Mitogen-activated Protein Kinase Signaling in Inflammation-related Carcinogenesis

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The role of mitogen-activated protein kinases (MAPKs) in regulation of inflammation is well known. MAPK family is activated by various stimuli and involved in transmitting extracellular signals to nucleus leading to gene regulation. Inflammation is primary defensive response of host against microbes. Controlled inflammation is helpful and indispensable for host defense. However, uncontrolled inflammatory response leads to various inflammatory diseases and cancer. Persistent inflammation leads to cell proliferation and survival that plays crucial role in tumorigenesis. In this review, we recapitulate the recent knowledge of MAPK signaling and its roles in inflammation-associated carcinogenesis.

Key Words: Cancer, Inflammation, Mitogen-activated protein kinase

INTRODUCTION

The innate immune system of mammals recognizes microbial infection and plays its role in elimination of microbes. Inflammation is primary protective response of host to eradicate destructive stimuli and repair injured tissue (1). Inflammation is characterized by five fundamental symptoms: heat, redness, pain, swelling, and loss of tissue function (2). Dendritic cells (DCs) and macrophages are the key player in innate immunity. In addition, endothelial cells, epithelial cells, and fibroblasts also take part in innate immunity. Controlled inflammatory response is indispensable and protects against destructive stimuli. However, deregulated inflammation is injurious because it leads to different diseases such as rheumatoid arthritis, atopic dermatitis, septic shock and cancer (1). Tissue macrophages and mast cells are implicated in recognition of primary infection. Upon detection of invading microorganisms, these cells produce inflammatory mediators such as pro-inflammatory cytokines and chemokines. The immediate effect of these mediators educes inflammatory exudates containing plasma proteins and neutrophils (1).

The germ line-encoded pattern recognition receptors are responsible for sensing the pathogen associated molecular patterns of the microbes (2, 3). The pattern recognition receptors are divided into four different groups; Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLR) and Retinoic acid inducible gene (RIG)-1-like receptors (RLRs) (2–8). Recent reports have highlighted the role of TLRs in the development of inflammation-associated carcinogenesis. Especially, it has been reported...
that endogenous TLR ligands involved in solid tumor progression and leukemic cell growth were released from dying tumor cells (9–11).

TLR activation leads to phosphorylation of mitogen activated protein kinases (MAPKs) and IκB kinases (IKK) complex which ultimately result in activation of activator protein 1 (AP-1) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), respectively (12). Activated AP-1 and NF-κB is responsible for production of pro-inflammatory cytokines and chemokines (13). The activation of MAPKs and NF-κB tightly controlled by phosphorylation and ubiquitination and results in production of tumor necrosis factor (TNF-α) and interleukins (ILs) (14). TNF-α is recognized as a pro- or anti-tumorigenic protein. TNF-α also promotes the production of inflammatory mediators including cyclooxygenase-2 (COX-2), IL-6, IL-8, and TNF-α and results in tumorigenesis through MAPK and NF-κB signaling cascade (15, 16).

Inflammation plays important role in pathogenesis of diverse types of malignancies (17, 18). Different stimulators including growth factors, stress, chemokines, cytokines and reactive oxygen species (ROS) result in activation of MAPK signaling pathways (19). MAPK pathways are the key regulator in the activation of various pro-inflammatory cytokines in different cell types, including dendritic cells, macrophages, epithelial cells, and T cells (18).

Cancer is essentially a disease of failure in tissue growth regulation (17). The feature of cancer is unregulated proliferation of cells (17). Cancer cells can subvert c-Jun amino-terminal kinases (JNKs), ERKs and p38 MAPK pathways to facilitate proliferation, survival, and invasion (17). Over-activation of ERK1 and ERK2 MAPKs has been associated with tumors (17, 20). In addition, deregulations of JNK and p38 MAPK pathways are also related with cancer developments (17, 20). As MAPKs have key roles in inflammation and cancer, it has been focused and shown by different scientists that targeting MAPK inhibition is beneficial for cancer therapy (17, 21).

**Mitogen-activated Protein Kinases**

MAPKs were originally termed as extracellular signal-regulated kinases (ERKs). MAPKs are chain of proteins in the cell and regulate communication of signals in the eukaryotic cells. MAPK pathway is evolutionarily conserved in eukaryotic cells. MAPK pathway plays serious role in communication of signal from cell surface to the nucleus and manages cellular functions such as growth, differentiation, proliferation, migration and death (21, 22). Some defects in MAPK signaling are associated with carcinogenesis by increased production of pro-inflammatory cytokines, growth factors, anti-apoptotic factors and cell proliferation (21) (Fig. 1). Generally growth factors are involved in activation of ERK1 and ERK2, whereas stress stimuli activates the JNK and p38 MAPK (23). In mammals ERKs, JNKs, p38, ERK3 and 4, and ERK5 are well characterized (24).

**ERK MAPKs and Cancer Development**

ERKs are the well characterized members of the MAPK family. The mammalian ERK1 and ERK2 are profusely present in all tissues and share almost 80% amino acid identity (23). ERK1 and ERK2 are controlled by analogous molecular signals and have numerous overlapping roles in various aspects (21). However, ERK1 and ERK2 knockout mice have shown different phenotypes (21). In ERK1 knockout mice, impaired thymocyte maturation and adipocyte differentiation is reported (25). In contrast, ERK2 knockout mice show different phenotype highlighted by lethality at day 6.5~8.5 due to impaired placental development (26). Rapidly accelerated fibrosarcoma (Raf) kinases (A-Raf, B-Raf, and C-Raf) play vital role in the Ras-Raf-MEK-ERK signaling pathway (27). Rat sarcoma (Ras) family function as molecular switches and involved in regulating intracellular signaling networks.

Stimulation of cell surface receptors tyrosine kinases activates Raf/MEK/ERK signaling cascade (28). Activation of Raf results in phosphorylation of MEK1/2 which in turn phosphorylate ERK1 and ERK2 (29). ERK1 and ERK2
MAPKs, Inflammation and Cancer

Figure 1. MAPK signaling pathways and cancer development. The MAPK signaling pathways are stimulated by pathogen-associated molecular patterns (PAMPs), growth factors (GF), tumor necrosis factor (TNF), stress, and inflammatory cytokines. Stimulation of MEK1/2 results in activation of MAPK (ERK1/2) activity. Activation of MEK4/7 is responsible for the activation of JNKs. The phosphorylation of p38 is regulated by MKK3/6. ERK1/2, JNK, and p38 activate various transcription factors such as TCF/ELK1, AP-1, ATF-2, and others. Different transcription factors including these result in expression of genes encoding pro-inflammatory cytokines, cell proliferation related proteins, growth factors, and anti-apoptotic factors. These factors are related with cancer development.

Signaling are involved in regulation of cell proliferation, motility and shape. In addition ERK1/2 activates cyclin-dependent kinase (CDK) proteins which play role in regulating cell cycle (30). Activation of ERK1/2 leads to phosphorylation of various substrates including some cytoskeletal proteins, nuclear substrates, membrane proteins and numerous MKs (24).

Sustained activation of Ras-ERK signaling pathway is associated with various cancers (31). B-Raf is critical component in Ras-Raf signaling pathway and involved in cell growth. Mutation of B-Raf, which results in excessive signaling in the pathway, has been linked to cancer development in humans. B-Raf shows high incidence of activating mutations and frequently associated with cancers (31). More than 50% melanoma harbors B-Raf mutations and selective B-Raf inhibitors have been shown effective for the treatment of tumors harboring B-Raf mutations (32, 33). Vemurafenib has been approved for the treatment of B-Raf V600 mutation positive metastatic melanoma (32). B-Raf mutations are also linked to papillary thyroid carcinoma.
and colorectal cancer (34, 35).

Pancreatic ductal adenocarcinoma is the most prevalent pancreatic carcinoma and deadliest human cancer (36). Pancreatic ductal adenocarcinoma is associated with chronic pancreatitis (36). Both K-Ras oncogenes and chronic pancreatitis are associated for development of pancreatic ductal adenocarcinoma in adult mice (36). During embryonic development, if K-Ras oncogenes are expressed, chronic pancreatitis facilitates the multistep transformation process in adult mice. Recently, one research group showed that IL-22 has very important role in breast carcinogenesis through increase activation of ERK, JNK-c-Jun, and STAT3 signaling pathways (37).

**JNK MAPKs and Cancer Development**

JNKs were initially known as stress-activated kinases (SAPKs) (38, 39). JNK1, JNK2, and JNK3 are commonly referred as SAPK γ, SAPK α, and SAPK β, respectively (23, 38). The JNKs are activated by growth factor deprivation, UV irradiation and DNA-damaging agents. The JNKs regulate the phosphorylation of various transcription factors including activating transcription factor-2 (ATF-2), Signal transducer and activator of transcription 3 (STAT3) and heat shock factor protein 1 (HSF-1) (24, 38, 40). JNKs can enhance the expression of AP-1 controlled specific genes (41). JNKs are involved in regulating various cellular processes including proliferation, survival and apoptosis (42).

Pro or anti-apoptotic functions of JNK are dependent upon duration of JNK activation (43). JNK regulate the expression and activation of inflammatory mediators such as TNF-α and IL-2 (44). Hyperactivation of JNK is linked with inflammatory bowel disease and its inhibition by SP600125 has been shown to significantly reduce clinical and pathological condition in rats (45). Inflammatory bowel disease is also associated with colorectal cancer development (46). Many researchers have shown the role of JNKs in cancer development (44). Hepatocellular carcinoma (HCC) is commonly observed in human population and major cause of cancer death worldwide (47). Mice lacking JNK1 showed decreased tumor cell proliferation in liver carcinogenesis (47). JNK1 regulates the proliferation of HCC cells by controlling the expression of p21 (47). Increased activity of JNK has been associated with enhanced liver cancer development in mice lacking p38α in hepatocytes (47, 48).

JNK deficient mice showed reduced liver damage in response to concanavalin-A treatment revealing the role of JNK in hepatitis (49, 50). JNK1 activation correlates with up-regulation of histone H3 methylation in HCC tissue, which augment the expression of genes involved in cell growth (51). JNK1/2 deficient mice showed reduced hepatitis which is linked with reduced TNF-α expression in hematopoietic cells (52). Carcinoma of the prostate is a common neoplasm in ageing males and severe health problem. JNKs play critical role in its development (17). In human prostate cancer, c-Jun proteins are up-regulated in advance disease (53). Phosphatase and tensin homolog (PTEN) is tumor suppressor and frequently mutated in prostate cancer. Loss of PTEN is attributed to AKT activation and higher JNK activity (54).

**p38 MAPKs and Cancer Development**

Four p38 isoforms are identified in mammals: p38α, p38β, p38γ, and p38δ. Generally most of the tissues express p38α and p38β isoforms, however, p38γ and p38δ isoforms are expressed by skin, kidney and muscle cells (55). The stress and inflammatory cytokines activate the p38 isoforms in mammalian cells (56). Upon stimulation, p38 is phosphorylated by MKK3 and MKK6, which are controlled by MTK1 and ASK1 (57, 58). Activated p38 results in phosphorylation of various cellular targets including cytosolic phospholipase A2, the microtubule-associated protein Tau, and the transcription factors MEF2A, Sap-1, Elk-1, ATF1, ATF2, NF-κB, p53, and Ets-1. The p38 is also responsible for activation of some MKs, which includes MSK1, MSK2 and MNK1 (38). The p38 is a critical regulator in inflammatory responses and cell proliferation (59). The specific function of p38 MAPK is dependent on the cell type and also on the external stimulus (60). The p38 MAPK play a key role in breast, liver, prostate, bladder and lung cancer (60). The p38 MAPK has been reported to
regulate TNF-α expression in macrophages and rheumatoid synovial cells (61). The p38α is involved in transcriptional activation of TNF in macrophages by lipopolysaccharide-stimulation (59). TNF-α is a pleiotropic cytokine and involved in variety of inflammatory diseases (61). TNF can induce diverse effects such as immune cell activation, angiogenesis and cell differentiation. Such processes are relevant to tumor immune surveillance and play critical roles in tumorigenesis (62). Neutralization of TNF-α has been reported to inhibit tumor formation during early tumorigenesis (63). In addition, neutralization of TNF-α result in inhibition of murine breast carcinoma development, so TNF-α antagonist may be useful in treating cancer (63).

The p38α is also involved in up-regulation of heat shock protein 25 expression, which inhibits ROS accumulation (64, 65). p21 is also called as cyclin-dependent kinase inhibitor 1 which binds to and inhibits the activity of cyclin and results in cell growth arrest. Inhibition of p38 results in enhanced p21 gene transcription in rat chondrosarcoma (66). Pharmacological inhibition of p38 MAPK has been shown to reduce cell proliferation in rat chondrosarcoma (66). In addition, in prostate cancer patients, the percentages of cells that were immunoreactive for p-ATF-2 or p-Elk-1 (downstream components of p38) were higher than in normal. It has been shown that inhibition of p38 might be helpful in the treatment of prostate cancer (67).

**Inflammation and Caner Development**

Various epidemiological studies have shown that chronic inflammation, triggered by microbial infection and autoimmune diseases, is involved in the increased risk of tumorigenesis (21, 68). In human chronic Salmonella typhi infection is allied with gallbladder cancer (69). Chronic inflammation by Helicobacter pylori infection is associated with development of gastric cancer (70). Streptococcus bovis has been linked to promote intestinal carcinogenesis by increasing the production of IL-8, which is promoter of angiogenesis (71). Inflammatory bowel diseases come under the class of autoimmune diseases and associated with colorectal cancer development (46).

The hallmark of cancer-related inflammation is the presence of inflammatory mediators such as cytokines and chemokines in tumor microenvironment (68). During chronic inflammation, ineffective clearance of destructive stimuli leads to accumulation of ROS which results in DNA damage and mutation (21, 72). If this mutation is in critical genes such as tumor suppressors, then it leads to initiation or progression of cancer (72).

To maintain tissue homeostasis, cells are continuously proliferating under inflammatory conditions, which is the driving force for transformation of initial tumor cells (21, 68). The chronic inflammation is responsible for persistent undue production of inflammatory cytokines including TNF-α and IL-6 which are important triggers for endorsing malignant tumor (21, 68). IL-6, TNF and IL-1 have been reported to promote colitis-associated tumor development (73). Up-regulation of ERK1 and ERK2 MAPKs has been associated with human tumors (17, 73). The JNK and p38 MAPK pathways are also often deregulated in cancers (17, 20). MAPK activates transcription factors such as AP-1 and C/EBP which results in up-regulation of COX-2 (74). Activation of AP-1 in tumoral microenvironment cells directly regulates the pro-angiogenic genes, including IL-8, CXCL1 and CXCL8 (68). COX-2 has critical role in pathogenesis of carcinogenesis because it's over expression results in dysplasia, metaplasia, hyperplasia, proliferation, immune surveillance, angiogenesis and metastasis (75). Elevated level of COX2 expression has been reported in different type of cancers including breast, colorectal, cervical, lung and prostate cancer (74).

**Conclusion and Perspective**

Roles of MAPKs in inflammation and host protection against microbial infection have been well characterized. MAPK signaling regulates key cellular functions including gene expression, cell proliferation, cell motility and cell survival. However, uncontrolled MAPK signaling is linked with cancer development. Currently, various MAPK signaling pathway inhibitors are available and many scientists have focused on identification of natural MAPK inhibitors which
will help in the treatment of inflammatory diseases and cancer. MAPK inhibitors might be combined with conventional therapies of cancer to improve their efficacy.

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