In vivo gene manipulation reveals the impact of stress-responsive MAPK pathways on tumor progression

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It has been widely accepted that tumor cells and normal stromal cells in the host environment coordinately modulate tumor progression. Mitogen-activated protein kinase pathways are the representative stress-responsive cascades that exert proper cellular responses to divergent environmental stimuli. Genetically engineered mouse models and chemically induced tumorigenesis models have revealed that components of the MAPK pathway not only regulate the behavior of tumor cells themselves but also that of surrounding normal stromal cells in the host environment during cancer pathogenesis. The individual functions of MAPK pathway components in tumor initiation and progression vary depending on the stimuli and the stromal cell types involved in tumor progression, in addition to the molecular isofoms of the components and the origins of the tumor.

Recent studies have indicated that MAPK pathway components synergize with environmental factors (e.g. tobacco smoke and diet) to affect tumor initiation and progression. Moreover, some components play distinct roles in the course of tumor progression, such as before and after the establishment of tumors. Hence, a comprehensive understanding of the multifaceted functions of MAPK pathway components in tumor initiation and progression is essential for the improvement of cancer therapy. In this review, we focus on the reports that utilized knockout, conditional knockout, and transgenic mice of MAPK pathway components to investigate the effects of MAPK pathway components on tumor initiation and progression in the host environment.

Eukaryotic cells sense a wide range of biological and physicochemical stressors from both external and internal environments. Stress-responsive signaling cascades convert these stressors to appropriate physiological cellular responses (e.g. apoptosis and proliferation). Dysfunction in stress-responsive signaling cascades may often result in the acquired hallmarks of cancer, which are necessary for tumor progression. Thus, the components of stress-responsive signaling cascades could be very promising drug targets for cancer therapy.

Mitogen-activated protein kinase pathways are one of these stress-responsive cascades. In response to various stressors, upstream MAP3K phosphorylates and activates MAP2K, and MAP2K subsequently activates MAPK. Activated MAPK regulates the dynamics of intranuclear transcription factors that induce physiological responses (Fig. 1). In mammals, MKK4 (MAP2K4) and MKK7 (MAP2K7) phosphorylate and activate JNK, whereas MKK3 (MAP2K3), MKK4, and MKK6 (MAP2K6) activate p38. Both JNK and p38 regulate the expression of numerous genes related to cell survival (e.g. Bcl-2 and BAX), cell cycle (e.g. p53 and cyclin D1), and cell proliferation (e.g. JUN and MYC). Stress-responsive signaling cascades have been shown to regulate features of tumor cells themselves, such as hyperproliferation, replicative immortality, and resistance to cell death. Meanwhile, it has been recently revealed that tumors are more than homogenous masses of proliferating malignant cells. Rather, they consist of heterogeneous cell types, including recruited monocytes, lymphocytes, and fibroblasts, interacting with one another in the host environment. Moreover, stress-responsive signaling cascades also play pivotal roles in these heterotypic interactions, and a plethora of evidence has been accumulated that suggests the involvement of MAPK pathways in these interactions.

This review outlines the reports that establish the impact of MAPK pathways on tumor progression, focusing not only on malignant cells but also on the host environment, including stromal cells. We summarize the findings obtained by using KO, cKO, and Tg mice of MAPK pathway components.
MAPK pathways and tumorigenesis

A wide variety of GEMMs and chemically induced tumorigenesis models have been developed because they are useful to assess the functions of specific proteins in tumorigenesis. In this section, we will classify the results from current studies of the roles of MAPK pathway components in tumorigenesis based on the tissues and the organs of origin.

Skin tumorigenesis

Various chemically induced tumorigenesis models as well as GEMMs have been established for the analysis of skin tumorigenesis. The most widely used model is the two-stage skin tumorigenesis model. In this chemically induced tumorigenesis model, murine dorsal skin is treated once with DMBA to induce DNA damage in keratinocytes (initiation), followed by repeated applications of TPA, which enhances the inflammatory responses (promotion).

Several MAPK pathway components have been reported to regulate skin tumorigenesis in this model. \( \text{Cot/Tpl2} \) (\( \text{Map3k8} \)) KO mice developed a higher number of tumors than WT mice through the induction of keratinocyte hyperproliferation triggered by elevated expression of inflammatory cytokines.\(^6\) In contrast, \( \text{Erk1} \) (\( \text{Mapk3} \)) KO mice,\(^7\) \( \text{p38} \) d (\( \text{Mapk13} \)) KO mice,\(^8\) and tamoxifen-inducible, keratinocyte-restricted \( \text{Mkk4} \) cKO mice\(^9\) showed resistance to skin tumorigenesis in this model due to defects in keratinocyte proliferation.

Here, we introduce one of the MAPK pathway components: the ASK family. The ASK family is a member of the MAP3Ks in the JNK and p38 MAPK pathways and is activated in response to various stressors, such as cytokines and oxidative stress.\(^{10,11}\) The mammalian ASK family is composed of three isoforms, \( \text{ASK1} \) (\( \text{MAP3K5} \)), \( \text{ASK2} \) (\( \text{MAP3K6} \)), and \( \text{ASK3} \) (\( \text{MAP3K15} \)), which have high homology, especially in the serine/threonine kinase domain.\(^{12}\) We have previously investigated the roles of \( \text{ASK1} \) and \( \text{ASK2} \) in skin tumorigenesis with the DMBA/TPA model. \( \text{ASK2} \) KO mice formed more skin tumors than WT mice, whereas \( \text{ASK1} \) KO mice showed comparable number of skin tumors to WT mice. The DMBA-induced apoptosis of epidermal keratinocytes was attenuated both in \( \text{ASK1} \) KO and \( \text{ASK2} \) KO mice to a similar extent. In contrast, TPA-dependent inflammatory responses, such as epidermal thickening and cytokine production, were impaired in \( \text{ASK1} \) KO mice but not in \( \text{ASK2} \) KO mice. These results suggest that \( \text{ASK1} \) and \( \text{ASK2} \) in keratinocytes cooperatively inhibit tumorigenesis by inducing apoptosis triggered by DNA damage, whereas only \( \text{ASK1} \) is distinctively pro-tumorigenic by evoking inflammation through the promotion of pro-inflammatory cytokines (Fig. 2).\(^{13}\) Considering the fact that \( \text{Ask1};\text{Ask2} \) doubleKO mice showed a similar phenotype to \( \text{Ask2} \) KO mice, the antitumorigenic role of \( \text{ASK1} \) is thought to compete with its tumorigenic role. This may explain why there was no difference in the extent of skin tumorigenesis between WT and \( \text{Ask1} \) KO mice.

In addition to the DMBA/TPA model, models using gene manipulation and the induction of skin tumorigenesis with other chemicals show that several other MAPK pathway components also have pro-tumorigenic or antitumorigenic functions. Keratinocyte-specific \( \text{Mek1} \) (\( \text{Map2k1} \)) cKO mice had fewer and smaller papillomas after DMBA/TPA treatment compared with WT or \( \text{Mek2} \) (\( \text{Map2k2} \)) KO mice.\(^{14}\) Meanwhile, keratinocyte-specific overexpression of a constitutively active \( \text{Mek1} \) mutant showed spontaneous skin tumor development out of hyperplasia.\(^{15}\) These reports suggest that MEK1...
in the host environment has essential and isoform-specific pro-tumorigenic roles in skin tumorigenesis. Alternatively, Jnk1 (Mapk8) deficiency promoted skin tumorigenesis in the DMBA/TPA model(16) as well as the DMBA/UVA exposure model, probably due to reduced apoptosis.(17) By contrast, Jnk2 deficiency suppressed skin tumorigenesis in both models, presumably owing to a defect in cell proliferation and tumor vascularization.(17,18) Collectively, individual JNK isoforms play unique roles in skin tumorigenesis. Epidermis-specific c-Raf (Raf-1) cKO mice were resistant to the formation of skin tumors in the DMBA/TPA model through reduced proliferation and enhanced apoptosis of keratinocytes.(19) DMBA evokes skin tumorigenesis by introducing mutations to the ras gene.20) However, most human squamous skin carcinomas have elevated oncogenic Ras signaling without the presence of activating mutations in ras.21) Thus, Ehrenreiter et al. used another model of skin tumorigenesis to imitate the progressive stages of skin tumorigenesis in humans. When crossed with the mice that express a dominant active form of SOS-F in the epidermis in a 4-OHT-inducible manner, c-Raf cKO showed resistance to skin tumorigenesis by inducing the differentiation of keratinocytes. In addition, keratinocyte-restricted overexpression of a dominant negative form of p38α impaired skin tumorigenesis in a UV-induced skin tumorigenesis model.22,23) Dickson et al.24) suggested that the skin phenotype of UV-B-induced skin tumorigenesis in these mice was due to a reduction in the chronic hyperproliferation of keratinocytes. Liu et al.25) suggested that solar UV-induced skin tumorigenesis in these mice was suppressed through defects in inflammation.

As described above, different isoforms of MAPK pathway components have the following features during the course of skin tumorigenesis: (i) isoform specificity (i.e., MEK1, but not MEK2 has pro-tumorigenic roles); (ii) bidirectionality (JNK1 functions as a tumor suppressor, whereas JNK2 functions as an oncogene); and (iii) distinctiveness (both ASK1 and ASK2 induce apoptosis, whereas only ASK1 evokes inflammatory responses). In summary, different MAPK pathway components, including different isoforms, play diverse roles in skin tumorigenesis. A previous clinical report suggested that, for example, a combination of trametinib, a MEK inhibitor, and dabrafenib, a Raf inhibitor, has significant clinical benefits for skin cancer patients.24) This is a convincing example showing the effectiveness toward target MAPK pathway components for cancer therapy.

Lung tumorigenesis

Oncogenic K-ras mutations have been identified in many lung cancer patients, and lung tumorigenesis GEMMs have been established by expressing these mutants in mice. Various KO or cKO mice of MAPK pathway components were crossed with the mice that have conditional expression of oncogenic K-ras (G12D or G12V, for example) to investigate the involvement of MAPK pathway components in lung tumorigenesis. For example, 4-OHT-inducible KO of p38α in adult mice fostered lung tumorigenesis triggered by K-RasG12V by interrupting maturation and promoting hyperproliferation of lung epithelium.25) By contrast, p38α deletion attenuated lung tumorigenesis triggered by K-RasG12D through an unknown mechanism,26) illustrating the distinct roles of p38 isoforms in lung tumorigenesis. Bronchial epithelium-specific deletion of Mkk427) and epidermis-specific ablation of Mkk728) were revealed to deteriorate lung tumorigenesis combined with K-RasG12D. Schramek et al. further investigated whether MKK7 affects lung tumorigenesis through its well-known downstream effectors, JNKs. Jnk1+/−; Jnk2−/− mice were also sensitive to K-rasG12D-induced lung tumorigenesis. Although there is still a need for further detailed analysis, it is conceivable that MKK7 modulates lung tumorigenesis through JNK1 and JNK2 and that the MKK7--JNK signaling axis acts as an antitumor barrier.

However, Blasco et al.29) showed that the c-Raf--MEK--ERK axis is oncogenic during lung tumorigenesis in the same manner as in skin tumorigenesis. K-rasG12V-induced lung tumorigenesis was mitigated when crossed with c-Raf+/lox/lox; Mkk7+/lox/+; Mek1+/lox/lox; Mek2−/−, and Erk1−/−; Erk2 (Mapk1)+/lox/lox mice that were treated with Cre recombinase. The Cre-mediated recombination was induced by intratracheal infusion of a non-replicative adenovirus that encodes Cre recombinase (Ad-Cre).

Intriguingly, Ad-Cre-induced ablation of c-Raf, but not B-Raf, specifically attenuated lung tumorigenesis evoked by K-rasG12D.29) Combined with the fact that the mice with lung-selective overexpression of c-Raf spontaneously developed lung adenomas,30) it is possible that c-Raf may be a suitable therapeutic target for lung cancer.

Mice overexpressing the B-Raf V600E mutant, which has constitutive kinase activity and a transforming ability, were shown to spontaneously develop hyperproliferative lung adenocarcinomas.31,32) The transgene was induced with Ad-Cre31) or with DOx32) in a lung-specific manner. As mentioned above, however, B-Raf deletion did not affect oncogenic K-ras-induced lung tumorigenesis, even though both c-Raf and B-Raf are downstream signaling components of ras. Collectively, ras may dominantly cultivate lung cancer in a c-raf-dependent manner. However, excessive activation of B-Raf caused by gene mutations may also trigger lung tumorigenesis.

Lung tumors can also be induced with chemical carcinogens. When Cot/Tpl2 KO mice were exposed to urethane, a chemical carcinogen, the mice were susceptible to the development of lung tumors exhibiting hyperproliferation, aggressive invasion, and cytologic atypia of lung epithelial cells through a defect in Jnk--p53 activation.33) Based on the fact that lung cancers are caused by environmental factors, such as tobacco smoke and asbestos, a more detailed understanding of the function of MAPK pathway components should be explored in chemically induced lung tumorigenesis models.

Mammary tumorigenesis

Manipulation of gene expression, such as overexpression of oncogenes and downregulation of tumor suppressor genes, can drive mammary tumors. Mammary tumorigenesis driven in mice by overexpression of NeuT, an activated form of human epidermal growth factor receptor 2, was facilitated when Mkk7 was ablated in mammary epithelial cells.27) Further analysis suggested that MKK7 maintains p53 protein stability and p53-mediated responses to genotoxic stresses, such as cell senescence. Alternatively, deficiency in either Jnk1 or Jnk2 reduced breast tumor-free survival when crossed with Tprp53+/− mice, which exhibit mild mammary tumorigenesis compared with Tprp53−/− mice.34) Moreover, the absence of Jnk2 exacerbated mammary tumorigenesis triggered by the PyVmT transgene through the dysregulation of DNA damage responses and a consequent increase in tumor aneuploidy.35) Previous reports proposed that Jnk regulates protein phosphorylation and the stability of p53.36) Considering that p53 is one of the genes responsible for mammary tumors and that mutations in p53 are
Colon tumorigenesis

Mutations in the Apc gene are observed in almost all colon tumor patients in the relatively early stage of tumorigenesis, and these mutations are known to induce the formation of premalignant adenomatous polyps. Thus, GEMMs harboring mutations in the Apc gene are used to explore the mechanism of colon tumorigenesis. Col/Tpl2 KO mice crossed with Apcmin/+ mice, which express a dysfunctional truncated Apc protein without sufficient tumor suppressive functions, formed more aggressive colon tumors compared with control Apcmin/+ mice. This may be because Apcmin/+ Tpl2−/− mice showed enhanced intestinal inflammation associated with a decreased number of immunosuppressive regulatory T cells (30) Jnk2 deletion in Apc1638/+ mice, which express undetectable amount of Apc protein, promoted colon tumorigenesis through accelerated inflammation and aberrant β-catenin expression. Intriguingly, Jnk2 deletion enhanced the burden of colon tumors only if combined with a “high-risk, Western-style” diet containing high fat, high phosphorous, low calcium, and low vitamin D. These results imply that Jnk2 and dietary factors may cooperatively regulate colon tumorigenesis under certain circumstances.

Chemically induced tumorigenesis models have also been widely used for studying colon tumorigenesis. One typical model of colon tumorigenesis is established by single i.p. injection of AOM and continuous administration of DSS through drinking water. It has been already shown that deletion of p38α in IECs fostered colon tumorigenesis in this model through an unknown mechanism. However, using this model, Gupta et al. (42) recently showed that p38α has both a tumor-suppressive role in colon tumorigenesis and a tumor-promoting role in tumor progression. Deficiency in p38α in IECs augmented sensitivity to colitis-associated colon cancer due to enhanced infiltration of inflammatory cells and increased apoptosis of IECs followed by compensatory hyperproliferation. Notably, p38α ablation in IEC triggered colon tumorigenesis when the mice received only DSS treatment. These data also indicate that p38α has a tumor-suppressive role in colon tumorigenesis. However, 4-OHT-inducible, IEC-specific cKO of p38α in tumor-established mice alleviated tumor burden, suggesting that p38α also has a supportive effect on tumor cell proliferation. Considering the dual functions of p38α in the course of colon cancer progression, targeting p38α should be examined carefully. In addition, other p38 isoforms, p38β and p38γ (MAPK12), have been revealed to regulate colon tumorigenesis in the same model. It was reported that p38β−/− p38γ−/− and p38δ−/− p38β−/− mice showed mitigation in colon tumorigenesis. Deletion of p38δ and/or p38γ in mice attenuated IEC proliferation and enhanced IEC apoptosis. It also reduced the expression of pro-inflammatory chemokines and cytokines such as monocyte chemotactic and activating factor-1 and TNF-α, resulting in defective recruitment of macrophages and neutrophils. Importantly, p38δ−/−; p38γ−/− mice were more resistant to colon tumorigenesis compared with p38δ−/− or p38γ−/− mice, which is indicative of the synergistic impact of p38δ and p38γ on colon tumorigenesis.

Ask1 KO mice were reported to show susceptibility to AOM/DSS colon tumorigenesis coinciding with severe inflammation. In vitro experiments have revealed that macrophages of Ask1 KO mice had impaired cytotoxicity against enteric bacteria and were vulnerable to bacteria-induced apoptosis owing to a defect in p38 activation. These phenomena may be attributed to decreased messenger RNA expression of anti-apoptotic genes, such as cellular inhibitor of apoptosis protein 1 and 2 and serpin B2.

It was also revealed that Jnk1 regulates colon tumorigenesis. Overexpression of a constitutively active form of Jnk1 in an IEC-specific manner increased colitis-associated colon cancer susceptibility to AOM/DSS-induced colon tumorigenesis owing to enhanced proliferation of progenitor cells. However, there is also a report that Jnk1 deletion can spontaneously induce colon tumorigenesis. Although compensation by other Jnk isoforms and the cell type-specific roles of Jnk1 may account for this discrepancy, further analysis is needed to clarify the divergent functions of Jnk1 and p38x in colon tumorigenesis. Compared with other organs, the colon is incessantly exposed to innumerable enteric bacteria, which can trigger divergent inflammatory responses. This peculiarity may explain why MAPK pathway components in the host environment have contrasting functions in colon tumorigenesis.

Liver tumorigenesis

There are many mouse models for liver tumorigenesis that recapitulate human liver cancers. Single i.p. injection of DEN is a common chemically induced model of liver tumorigenesis. Loss of Jnk1 mitigated liver tumorigenesis with a reduction of DEN-induced cell death, compensatory proliferation, and neovascularization. Moreover, cell type-dependent effects of Jnks in liver tumorigenesis were investigated using Jnk2−/− mice crossed with hepatocyte-specific (Alb−Cre−; Jnk1flx/flx), and hepatocyte/non-parenchymal cell-specific (Mx1−Cre−; Jnk1flx/flx) Jnk1 cKO mice (49) hereafter referred to as H−/− and Mx−/− mice, respectively. H−/− mice developed more tumors, whereas Mx−/− mice developed fewer tumors compared with control mice, which express only Cre recombinase in the corresponding tissues. In-depth analysis of these opposing phenotypes by Das et al. revealed that the expression and release of inflammatory cytokines, such as IL-6, IL-1α, IL-1β, and TNF-α, were enhanced in H−/− mice but were decreased in Mx−/− mice. Consequently, hepatocyte death and compensatory proliferation were accelerated in H−/− mice but attenuated in Mx−/− mice.

Meanwhile, hepatocyte-specific p38α cKO resulted in elevated HCC with this model due to an increase in reactive oxygen species production, liver damage, and compensatory proliferation. Further demonstrated that p38α inhibited liver fibrogenesis and subsequent HCC in another liver tumorigenesis model with thioacetamide added to drinking water. Hui et al. also reported the suppressive functions of p38α in liver tumorigenesis with another similar tumorigenesis model; liver-specific p38α-deficient mice were injected with DEN i.p. and subsequently fed with phenobarbital mixed with their diet. Notably, p38α is hypothesized to modulate hepatocyte proliferation by antagonizing the JNK–c-Jun pathway, suggesting the crosstalk between MAPK pathway components in liver tumorigenesis.

It has also been proposed that Ask1 regulates liver tumorigenesis; Ask1 KO mice formed more tumors in DEN-induced liver tumorigenesis model. ASK1 appeared to have an influence on death receptor-mediated apoptosis through JNK activation and DNA damage responses in p38 activation.
In addition to chemically induced tumorigenesis models, manipulating gene expressions of MAPK pathway components have been shown to induce spontaneous formation of liver tumors. Ablation of Tak1 (Map3k7) in hepatocytes generated HCC through various mechanisms: deregulated hepatic inflammation and hepatocyte injury that cause liver fibrosis, enhancement of TNF-α-dependent hepatocyte death, and compensatory proliferation. (34,55) Alternatively, Tak1 ablation specifically in liver parenchymal cells also led to spontaneous formation of liver tumors with hepatic inflammation, liver fibrosis, cholestasis, and ductopenia. (56) Furthermore, Tak1 was shown to regulate hepatocyte apoptosis and necrosis through a nuclear factor-κB-dependent and -independent manner, respectively. It is conceivable that gene manipulation of other MAPK pathway components may affect liver tumorigenesis, and further study is warranted to comprehensively understand the mechanism of how these components contribute to it. Taking p38α and JNK as an example, we have to pay close attention to the interaction between MAPK pathway components and their distinct functions according to cell types.

**Gastric tumorigenesis**

Similar to lung and liver tumorigenesis, gastric tumorigenesis can be evoked by environmental factors such as Helicobacter pylori and alcohol intake. A typical chemically induced model of gastric tumorigenesis that mimics those environmental factors is to challenge mice with N-methyl-N-nitrosourea in drinking water. Genetic disruption of Jnk1 attenuated the growth of GC, probably owing to decreases in apoptosis and in compensatory cell proliferation in a reactive oxygen species-dependent manner. (57) Ask1 KO mice were reported to be resistant to gastric tumorigenesis induced with N-methyl-N-nitrosourea. (58) ASK1 was shown to participate in a positive feedback loop with cyclin D1 through JNK activation and to promote the proliferation of gastric cells. It was further shown that K811, an ASK1 inhibitor, prevented the growth of GC, suggesting that ASK1 might be a promising drug target for GC therapy. (59) As MAPKs, such as p38, JNK, and ERK, were shown to be activated by H. pylori (60) investigating the effect of MAPK pathway components on gastric tumorigenesis induced by environmental factors would clarify the clinical significance of MAPK pathway components.

**Pancreatic tumorigenesis**

Human pancreatic cancer has a linear progression associated with accumulated gene mutations. Its premalignant lesion is referred to as PanIN and is classified into multiple stages based on cellular morphology and polarity, micropapillary structure, and chromosomal composition. Some GEMMs spontaneously generate PanIN in the course of developing pancreatic cancers. (61) For example, RIP1Tag2 Tg mice express Simian vacuolating virus 40 large T antigen transgene under RIP1 and spontaneously develop pancreatic islet carcinoma. The impact of genetic deletion of MAPK pathway components has been investigated in these models. In RIP1Tag2 Tg mice, pancreatic β cell-specific deletion of B-Raf delayed tumor progression with attenuated cell proliferation and suppressed angiogenesis. (62) Pancreas-specific ablation of Mkk4 and Mkk7 drastically facilitated PanIN progression in KrasG12D-triggered pancreatic tumorigenesis. (63) The detailed molecular mechanism is yet to be elucidated, but these results were presumably due to additional effects of M KK4 and M KK7 on the activity of JNK. Davies et al. further suggested that M KK4 and M KK7 may promote the transdifferentiation of pancreatic cell types from acinar to ductal cells and that they have synergistic impacts on pancreatic tumorigenesis. As pancreatic tumors are known to have a very poor clinical outcome, it will be important to identify the precise molecular mechanisms involved in pancreatic tumor progression, including the roles of M KK4 and M KK7, to develop effective therapies for pancreatic cancer.

**Hematological malignancies and tumorigenesis in other tissues**

The MAPK pathway components are known to govern non-epithelial cancers, such as hematological malignancies, as well as epithelial solid tumors. Manipulating the gene expression of specific MAPK pathway components can spontaneously trigger leukemia. Lymphocyte-specific overexpression of a Cot/Tpl2 mutant, which has a truncation in the carboxyl terminus leading to increased catalytic activity and hyperactivation of the MAPK pathway, gave rise to spontaneous lymphoma in mice. (64) By contrast, Cot/Tpl2 deficiency resulted in T-cell lymphoma by promoting T cell proliferation when crossed with TCR2C Tg mice, which express the specific T cell receptors to MHC class I. (65) This discrepancy is informative because it suggests that appropriate kinase activity of Cot/Tpl2 is vital for inhibition of lymphoma and that either excessive or defective kinase activity results in the formation of lymphoma.

Myeloid lineage-specific deletion of Tak1 resulted in the development of myelomonocytic leukemia with splenomegaly due to a lack of myeloid cell maturation and genomic instability. (66) B-Raf is oncogenic also in hematological malignancies, and interferon-inducible overexpression of the B-Raf V600E mutant in somatic tissues led to the spontaneous development of non-lymphocytic leukemia. (67) B-Raf is thought to be an oncogene in other epithelial solid tumors (68) including thyroid (69) and prostate (70) cancers. Overexpression of the B-Raf V600E mutant in thyroid cells evoked poorly differentiated papillary thyroid carcinomas. (69) Similarly, DOX-induced overexpression of the B-Raf V600E mutant in prostate basal epithelial cells led to the development of invasive prostate adenocarcinomas with aberrant cell proliferation. (70) Jeong et al. also showed that DOX withdrawal eliminated the expression of B-Raf within established adenocarcinomas, but those established tumors did not regress. From these data, Jeong et al. suggested that stimulation of B-Raf is sufficient to initiate prostate adenocarcinoma growth, but unnecessary to maintain these tumors once they were established. As we have previously mentioned, p38α also has distinctive roles during the establishment and the maintenance phases of colon cancer growth. (42) These reports suggest that the divergent roles of MAPK pathway components in tumor progression should be analyzed separately prior to and after the establishment of tumors.

**Mitogen-activated protein kinase pathways and tumor metastasis**

Tumor cells from primary lesions gradually invade into the local surrounding stroma, enter circulation (intravasate) and disseminate, exit circulation (extravasate), and finally adapt to foreign environments of distant sites (colonize). (71) This sequential transition is called tumor metastasis. It is of paramount importance from a clinical perspective to elucidate the
| Molecule | Gene manipulation of MAPK pathway components | Tumorigenesis model | Tumor type | Phenotype and concomitant phenomena | Role | References |
|----------|---------------------------------------------|-------------------|------------|------------------------------------|------|-----------|
| B-Raf   | Lung-Tg (V600E, adenovirus with Cre-inducible) | Spontaneous       | Lung adenocarcinoma | Enhanced (mechanism unknown) | Pro  | (30)      |
|         | Lung-Tg (V600E, DOX-inducible)             | Spontaneous       | Lung adenocarcinoma | Enhanced (mechanism unknown) | Pro  | (31)      |
|         | Pancreatic β cell-cKO                      | Spontaneous       | Pancreatic adenoma | Enhanced (cell proliferation ↓, angiogenesis ↓) | Pro  | (61)      |
|         | Prostate basal epithelial-Tg (V600E, DOX-inducible) | Spontaneous       | Prostate adenocarcinoma | Enhanced (aberrant cell proliferation) | Pro  | (69)      |
| Thyroid cell-Tg (V600E) | Spontaneous | Papillary thyroid carcinoma | Enhanced (mechanism unknown) |             | Pro  | (68)      |
| Somatic tissues-Tg (IFN-inducible) | Spontaneous | Nonlymphoid leukemia | Enhanced (mechanism unknown) |             | Pro  | (66)      |
| C-Raf (Raf-1) | Lung-Tg | Spontaneous | Lung adenoma | Enhanced (mechanism unknown) | Pro  | (29)      |
|         | cKO (adenovirus with Cre-inducible)        | K-ras^{G12V} Tg   | NSCLC      | Attenuated (mechanism unknown) | Pro  | (27)      |
|         | cKO (adenovirus with Cre-inducible)        | K-ras^{G12D} Tg   | Lung adenocarcinoma | Attenuated (mechanism unknown) | Pro  | (28)      |
|         | Epidermis-cKO                             | Induced with DMBA + TPA | Cutaneous papilloma | Enhanced (proliferation ↓, apoptosis ↑ of keratinocyte) | Pro  | (19)      |
|         | Epidermis-cKO                             | 4OHT-inducible SOS-F Tg | Cutaneous papilloma | Attenuated (keratinocyte differentiation ↑) | Pro  | (19)      |
| ASK2 (MAP3K6) | KO | Induced with DMBA + TPA | Cutaneous papilloma | Enhanced (apoptosis of keratinocyte ↓ [cooperating with ASK1]) | Anti | (13)      |
| TAK1 (MAP3K7) | Liver parenchymal-cKO | Spontaneous | HCC | Enhanced (hepatic inflammation, liver fibrosis, cholestasis, ductopenia, hepatocyte apoptosis and necrosis) | Anti | (55)      |
|         | Hepatocyte-cKO                            | Spontaneous       | HCC         | Enhanced (dysregulated hepatic inflammation, hepatocyte injury, liver fibrosis, hepatocyte death, compensatory proliferation) | Anti | (53,54) |
| MEK1 (MAP2K1) | Myeloid-cKO | Spontaneous | Leukemia | Enhanced (immaturity of myeloid cells, genomic instability, splenomegaly) | Anti | (65)      |
|         | Keratinocyte-Tg (constitutively active mutant) | Spontaneous | Cutaneous papilloma | Enhanced (hyperplasia ↑) | Pro  | (15)      |
|         | Keratinocyte-cKO                          | Induced with DMBA + TPA | Cutaneous papilloma | Attenuated (mechanism unknown) | Pro  | (14)      |
| MKK7 (MAP2K7) | Epidermis-cKO | K-ras^{G12D} Tg | Lung adenocarcinoma | Enhanced (p53 stability ↑, cellular senescence ↑, cell proliferation ↓) | Anti | (26)      |
| ERK1 (MAP3K) | MEC-cKO | NeuT Tg | Mammary carcinoma | Enhanced (p53 stability ↑, p53-mediated responses to genotoxic stresses ↓) | Anti | (26)      |
mechanism of tumor metastasis because over 90% of tumor mortality is attributed to metastatic spread. However, deciphering the complexity of tumor metastasis is a challenging task because this process is very inefficient. Only a tiny fraction of tumor cells can adapt to foreign sites and form metastatic foci. Although there are a few reports that show that MAPK pathway components in the host environment participate in tumor metastasis, we will present the collection of studies on the relationships between MAPK pathway components and tumor metastasis using our unpublished data as well.

*Mekk1* (Map3k1) KO mice were examined in a PyVmT transgene-induced mammary tumorigenesis model, which is also known to develop lung metastasis. Ablation of Mekk1 did not affect the frequency and growth of primary mammary tumors, but it mitigated tumor cell dissemination and lung metastasis. Melanocyte-specific Tg mice of the B-Raf V600E mutant developed melanoma and tumor metastasis to draining lymph nodes. They even developed lung metastases when crossed with *Cdkn2a* KO mice. They even developed lung metastases when crossed with *Jnk2* KO mice to i.v. injection of 3LL-Luc2 cells, which have constitutive luciferase expression so that lung micrometastases can be detected by measuring the luciferase activity of lung lysates. As a result, Ask1 KO mice had a markedly reduced number of tumor cell lineages. In addition, Jnk2 KO mice showed resistance to lung and bone metastasis caused by intracardiac injection of 4T1 mammary tumor cell line. Through osteolysis mediated by mature osteoclasts, a variety of growth factors, such as transforming growth factor-β, fibroblast growth factors, and bone morphogenetic proteins, are released and stimulate the proliferation of tumor cells.

Once intravasated, circulating tumor cells face several risks for death, such as the shear stress of the blood or the lymphatic flow and immunosurveillance by antitumor immune cells. Platelets can shield tumor cells from those threats in the case of hematogenous metastasis by forming platelet–tumor cell (that sometimes include leukocytes) aggregates. The formation of these aggregates is mediated by the release of factors (e.g., chemokines and growth factors) and adhesive molecules (e.g., integrins and selectins). These aggregates support tumor cell attachment to and interaction with endothelial cells, and aid tumor cells in crossing endothelial walls to extravasate.

Subcutaneous injection of B16F10 melanoma and Lewis lung carcinoma cell lines into p38α−/− mice did not affect the penetrance or growth of primary tumors compared with WT mice. However, when p38α−/− mice were challenged with i.v. injection of B16F10 and Lewis lung carcinoma, lung metastasis was reduced in p38α−/− mice. Matsuo et al. claimed that tumor cell-dependent upregulation of P-selectin in platelets can shield tumor cells from those threats in the case of hematogenous metastasis by forming platelet–tumor cell aggregates. This phenomenon is mediated by the release of factors (e.g., chemokines and growth factors) and adhesive molecules (e.g., integrins and selectins).

### Table 1 (continued)

| Molecule | Gene manipulation of MAPK pathway components | Tumorigenesis model | Tumor type | Phenotype and concomitant phenomena | Role | References |
|----------|---------------------------------------------|---------------------|------------|-----------------------------------|------|------------|
| p38γ (MAPK12) | KO | Induced with AOM + DSS | Colon adenocarcinoma | Attenuated (expression of pro-inflammatory cytokine and chemokine, recruitment of macrophage and neutrophil, proliferation, apoptosis of IEC) | Pro | (42) |
| p38δ (MAPK13) | KO | Induced with DMBA + TPA | Cutaneous papilloma | Attenuated (keratinocyte proliferation ↓) | Pro | (8) |
| KO | *K-ras*G12D | Lung adenocarcinoma | Attenuated (mechanism unknown) | Pro | (8) |

For B-Raf, see the referenced review. Anti, antitumorigenic role of MAPK pathway; AOM, azoxymethane; cKO, conditional knockout; DMBA, 7,12-dimethylbenz(a)anthracene; DOX, doxycycline; DSS, dextran sodium sulfate; IEC, intestinal epithelial cell; MEC, mammary epithelial cell; NSCLC, non-small cell lung cancer; Pro, pro-tumorigenic role of MAPK pathway; Tg, transgenic. Upright arrows indicate the enhancement of the phenomena and down arrows indicate the attenuation of the phenomena.
Table 2. Mitogen-activated protein kinase pathway components reported to have both tumorigenic and antitumorigenic functions

| Molecule | Gene manipulation of MAPK pathway components | Tumorogenesis model | Tumor type | Phenotype and concomitant phenomena | Function | References |
|----------|---------------------------------------------|---------------------|------------|-------------------------------------|----------|------------|
|ASK1 (MAP3K5) | KO Induced with AOM + DSS | Colon adenocarcinoma | Enhanced (cytotoxicity and apoptosis of macrophage ↓, inflammation ↑) | Anti | (43) |
| | KO Induced with DEN | HCC | Enhanced (death receptor-mediated apoptosis ↓, DNA damage responses ↑) | Anti | (52) |
| | KO Induced with MNU | Gastric carcinoma | Attenuated (cell proliferation ↓, cell cycle progression ↓) | Pro | (57,58) |
| | KO Induced with DMBA + TPA | Cutaneous papilloma | Comparable in overall (inflammatory responses ↓) | Pro | (13) |
| | KO Induced with DMBA + TPA | Cutaneous papilloma | Comparable in overall (apoptosis of keratinocyte ↓ [cooperating with ASK2]) | Anti | (13) |
| | KO Induced with DMBA + TPA | Cutaneous papilloma | Comparable in overall (apoptosis of keratinocyte ↓) | Anti | (13) |
| | KO Induced with DMBA + UVA | Cutaneous papilloma | Attenuated (mechanism unknown) | Pro | (6) |
| | KO Induced with DMBA + UVA | Cutaneous papilloma | Attenuated (mechanism unknown) | Pro | (6) |
| | KO Induced with DMBA + TPA | Cutaneous papilloma | Enhanced (mechanism unknown) | Anti | (6) |
| | KO Induced with DMBA + TPA | Cutaneous papilloma | Enhanced (mechanism unknown) | Anti | (6) |
| | KO Induced with DMBA + UVA | Cutaneous papilloma | Attenuated (mechanism unknown) | Anti | (6) |
| | KO Induced with DMBA + UVA | Cutaneous papilloma | Attenuated (mechanism unknown) | Pro | (17) |

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In summary, the reports shown above imply that MAPK pathway components in the host environment could perform multifaceted functions in tumor metastasis, sometimes with little effect on tumor initiation. Thus, it is necessary to dissect the functions of MAPK pathway components in tumor initiation and metastasis separately while keeping the comprehensive system in mind.

**Conclusion**

Tumor cells and normal stromal cells in the host environment are revealed to communicate each other, and their complex network appears to influence every aspect of tumor progression. Although the fully detailed picture of this heterotypic interaction is still enigmatic, ever-progressing technologies such as high-resolution single-cell imaging, high accuracy gene manipulation both in vivo and in vitro (e.g. CRISPR/Cas9, TALEN), and GEMMs with temporally or spatially increased specificity will reveal the comprehensive frameworks of tumor biology.

A myriad of evidence has been accumulated to suggest that MAPK pathway components in tumor cells as well as normal stromal cells in the host environment greatly influence tumor progression. Some MAPK pathway components such as B-Raf and M KK7 seem to have pro-tumorigenic or antitumorigenic functions, respectively (Table 1). Many MAPK pathway components seem to have both pro-tumorigenic and antitumorigenic functions, depending on the context. Anti, antitumorigenic function of MAPK pathway; AOM, azoxymethane; cKO, conditional knockout; DEN, diethylnitrosamine; DMBA, 7,12-dimethylbenz(a)anthracene; DSS, dextran sodium sulfate; HCC, hepatocellular carcinoma; IEC, intestinal epithelial cell; LEC, lung epithelial cell; MNU, N-methyl-N-nitrosourea; NSCLC, non-small cell lung carcinoma; 4-OHT, 4-hydroxy-tamoxifen; Pro, pro-tumorigenic function of MAPK pathway; ROS, reactive oxygen species; TAA, thioacetamide; TAM, tamoxifen; Tg, transgenic; Treg, regulatory T cell.

### Table 2 (continued)

| Molecule | Gene manipulation of MAPK pathway components | Tumorigenesis model | Tumor type | Phenotype and concomitant phenomena | Function | References |
|----------|---------------------------------------------|---------------------|------------|------------------------------------|----------|------------|
| JNK2 (continued) | KO | Trp53<sup>−/−</sup> | Mammary gland tumor | Enhanced (mechanism unknown) | Anti | (33) |
| KO | PyVmT Tg | Mammary carcinoma | Enhanced (dysregulated DNA damage responses, aneuploidy †) | Anti | (34) |
| KO | Apc1638<sup>−/−</sup>; “Western-style” diet | Intestinal adenoma | Enhanced (inflammation †, aberrant β-catenin expression) | Anti | (39) |
| KO (4OHT-inducible) | K-ras<sup>G12V</sup> Tg | NSCLC | Enhanced (maturation †, hyperproliferation † of lung epithelium) | Anti | (24) |
| Liver-cKO | Induced with DEN + Pb | HCC | Enhanced (hepatocyte proliferation †) | Anti | (51) |
| Hepatocyte-cKO | Induced with DEN | HCC | Enhanced (ROS production †, liver damage †, compensatory cell proliferation †) | Anti | (49) |
| Hepatocyte-cKO | Liver (TAA) | HCC | Enhanced (liver fibrogenesis †) | Anti | (50) |
| IEC-cKO | Colon (DSS) | Colon adenocarcinoma | Enhanced (mechanism unknown) | Anti | (41) |
| IEC-cKO | Colon (AOM + DSS) | Colon adenocarcinoma | Enhanced (inflammatory cell infiltration †, IEC apoptosis †, compensatory hyperproliferation) | Anti | (40, 41) |
| IEC-cKO (4-OHT-inducible) | Colon (AOM + DSS) | Colon adenocarcinoma | Attenuated (cell proliferation †, apoptosis † [tumor maintenance]) | Pro | (41) |
| Keratinocyte-Tg (dominant negative mutant) | Induced with SUV | Cutaneous papilloma | Attenuated (inflammation †, epidermal thickening †) | Pro | (23) |
| Keratinocyte-Tg (dominant negative mutant) | Induced with UVB | Cutaneous papilloma | Attenuated (chronic hyperproliferation †) | Pro | (22) |

Many MAPK pathway components seem to have both pro-tumorigenic and antitumorigenic functions, depending on the context. Anti, antitumorigenic function of MAPK pathway; AOM, azoxymethane; cKO, conditional knockout; DEN, diethylnitrosamine; DMBA, 7,12-dimethylbenz(a)anthracene; DSS, dextran sodium sulfate; HCC, hepatocellular carcinoma; IEC, intestinal epithelial cell; LEC, lung epithelial cell; MNU, N-methyl-N-nitrosourea; NSCLC, non-small cell lung carcinoma; 4-OHT, 4-hydroxy-tamoxifen; Pro, pro-tumorigenic function of MAPK pathway; ROS, reactive oxygen species; TAA, thioacetamide; TAM, tamoxifen; Tg, transgenic; Treg, regulatory T cell.
Manipulating multiple MAPK pathway components allows us to examine the combined effects on tumor progression and various biological responses. Anti, antitumorigenic role of MAPK pathway; AOM, azoxymethane; cKO, conditional knockout; DEN, diethylnitrosamine; DSS, dextran sodium sulfate; HCC, hepatocellular carcinoma; IEC, intestinal epithelial cell; NSCLC, non-small cell lung cancer; Pro, pro-tumorigenic role of MAPK pathway; Tg, transgenic.

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### Disclosure statement

The authors have no conflict of interest.

### Abbreviations

| Abbreviation | Description |
|--------------|-------------|
| AOM          | azoxymethane |
| ASK          | Apoptosis Signal-regulating Kinase |
| cKO          | conditional knockout |
| DEN          | diethylnitrosamine |
| DMBA         | 7,12-dimethylbenz(a)anthracene |
| DOX          | doxycycline |
| DSS          | dextran sodium sulfate |
| GC           | gastric cancer |
| GEMM         | genetically engineered mouse model |
| HCC          | hepatocellular carcinoma |
| IEC          | intestinal epithelial cell |
| IL           | interleukin |
| KO           | knockout |
| MAP2K        | MAPK kinase |
| MAP3K        | MAPK kinase kinase |

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Table 3. Reports regarding the combined effects of multiple MAPK pathway components in tumorigenesis

| Molecule | Gene manipulation of MAPK pathway components | Tumorigenesis model | Tumor type | Phenotype and concomitant phenomena | Role | References |
|----------|---------------------------------------------|---------------------|------------|-----------------------------------|------|------------|
| MEK1     | Mek1 cKO; Mek2 KO (adenovirus with Cre-inducible) | K-ras<sup>G12V</sup> Tg | NSCLC      | Attenuated (mechanism unknown)    | Pro  | (27)       |
| MEK2     |                                             |                     |            |                                   |      |            |
| (MAP2K2) |                                             |                     |            |                                   |      |            |
| MKK4     | Pancreas Mkk4 cKO; Mkk7 cKO                  | K-ras<sup>G12D</sup> Tg | Pancreatic ductal adenocarcinoma | Enhanced (trans-differentiation of acinar cell into duct-like cell) | Anti | (62)       |
| MKK7     |                                             |                     |            |                                   |      |            |
| JNK1     | Jnk1<sup>+/−</sup>; Jnk2<sup>−/−</sup>       | K-ras<sup>G12D</sup> Tg | Lung adenocarcinoma | Enhanced (mechanism unknown)    | Anti | (26)       |
| JNK2     |                                             |                     |            |                                   |      |            |
| JNK1     | Hepatocyte-Jnk1 cKO; Jnk2 KO                | Induced with DEN    | HCC        | Enhanced (release of inflammatory cytokines, hepatocyte death, compensatory proliferation) | Anti | (48)       |
| JNK2     |                                             |                     |            |                                   |      |            |
| p38α     | p38α KO; p38γ KO                            | Induced with AOM + DSS | Colon adenocarcinoma | Attenuated (expression of pro-inflammatory cytokine and chemokine, recruitment of macrophage and neutrophil, proliferation, apoptosis of IEC) | Pro  | (42)       |
| p38γ     |                                             |                     |            |                                   |      |            |
| ERK1     | Erk1 KO; Erk2 cKO (adenovirus with Cre-inducible) | K-ras<sup>G12V</sup> Tg | NSCLC | Attenuated (mechanism unknown) | Pro  | (27)       |
| ERK2     |                                             |                     |            |                                   |      |            |
| (MAP1/2) |                                             |                     |            |                                   |      |            |

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