

Extra View

Mig-6, Signal Transduction, Stress Response and Cancer

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ABSTRACT

The mitogen-inducible gene-6 (*Mig-6*) is an immediate early response gene encoding a nonkinase scaffolding adaptor protein. *Mig-6* gene expression can be rapidly and robustly induced under both normal and pathological scenarios by factors including hormones, growth factors, and stresses. However, the precise role of *Mig-6* has virtually been a mystery until recently, when we and others discovered that *Mig-6* may play important roles in regulating stress response, maintaining homeostasis in tissues like joints or cardiac muscle, and functioning as a tumor suppressor. The discovery that *Mig-6* acts as a negative feedback inhibitor of EGF receptor signaling through a direct, physical interaction with the EGF receptor opens a door for understanding the mechanism underlying *Mig-6* function. Yet how *Mig-6* fine tunes or integrates signal transduction in many pathophysiological situations remains to be determined. Here we will highlight recent discoveries on the role of *Mig-6* in stress response, tissue homeostasis, and cancer development; review the transcriptional regulation of *Mig-6* expression; share insight into its mechanism in regulating signal transduction; and discuss the paradox of its action modes under different pathophysiological conditions.

INTRODUCTION

Signal transduction mediated through receptor tyrosine kinases (RTKs) and other signaling molecules is crucial in regulating cell growth, differentiation, and survival, and in determining cell fate. The intensity and duration of a cellular signal defines how the cells will respond and what biological event will occur. In a normal physiological milieu, signaling requires a timely attenuation, and several mechanisms are employed by the cells to ensure this occurs. One such mechanism is by establishing a negative feedback loop, in which the activated signal up-regulates certain negative inhibitors that are involved in the attenuation of the signaling.^{1,2} From identification and characterization of a list of such inhibitors, compelling evidence reveals that negative feedback regulation of signal transduction is crucial in many processes, including development and other biological events.^{1,2} The emergence of mitogen-inducible gene-6 (*Mig-6*)³ as a negative feedback regulator of the epidermal growth factor receptor (EGFR) and other RTK signals has brought a renewed attention to this little-known molecule.⁴⁻⁷

Mig-6 (also known as receptor-associated late transducer (RALT) or ErbB receptor feedback inhibitor 1 [Errfi1]) was initially described as gene-33, and was first identified from a hydrocortisone-induced rat liver cDNA library.⁸ *Mig-6* is mapped to human chromosome 1p36 and to the distal region of mouse chromosome 4. In rat, its 2970-bp mRNA consists of 4 exons spanning a 13,500-bp chromosomal region, and it encodes a 50-kDa polypeptide.⁹⁻¹¹ *Mig-6* contains several important protein-protein interaction domains/motifs, including a Cdc42/Rac-interaction and binding (CRIB) domain, a Src-homology-3 (SH3) domain binding motif, and a 14-3-3 protein binding motif.¹² Further, the carboxyl terminus of *Mig-6* shares a striking homology with the nonreceptor protein tyrosine kinase Ack-1, another CRIB domain-containing protein.¹²

The *Mig-6* gene has the following features. First, it is present in the higher-order species such as *Xenopus*, rodents, and humans, but not in relatively less complex organisms like *S. cerevisiae*, *C. elegans*, or *Drosophila*,¹³ suggesting that *Mig-6* has been acquired during evolution for the needs of more-complex signaling circuits. Second, it is an immediate early response gene whose expression can be rapidly and robustly induced by many factors including hormones, growth factors, and various stresses (Table 1), indicating that *Mig-6* may play a critical role in the early regulation of many cellular responses. However, despite

Table 1 Summary of the transcriptional inducers for Mig-6 expression

Growth Factors	Stresses	Hormones & Others
EGF ^{4,6,21,24}	Diabetic nephropathy ¹²	Alkylating agent ¹⁴
FGF ²³	Hepatocyte isolation or partial hepatectomy ²⁶⁻²⁸	Calcium ionophores ¹⁹
HGF/SF ^{7,21}	Hypoxia ^{24,25}	cAMP ⁸
IGF-1 ²²	Joint mechanical impact ²⁹	Glucocorticoids ^{6,8}
NDF ⁴	Live staphylococcus infection ⁶⁰	GPCR agonists LPA & thrombin ⁵
NRG1 ³⁷	LPS- or ventilator-induced lung injury ⁶¹	Insulin ^{8,15-20,26,31-33}
PDGF ^{6,24}	Mechanical strain ¹²	Phorbol ester ^{18,23}
TGF- α ^{4,23}	Myocardial ischemic injury or infarction ²⁴	Plant lectins ^{16,17}
	Sorbitol-induced osmotic stress ^{12,23,30}	Retinoic acid ²⁰
	Vasoactive peptide ¹²	Serum ^{3-6,23,26}

nearly two decades since its identification, its biological function and pathophysiological role remained largely unknown until a recent wave of new discoveries.

Mounting evidence suggests that *Mig-6* plays an important role in many pathophysiological conditions. Here, we will review the transcriptional regulation of *Mig-6* expression, its mechanism in regulating signal transduction, and the paradox of its action modes. We also highlight recent new discoveries on its role in stress response and tissue homeostasis, as well as its function as a tumor suppressor.

THE TRANSCRIPTIONAL REGULATION OF MIG-6

Since the first description of *Mig-6* gene-33 as a (multi)hormone-inducible gene in 1985 by Kenney and colleagues,⁸ a number of factors have been reported to induce *Mig-6* gene expression. These factors include hormones, chemical agents, growth factors, and various stresses (Table 1). Hormones and chemical agents that can induce *Mig-6* expression include insulin, glucocorticoids, cyclic adenosine monophosphate (cAMP), the alkylating agent methyl methane-sulfonate, plant lectins, phorbol esters, calcium ionophores, and retinoic acid.^{8,14-20} The induction of *Mig-6* by growth factors has been demonstrated with EGF, new differentiation factor (NDF), transforming growth factor alpha (TGF- α), hepatocyte growth factor/scatter factor (HGF/SF), insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF).^{4,7,21-24} The stress stimuli that have been reported to induce *Mig-6* expression include mechanical strain and joint mechanical impact, vasoactive peptide, and conditions such as diabetic nephropathy, myocardial ischemic injury and infarction, hypoxia, partial hepatectomy, and hepatocyte isolation.^{12,25-29}

Mig-6 transcription has been shown to be regulated during cell cycle progression; its induction by serum seems to be G₁ phase-specific and reaches peak level around mid G₁.³ As an immediate early response gene, *Mig-6* expression can be rapidly and robustly up-regulated upon stimulation by inducers. This event is mainly regulated at the transcriptional level and requires de novo synthesis of *Mig-6* mRNA. Various inducers can differentially activate the intracellular signaling pathways that mediate their transcriptional regulation of *Mig-6* and may use different intracellular pathways to accomplish this regulation. The induction of *Mig-6* by growth factors such as EGF is mediated via the Ras-MEK-ERK pathway (but not the PI-3K, p38/SAPK, Src, or PKC pathways), as shown by the fact that only the specific MEK-1 inhibitors U0126 and PD98059 can block the induction of *Mig-6* by EGF.^{5,21,30} The MEK-ERK pathway

is also responsible for *Mig-6* induction by serum, phorbol ester 12-*O*-tetradecanoylphorbol-13 acetate (TPA), or sorbitol-induced osmotic stress.³⁰ Meanwhile, the TPA-induced *Mig-6* expression can be blocked completely by the PKC inhibitor Go6983, indicating that PKC may be a molecule upstream of the MEK-ERK pathway for the *Mig-6* induction by phorbol esters.³⁰

The regulation of insulin-induced *Mig-6* expression seems to be different from cell to cell.³¹⁻³³ In Chinese hamster ovary cells (CHONewIRa), wortmannin has some inhibitory effect on insulin-induced *Mig-6* expression, while the MAPK inhibitor PD98059 shows only a minimal effect.³² In contrast, it has also been reported that insulin-induced *Mig-6* expression occurs via the activation of the MEK-ERK pathway, because PD98059 (but not the PI-3K specific inhibitor LY294002) abolishes insulin-induced *Mig-6* transcription in H4II hepatoma cells.³³ In H4II hepatoma cells, wortmannin also inhibits insulin-induced *Mig-6* expression.³¹ This inhibition appears to be partially through blocking insulin-induced ERK activation,³³ raising the question of whether wortmannin is a specific inhibitor for PI-3K. However, both the PI-3K and ERK pathways play roles in *Mig-6* induction by hypoxia in cardiomyocytes.²⁴ In isolated hepatocytes, the induction of *Mig-6* transcripts seems to be mediated via the p38/SAPK pathway.²⁸

The *Mig-6* promoter is GC-rich and contains many CpG islands, which are common in the promoters of many housekeeping genes.¹¹ The insulin regulatory element is located between the nucleotides -480 to +27 relative to the transcription start point of *Mig-6* gene.³⁴ The promoter regulatory region also contains putative AP-1 and SP-1 binding sites, cAMP regulatory elements, and glucocorticoid regulatory elements.¹¹ Further analysis will be required to determine if these elements are truly functional and how they respond to the regulators that can induce *Mig-6* expression. Alternative splicing may play a role in regulating *Mig-6* expression, as two different isoforms have been identified.^{8,12} *Mig-6* gene expression also seems to be regulated at the posttranscriptional level through ubiquitinylation and degradation by the proteasome, as it contains two PEST sequences.³⁰

MIG-6 IN REGULATING SIGNAL TRANSDUCTION

Mig-6 is a nonkinase scaffolding adaptor protein¹³ found in the cytosol of cells.^{3,4,12} Adaptor proteins can modulate and integrate signal transduction by interacting with other signaling molecules through their protein-protein interaction motifs/domains.³⁵ Depending on the molecules or catalytic enzymes brought into the

signaling complex, adaptor proteins can positively or negatively influence the signaling output through either changing the conformation of the signaling complex or the phosphorylation states of kinases or phosphatases. Mig-6 possesses several well-known motifs/domains that allow it to interact with other signaling molecules such as Cdc42, 14-3-3, or SH3-domain-containing proteins^{4,12} and is likely involved in many aspects of signaling regulation.

Many important signaling molecules contain an SH3 domain, including nonkinase adaptor proteins like Grb2 and nonreceptor tyrosine kinases such as PI-3K and Src.³⁶ Mig-6, which contains a proline-rich motif in its center region, can bind Grb2 with high affinity;^{4,7,37} low-affinity binding with the SH3 domain of Src, p85 PI-3K, PLC- γ , or Fyn is also observed.⁴ However, the biological significance of such binding remains to be addressed. Grb2 forms a bridge between RTKs such as EGFR and the downstream Ras-MAPK pathway, and it plays a crucial role in transmitting extracellular signals into cells.³⁸ The binding of Mig-6 to Grb2 might sequester Grb2 from binding to other partner molecules that are critical for the activation of MAP kinase pathway, thereby disconnecting the signaling flow. In a different scenario, it might bring different signaling molecules into the RTK-Grb2 complex to influence signaling specificity.

CRIB is a small but important domain that has been identified in the NH₂-terminus of the Mig-6 protein.¹² CRIB domain-containing proteins form a large family including kinases and nonkinases, and they can bind to Cdc42 small GTPases to regulate cellular signaling and actin cytoskeleton remodeling.^{13,39} Mig-6 binds to active GTP-bound Cdc42,¹² and it negatively regulates HGF/SF-induced Cdc42 activation and cell migration in a CRIB domain-dependent fashion.⁷ Interestingly, it has been shown that full-length Mig-6 can induce transcriptional activation of nuclear factor κ B (NF κ B) by sequestering the inhibitor of κ B α (I κ B α), while the CRIB domain-deleted version shows no such activity.^{40,41} These data suggest that the CRIB domain in Mig-6 might have dual roles: negative regulation of Cdc42 signaling and positive regulation of the NF κ B pathway.

The interaction between Mig-6 and the 14-3-3 ζ protein has also been demonstrated.¹² The 14-3-3 proteins are a family of cytosolic adaptors that may play roles in many important cellular processes including cell cycle control, apoptosis, and stress response.⁴² Given that 14-3-3 proteins can interact with more than 100 different partner proteins,⁴² this will bring even more complexity to the problem of understanding Mig-6-mediated signal transduction.

Recent discoveries about the functional interactions between Mig-6 and the EGF receptor or the ErbB family have brought a new dynamics into Mig-6 research. Mig-6 was initially identified as an EGFR- or ErbB2-interacting partner by two independent groups using a yeast two-hybrid system.^{4,5} Later it was found that Mig-6 can interact with all four ErbB members, although its interaction with ErbB3 appeared to be indirectly mediated through their binding to ErbB2 upon ligand stimulation.⁴³ The binding of EGFR or ErbB2 to Mig-6 requires their catalytic activities, but the carboxyl-terminal region that contains the major autophosphorylation sites is dispensable.^{4,5,43} Mig-6 can be transcriptionally induced by EGF, and it

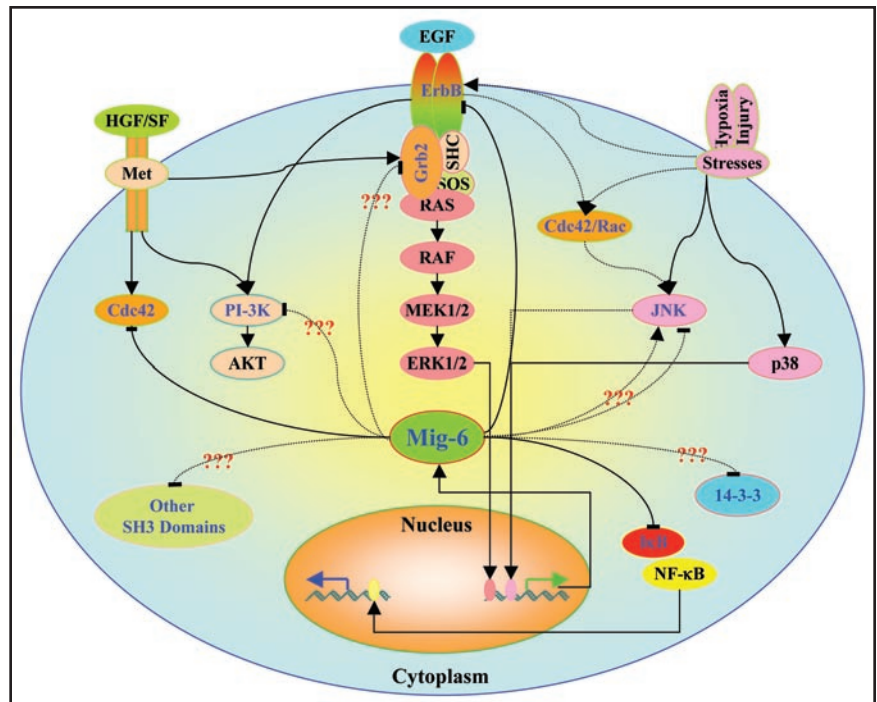


Figure 1. Diagram of the known and potential Mig-6 actions on the EGF-, HGF/SF- and certain stress-induced signal transduction. Both EGF and HGF/SF can induce Mig-6 expression through the activation of the RAS-MEK-ERK pathway, while certain stresses may also induce Mig-6 expression via JNK or p38/SAPK. Mig-6 protein is mainly localized in the cytoplasm and functions as a scaffolding adaptor for many signaling molecules. Mig-6 can directly bind to ErbB receptor and negatively regulate EGF signaling. It inhibits HGF/SF-Met-mediated activation of the Cdc42 small GTPase through directly interacting with the GTP-bound Cdc42. The activation of NF κ B pathway by Mig-6 is mediated through its ability to sequester the I κ B α . Mig-6 might also play a role in regulating the signal transduction mediated by the PI-3K, 14-3-3, and Grb2 or other SH3-domain-containing proteins, as physical interactions between Mig-6 and these molecules have been observed *in vitro*. In addition, Mig-6 might also influence JNK activity either positively or negatively.

acts as a negative feedback inhibitor of EGF receptor signaling by downgrading EGFR tyrosine phosphorylation and the downstream ERK, JNK, and Akt activation.^{4-6,24,43} The minimal EGF-receptor binding region (EBR) has been mapped to the amino acids 323-372 in Mig-6, which is within its Ack1 homology (AH) domain.^{4,43} Interestingly, Ack1 has been shown to be recruited as well to activated EGFR upon EGF stimulation,⁴⁴ although it is not clear whether this binding is mediated through the AH domain. Ack1 can also bind to clathrin through a portion of the AH domain and colocalizes with clathrin and AP-2 vesicles *in vivo*,⁴⁵ suggesting that Ack1 might play a role in clathrin/AP-2 vesicle-mediated receptor endocytosis.⁴⁶

Receptor endocytosis is an important mechanism by which activated RTKs such as EGFR can be internalized, degraded, or recycled, causing the attenuation of RTK activation.⁴⁷ It will be worthwhile to determine if the down-regulation of EGFR phosphorylation by Mig-6 is processed by clathrin-mediated EGFR endocytosis. While the EBR domain is required for Mig-6 to bind the ErbBs and to inhibit ErbB-dependent activation of ERK and Akt, EBR-deleted Mig-6 is still capable of inhibiting ErbB2/ErbB3 heterodimer-mediated proliferation induced by their high-affinity ligand neuregulin 1 (NRG1).⁴³ This phenomenon indicates that functional motifs/domains in Mig-6 other than the EBR domain are also involved in the negative regulation of ErbB signaling in a receptor-proximal fashion.

Although Mig-6 has been characterized as an ErbB-specific negative feedback inhibitor due to its ability to bind the receptor, a question exists to whether it is as simple as that (Fig. 1). Like EGF, other growth factors such as HGF/SF, FGF, or PDGF can also induce Mig-6 expression (Table 1). Mig-6 has also been shown to be an endogenous negative feedback inhibitor of HGF/SF-Met signaling.⁷ However, no physical interaction between Met and Mig-6 has been observed, and the inhibition of HGF/SF-induced cell migration by Mig-6 relies on CRIB domain-mediated suppression of Cdc42 activation.⁷ Mig-6 also shows an ability to inhibit HGF/SF-Met-mediated cell proliferation that is independent from its inhibitory activity on cell migration.⁷ This suggests that Mig-6 can be more than just an ErbB-specific inhibitor. It might exert its inhibitory function via different RTKs, or via the same RTK but mediating different biological activities through distinct motifs/domains.

Nonetheless, the Mig-6 mode of action seems to be different from that of sprouty, another important negative feedback inhibitor of RTK signaling.⁴⁸ Sprouty can suppress RTK signaling by acting on multiple common downstream signaling molecules such as Grb2, RAS, RAF, and ERK, rather than the RTK itself, and sprouty functions as a general inhibitor of many RTK signaling molecules like EGFR and FGFR.⁴⁸ It is still unknown whether the binding of Mig-6 to Grb2 has any biological significance. Therefore, it will be important to determine how Mig-6 influences Grb2-mediated RTK signal transduction.

Overexpression of Mig-6 has no effect on v-Ras-mediated transformation, or on FGF- or PDGF-induced proliferation of NIH3T3 cells.^{4,43} The ERK or Akt activation induced by FGF or PDGF is not affected by Mig-6 in Cos-7 cells, Rat 2 fibroblast cells, or cardiomyocytes.^{5,6,24} This raises questions of whether Mig-6 is or is not a negative inhibitor of FGFR or PDGFR, or whether only in certain cell types will it act as an inhibitor of these RTKs. The idea that Mig-6 might act differently in different cell types or in different signaling contexts also arises from its effect on the SAPK/JNK pathway, which plays interesting roles in many cellular events including stress response and apoptosis.⁴⁹ Mig-6 can activate JNK in human embryonic kidney 293 cells,¹² while it can suppress the EGF-induced JNK activation in Rat 2 fibroblast cells.⁶ Understanding how Mig-6 exerts its function under different physiological conditions will provide a further insight into its potential roles in many cellular and biological processes.

MIG-6 IN DEVELOPMENT, HOMEOSTASIS AND STRESS RESPONSE

Mig-6 is differentially expressed across tissues. A high level of Mig-6 expression is observed in the liver and kidney of both humans and rats, while varied levels of expression are detected in the lung, placenta, heart, brain, stomach, and skeletal muscle.^{14,25} Mig-6 expression is subjected to developmental regulation as well. While Mig-6 expression is low in the fetal liver, a sharp increase is observed in the newborn liver,^{50,51} suggesting that hepatocytes might be responding to environmental or hormonal changes accompanying birth. Mig-6 expression is also increased in the developing metanephric kidney during the early stage of mesenchymal-epithelial conversion.⁵² However, the role of Mig-6 in development has been unknown until recent reports from us and others on Mig-6 knock-out mice.^{21,53,54} Despite a normally high level expression in the liver and kidney, no obvious developmental abnormality was observed in these organs from Mig-6-deficient mice,^{21,53,54} indicating that Mig-6 is likely to play a role other than in the development of these organs.

It was surprising to find that homozygous disruption of Mig-6 in mice results in an early-onset degenerative joint disease resembling human osteoarthritis.⁵³ While born with normal joint structures, the Mig-6-deficient mice gradually develop joint abnormalities including degradation of the articular cartilage and the formation of large bony outgrowths, with the latter likely due to overproliferation of mesenchymal progenitor cells followed by chondrogenic differentiation.⁵³ The most severely affected joints are those that are heavily used—especially the knee, ankle, and temporal-mandibular joints,⁵³—indicating that mechanical stress might be a trigger for this phenotype. These data demonstrate that Mig-6 is essential for normal joint function. It has been shown that Mig-6 expression in the joints can be induced by mechanical impact in a canine model.²⁹ Together, these results suggest that Mig-6 might protect the joint from stress by counterbalancing the proliferative activity induced by mechanical forces, thereby maintaining normal joint homeostasis.

Mig-6 is also involved in skin morphogenesis.⁵⁴ Mig-6-deficient mice display a skin phenotype resembling that seen in mice transgenic for the EGFR ligand TGF- α .⁵⁵ Mig-6 deficiency causes a hyperactivation of endogenous EGFR and sustained MAP kinase signaling, leading to overproliferation and impaired differentiation of epidermal keratinocytes in the tail and footpad skin.⁵⁴ Strikingly, targeted expression of Mig-6 in the mouse skin results in a Waved-like phenotype recapitulating the skin abnormalities observed in mice with hypomorphic or antimorphic EGFR alleles.⁵⁶⁻⁵⁹ These two sets of data all lead to the conclusion that Mig-6 is a bona fide negative inhibitor of EGFR signaling in skin keratinocytes.

It should also be noted that Mig-6 may play a role in neuronal development by acting as a negative feedback inhibitor of HGF/SF-Met signaling-mediated cell migration and neurite growth.⁷ Given the many growth factors capable of inducing Mig-6 expression, it is conceivable that Mig-6 may act as a general feedback inhibitor for many RTK signals, rather than as an EGF receptor-specific inhibitor, even though the mechanism might be different from RTK to RTK.

Although many organs and tissues normally express Mig-6,^{14,25} most of them show no developmental defects in the Mig-6-deficient mouse. That does not necessarily mean that Mig-6 is dispensable for those organs or tissues. The fact that Mig-6 expression can be induced by many stress stimuli indicates that it may play a crucial role in either regulating stress response or maintaining homeostasis in organs and tissues. In liver, a sharp increase of Mig-6 expression shortly after partial hepatectomy parallels the transition of hepatocytes from a quiescent to proliferative state and might play a role in liver regeneration.²⁵ In the heart, hypoxia/ischemia-induced Mig-6 expression promotes cell death of cardiomyocytes by inhibiting PI-3K and ERK pathways and might contribute to myocardial infarction caused by ischemic stress.²⁴

Mig-6 is also implicated in several chronic pathologic conditions such as kidney dysfunction and hypertension, as its expression is induced by experimental diabetic nephropathy or vasoactive peptides like endothelin-1 or angiotensin-II.¹² Further, induction of Mig-6 by mechanical strain in the A549 human lung cell line,¹² by the live staphylococcus infection in human airway cells,⁶⁰ or by LPS- or ventilator-induced lung injury,⁶¹ suggest that Mig-6 may somehow take part in the regulation of pulmonary ventilation or inflammatory response in the lung.

The transient induction of Mig-6 by various types of stress might help in balancing signaling responses, as suggested by the Mig-6-deficient joint phenotype.⁵³ However, a sustained level of Mig-6 expression induced by chronic stress might promote certain

disease conditions through its ability to induce transcriptional activation of NF κ B,⁴⁰ which plays an important role in regulating stress response and in the pathogenesis of many human diseases.⁶² Finally, Mig-6 may be involved in glucose metabolism or hormonal regulation, because insulin can strongly induce its expression. In any event, whether Mig-6 is involved in the regulation of cell growth, differentiation, survival, stress response, metabolism, or homeostasis, further studies are required to determine the precise role of Mig-6 in individual organs or tissues under both physiological and pathological conditions.

TUMOR SUPPRESSOR ROLE OF MIG-6 IN CANCER

The *Mig-6* gene locus in the human is on chromosome 1p36, a locus that has been strongly associated with many human cancers by linkage analyses. Notably, loss of heterozygosity (LOH) on 1p36 is frequently observed in human lung cancer,⁶³⁻⁶⁵ and a microsatellite marker closely linked with *Mig-6* gene has been associated with smoking patients, squamous cell carcinoma patients, and late-stage patients with non-small cell lung cancer (NSCLC).⁶⁶ Mouse lung carcinogenesis is also strongly associated with LOH on the distal region of mouse chromosome 4, the orthologous region to human chromosome 1p36.^{67,68} More importantly, germline disruption of the *Mig-6* gene in mice can cause lung carcinogenesis (ranging from bronchi or bronchiole epithelial hyperplasia to adenoma or adenocarcinoma) as well as gallbladder, bile duct, gastrointestinal (GI) tract, and skin cancer.^{21,54}

Mutation analyses on human NSCLC cell lines have led to the identification of two homozygous mutations in the *Mig-6* coding region: a missense mutation at codon Asp109 to Asn in the NCI-H226 squamous cell carcinoma cell line, and nonsense mutation at codon Glu83 causing premature truncation in the NCI-H322 adenocarcinoma cell line.²¹ However, in 41 primary human lung cancer samples screened, only one heterozygous germline mutation was identified, in a squamous cell carcinoma (a change of codon Ala373 to Val).²¹ Nonetheless, our data suggest that *Mig-6* may function as a tumor suppressor gene in lung cancer development, even though mutation in *Mig-6* seems to be a rare event. Given that LOH on 1p36 is associated with smoking, squamous cell carcinoma, and late-stage lung cancer patients,⁶⁶ it is possible that a higher mutational rate might be observed in certain groups of lung cancer patients.

LOH on 1p36 is also frequently observed in breast cancer, even though only a single nucleotide polymorphism (SNP) at codon Asp109 in *Mig-6* has been identified in 3 out of the 92 breast carcinomas and 7 out of the 190 randomly selected healthy donors.³⁷ Interestingly, this SNP is identical to the mutation that we have identified in the NCI-H226 lung cancer cell line.²¹ Given that this SNP is also identified in the normal population,³⁷ it is not clear if it plays a role in breast carcinogenesis; the population carrying this SNP might be predisposed to the development of breast or other types of carcinogenesis. A follow-up examination on the 7 healthy donors could provide important information on the relevance of this SNP to carcinogenesis, as SNPs in the p53 tumor suppressor gene has been shown to play a role in cancer development.^{69,70} Other SNPs in the *Mig-6* gene have also been identified in lung cancer patients.²¹

Many tumor suppressor genes can be inactivated by either loss-of-function mutations or loss/reduction of their expression, or both.⁷¹ Even though genetic mutations in the *Mig-6* coding region do not seem to be frequent in the cancers analyzed so far,^{21,37} a reduction of Mig-6 expression has been observed in human breast,

skin, pancreatic, and ovarian cancers.^{37,54} Down-regulation of Mig-6 expression is correlated with poor survival of breast cancer patients.⁷² There are at least two distinct mechanisms that might account for the reduction of Mig-6 expression: genetic changes such as mutation or deletion in its promoter regulatory region, or epigenetic modification caused by hypermethylation in its promoter CpG island. Interestingly, we found that Mig-6 expression in NCI-H226 human lung cancer cells was almost undetectable and showed no response to the activated MAPK pathway induced by EGF or HGF/SF,²¹ implying a genetic or epigenetic change in the Mig-6 promoter regulatory region. Likewise, it was also reported that Mig-6 expression in the ERBB2-amplified breast cancer cell lines BT474 and SKBr-3 was low and responded poorly to various stimuli, although methylation seemed not to be the reason for its transcriptional repression in these two lines.³⁷ Further studies are required to determine how Mig-6 transcription is regulated to become less responsive (or unresponsive) to stimuli such as EGF and HGF/SF.

Loss of Mig-6 function can cause a prolonged activation of EGFR, Met, or other RTK signaling.^{4-7,21,54} Overexpression or inappropriate activation of EGFR or Met is observed in many human cancers and has been well demonstrated to play crucial roles in aspects of tumor malignancy including aberrant cell proliferation, apoptosis, angiogenesis, and metastasis,^{73,74} all of which are the hallmarks of cancer.⁷⁵ In the Mig-6-null mouse, it is clear that the lack of Mig-6 causes hyperproliferation of the cells in the skin and gallbladder with spontaneous tumor formation,^{21,54} as well as in joints developing degenerative joint disease.⁵³

The skin hyperplasia caused by Mig-6 deficiency appears to be mediated through the sustained activation of the EGFR-MAPK pathway, because the phenotype can be reversed by the EGFR inhibitor gefitinib (Iressa) or replacement of wild-type EGFR with the hypomorphic EGFR^{wa2} allele encoding a kinase-defective receptor.⁵⁴ Moreover, challenging Mig-6-deficient mice with the carcinogens 7,12-dimethylbenz[a]anthracene (DMBA) or TPA leads to increased incidence of skin carcinogenesis including melanomas and papillomas,⁵⁴ similar to what has been observed in TGF- α transgenic mice.⁷⁶ Gefitinib treatment can lead to regression of the carcinogen-induced skin tumors in Mig-6-deficient mice.⁵⁴ These data, together with the fact that EGFR-deficient mice or transgenic mice expressing a dominant-negative EGFR are resistant to TPA-induced carcinogenesis,⁷⁷ further confirm that endogenous EGFR signaling is responsible for the development of the carcinogen-induced skin cancer in Mig-6-deficient mice.⁵⁴

A question remains whether endogenous EGFR signaling is involved in the development of spontaneous tumors in organs like the lungs, GI tract, gallbladder, or bile duct in Mig-6-deficient mice.^{21,54} It will be interesting to see if spontaneous tumor formation will be blocked by the replacement of wild-type EGFR with EGFR^{wa2} alleles in these organs. In vitro, specific knockdown of Mig-6 expression by short interfering RNA (siRNA) increases EGFR phosphorylation and enhances EGF-induced proliferation in breast cancer cells,^{37,54} while overexpression of Mig-6 leads to suppression of the ErbB2- or EGFR-mediated fibroblast cell transformation^{4,5} and inhibits EGF-induced cell cycle entry.^{4,6} In addition, loss of Mig-6 expression increases the resistance of the ErbB2-amplified breast carcinomas cells to herceptin, the neutralizing antibody against ErbB-2 receptor.³⁷ However, it has also been shown that exogenous overexpression of Mig-6 inhibits apoptosis of MCF7 human breast cancer cells.⁷⁸ In contrast, another report shows that Mig-6 expression in cardiomyocytes promotes apoptotic cell death through

inhibition of the PI-3K and ERK pathways.²⁴ It is not clear if this discrepancy was due to the difference of cell types or other reasons. It is possible that different thresholds of Mig-6 protein may lead to different biological consequences.

ISSUES AND CHALLENGES

With the discoveries of more and more roles for Mig-6, it becomes increasingly important and challenging to determine the mechanism of how Mig-6 exerts its function under different pathophysiological situations. Even though the emerging of physical interaction between Mig-6 and the EGF receptor might indicate a mechanism for its function as a negative feedback inhibitor of that receptor, it might be difficult to attribute diverse biological events to one such mechanism. Given that many factors can induce Mig-6 expression, it is quite possible that Mig-6 might integrate or fine tune signal transduction under different scenarios through combinations of its functional motifs/domains via different signaling pathways. For example, EGFR signaling is responsible for the development of skin cancer in Mig-6-deficient mice,⁵⁴ but a different mechanism might be needed to explain the role of Mig-6 in joint homeostasis.⁵³ In the meantime, the early death of Mig-6-deficient mice has been an obstacle for understanding the Mig-6 role in organs like liver and kidney that have high levels of its expression. The generation and characterization of tissue-specific Mig-6 conditional knock-out mice will provide insight into its role in given tissues either as a tumor suppressor or in other functions.

Another major challenge will be to further understand the role of Mig-6 in various human cancers, given that loss-of-function mutations in Mig-6 seem to be rare genetic events and the involvement of genetic or epigenetic changes in the Mig-6 promoter regulatory region is still uncertain. New information will require the analysis of large cohorts of wide-spectrum human cancer samples that might have potential Mig-6 abnormalities, either genetic or epigenetic.

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