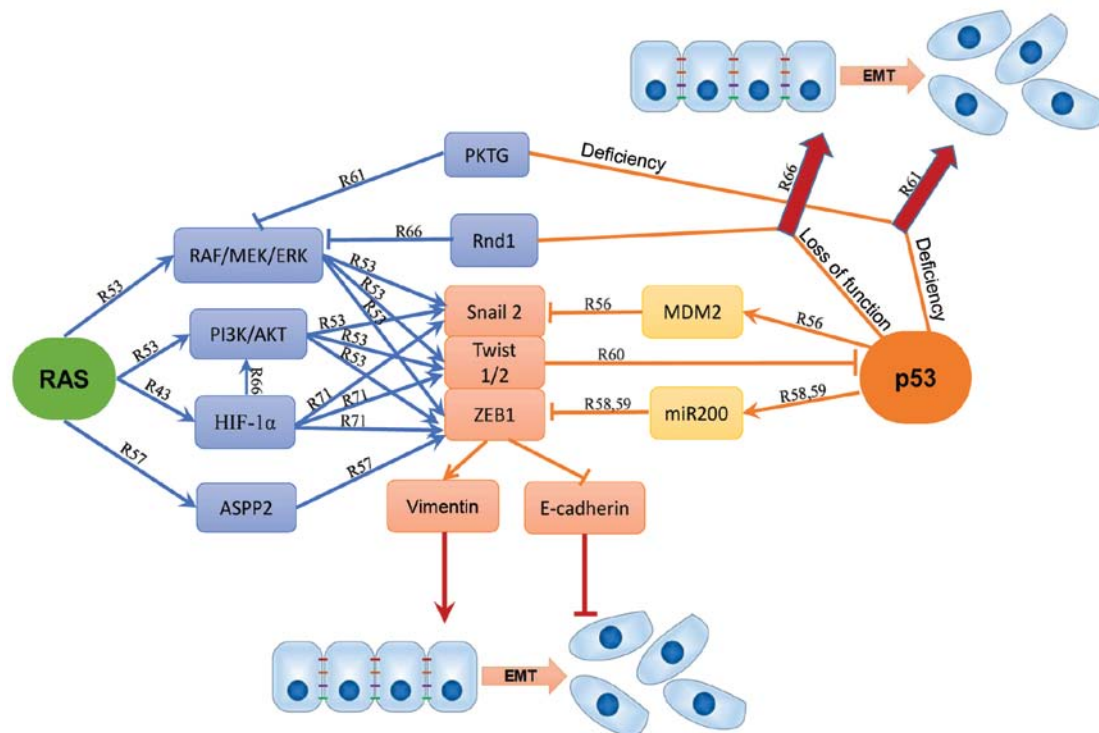


Figure 2. p53 and RAS participate in regulation of cancer cell EMT. R, reference.

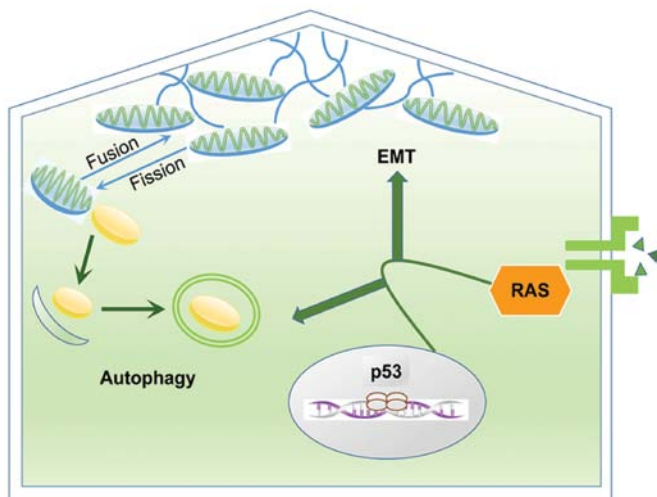


Figure 3. The relationship between autophagy and EMT in cancer.

indicated that autophagy deficiency increases the expression of mesenchymal markers such as N-cadherin, vimentin and Snail mainly through the ROS-NF- κ B-HIF-1 α pathway (74). Another research on human skin squamous cell carcinoma and melanoma described that autophagy deficiency facilitated EMT by stabilizing the pivotal mesenchymal marker TWIST1 (75). Autophagy stimulation downregulated two key regulators Slug and Snail in glioblastoma cells while inhibition of ATG5 and ATG7 led to overexpression of Slug and Snail (76). Studies on breast and colon cancers described that the death effector domain-containing DNA-binding protein (DEDD) negatively regulated EMT by activating autophagy and then inducing the autophagy-mediated lysosomal degradation of Snail and

Twist (77). Considering its special role in supporting cell viability during cancer progression and migration, autophagy also has a positive effect on EMT. Li and his colleagues (78) found that the inhibition of autophagy by silencing ATG3 or ATG7 also suppressed EMT and TGF- β /Smad3 signaling in hepatocellular carcinoma cells HepG2 and BEL 7402. While starvation-induced autophagy can promote EMT through the TGF- β /Smad3 signaling-dependent manner.

The correlation between autophagy and EMT is largely based on the close relationship between cytoskeleton and mitochondria and their pivotal function in modulating the two processes. Cytoskeleton structures are essential to facilitate cell movement and cytoskeleton remodeling is indispensable to accomplish the process of EMT (79,80). While mitochondria are responsible for ATP production and play fundamental roles in maintaining cellular metabolic homeostasis (81). Mitochondria are dynamic organelles that experience fusion and fission continuously (82). Fissile mitochondria are degraded through autophagy to be reused as source of energy and thus completed the recycling of metabolites (14,81). Mitochondria are reticular organelles characterized as high plasticity to move across the cells through the cytoskeleton (81). Amassing of mitochondria below the cell membrane is essential to provide an abundance of ATP to upgrade the formation of lamellipodia and filopodia, and then assuring the cellular motility during EMT (83,84).

Thus, the close relationship between mitochondria and cytoskeleton is correlated with both EMT and autophagy. During cancer progression, mitochondrial dynamics provide ATP for cytoskeleton remodeling to promote EMT while autophagy regulates mitochondrial dynamics by eliminating the damaged mitochondria. The relationship between autophagy and EMT in cancer is presented in Fig. 3.

5. Perspectives

Inactivation of tumor suppressor genes and activation of oncogenes may collaborate to induce cell malignant transformation. RAS and p53 have been found most frequently mutated in majority of human cancers. Early studies revealed that the activated H-RAS^{V12} cooperates with mutant p53 to induce tumor progression (85-87). Recent studies report that mutant p53 cooperates with activated RAS to stimulate highly invasive and metastatic tumors with poor prognosis (88-92). Since RAS and p53 pathways function as pivotal regulators in both cancer cells and tumor microenvironment (93), the associated genes including HIF-1 α , HDAC, EHF and VGLL and their functions may be thoroughly examined in autophagy and EMT. Retention of wt p53 can facilitate the sensitivity to chemotherapy in some tumor types and inhibition of the RAS downstream signaling factor AKT also represses survival, invasiveness and drug resistance of cancer cells (94-96). A variety of molecules and existing therapeutic agents targeting the RAS and p53 pathways are currently in clinical trials (97,98). Thus, identification of novel molecules or signaling cascades involved with p53 or RAS mutations may greatly contribute to precision medicine toward cancer treatment and prevention.

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