Review Article

Role of MIM/MIM–B in human tumor progression

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ABSTRACT The protein "missing in metastasis", known as MIM/MIM–B encoded by metastasis suppressor 1 (MTSS1), has been characterized as an actin–binding scaffold protein that may be closely related to tumor proliferation, invasion and metastasis. Recently, the role of MIM/MIM–B in tumor progression has gained a lot of attention. Therefore, a comprehensive review of the relationship between MIM/MIM–B and cancer development will surely benefit new cancer therapeutic strategies, and to embody the value of MIM protein in cancer diagnosis and treatment. In this paper, we summarized the role of MIM/MIM–B in the sonic hedgehog pathway and in cancer progression.

Key Words: carcinoma; metastasis suppressor 1; missing in metastasis B; invasion; metastasis

Introduction

Missing in metastasis gene, or MTSS1, is a newly discovered gene (1). It is a 5.3 kb transcript located at 8q24 (2). MTSS1 encodes an intracellular protein Missing in Metastasis (MIM). MIM–B is the human homology (3), which was also named basal cell carcinoma–enriched gene 4 (BEG4), mammalian metastasis suppressor 1, KIAA0429, and cf (4). Recently MIM has drawn a lot of attention as it is frequently regulated in a variety of cancers, including hepatocellular carcinoma (HCC), bladder carcinoma, breast cancer, and so on . MIM, Sufu and Zinc–finger transcription factors Glioblastoma–1, 2 (Gli1, Gli2) form a ternary complex, which activates Hh (Hedgehog)–Gli signaling pathway by enhancing Gli–induced transcription. MIM is believed to be a signaling molecule in the control of cell growth and morphogenesis. However, the regulation of MIM is unclear and the role of MIM in tumorigenesis and metastasis has not yet been well established (5 8).

Introduction of MTSS1 gene

MTSS1 gene was first discovered by Lee Young-Goo in 2002 using improved mRNA differential display method (1). Since it was not expressed in invasive-metastatic bladder cancer cell lines, it was named missing in metastasis (MIM). Multiple MIM splicing transcripts have been reported, including MIM–A, the prototype of MIM that encodes only 356 amino acids; MIM–B, which encodes a protein product of 759 amino acids; and MIM–C, which contains an alternative exon and predicts a protein of 734 amino acids (Table 1). MIM–A, MIM–C and MIM–B share > 50% amino acid sequence homology in the C–terminal domain.

However, analysis of a variety of human cells revealed only a dominant MIM–related immunoreactivity running at a position close to recombinant MIM–B, which indicated that MIM–B likely represents the primary protein product of MIM (2, 3, 9).

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Table 1 Amino acid encoded by MIM

Type of MIM	Amino acid encoded
MIM–A	356
MIM–B	759
MIM–C	734

MIM, consisting of 759 amino acids, has been characterized as a new actin–binding scaffold protein that may be closely related to tumor growth, invasion and metastasis (5, 8). It is involved in actin cytoskeleton rearrangements, signal transduction and transcriptional activation. MIM protein contains multiple function motifs, including a coiled–coil domain (CC), a lysine–rich domain (LRD), serine–rich domain (SRD), proline–rich domain (PRD) and a wiskoot–Aldrich syndrome protein homology 2 domain (WH2) at its C–terminus. WH2 domain inhibits the extention of F–actin by binding to GTP–G–actin; PRD interacts directly with the SH3 domain of cortactin,which ia an Arp2/3 complex activator, mediating actin polymerization in an SH3 dependent manner (10). The CC structure is involved with F–actin bundling (6, 11). IRSp53/MIM homology domain (IMD), 250 amino acids of the N–terminal of MIM, is an evolutionarily conserved F–actin bundling domain involved in filopodium formation (6, 11, 12). MIM–B binds and activates Rac through the IRSp53/MIM domain, enhancing actin polymerization and rearrangement of cell structure. MIM–B triggers RPTPδ (protein tyrosine phosphatase δ)–induced signalling pathway by binding to the D2 domain of RPTPδ, causing the loss of focal adhesion complex (10, 12, 13). Overexpression of MIM also activated Hh signalling pathway, enhancing tumor growth, invasion and matastasis (5, 7).

Role of MIM/MIM–B in tumor progression

Because MIM/MIM–B functions as metastasis suppressor in cancers like bladder cancer, prostate cancer, and breast cancer, MTSS1 was first recognized as tumor metastasis suppressor gene, (1, 2, 4, 14). Recent studies indicated that MIM is also expressed in normal tissue cells (11). Nixdorf Sheri suggested that the precise relationship between MIM/MIM–B and the invasive/metastatic behavior of bladder cancer cell lines remains to be elucidated (15). MIM, a new Shh responsive protein, takes part in signal transduction, transcription activation, and cytoskeleton rearrangements. Over expression of MIM enhances cell migration, resulting in tumorigenesis, invasion and metastasis (5, 6, 8, 16).

Downregulation of MTSS1 has also been found in some individual tumor matastasis cell line (like gastric cancer) (17, 18). However, MIM has not been identified as a tumor suppressor in most of the studies, because anything affecting stability of cytoskeleton could result in cell growth and migration, leading to aberrant cell development and migration (3, 19). On the contrary, recent researches demonstrated that MIM cannot be a metastasis suppressor factor for its high expression in various metastasis cancer cells (13, 20). It may have an important function in tumor metastasis (7, 11, 21). Chen Wan-nan reported the splicing–specific novel proteins (TPss and TPds) encoded by the 2.2 kb spliced variants of hepatitis B virus genome (TPds) enhanced MTSS1 expression in Huh7 hepatocytes, which interfered protein synthesis, signal transduction and cytoskeleton rearragements. There's statistically significant difference in the expression level of MIM-B between normal and tumor tissues obtained from the same subject. The expression of MIM-B in HCC tumor tissues is 379.25 (1.19–12555.22). The expression of MIM-B in the adjacent liver tissues is 137.26 (1–3655.87) (20). Moreover, MIM–B mRNA level was significantly higher in hepatocellular carcinoma tumor tissues comparing to normal liver tissue from healthy donors (median, 2.845;, 1.85–4.81) (P = .0005).

MIM–B expression in the tumor tissues was twice higher than that in normal tissues from 65% patients with hepatocellular carcinoma (26 of 40 patients). Overexpression of MIM–B was correlated with favorable clinical features including those of early tumor stage, presence of encapsulation, and absence of venous infiltration, suggesting that MIM–B plays a role in early carcinogenesis of hepatocellular carcinoma. MIM–B expression may serve as a biomarker for the prediction of early tumor development of hepatocellular carcinoma. It may be a critical regulator of carcinogenesis in different cancers. However, the function of MIM–B in cancer has not been clearly defined (20).

MIM/MIM–B is involved in tumorigenesis through Hedgehog (Hh) signalling pathway

Hedgehog (Hh)–Gli singnaling pathway not only controls the embryonic development, but also plays an important role in tumorigenesis. There are three types of Hh peptides (sonic hedgehog, SHh; indian hedgehog, IHh; desert hedgehog, DHh), membrane proteins (patched homolog 1, Ptch1; patched homolog 2, Ptch2; smoothened, Smo; human hedgehog inhibitory protein, HHIP; growth arrest–specific gene 1, GAS1), nuclear transcription factors (Gli1, Gli2, Gli3) and downstream target genes (Ptch, Gli1, Bcl2, CCND2, Snail, N–myc, Cyclins, etc.) (16, 22). SHh signal peptide is widely expressed in mammal Hh signal pathway, and N–myc is essential in cell proliferation mediated by SHh (22). Hh signaling is initiated by the binding of Hh protein (SHh, IHh, DHh) to its receptor Patched (Ptc), which in the absence of Hh protein

represses signal transduction by inhibiting Smo. Binding of Hh protein to the Ptc receptor abolishes the inhibitory effect of Ptc on Smo, allowing Smo to transduce the signal towards the nucleus via activating the Gli family of zinc finger transcription factors, which start the expression of target genes (Ptch, Gli1, Cyclins, etc, Figure 1).

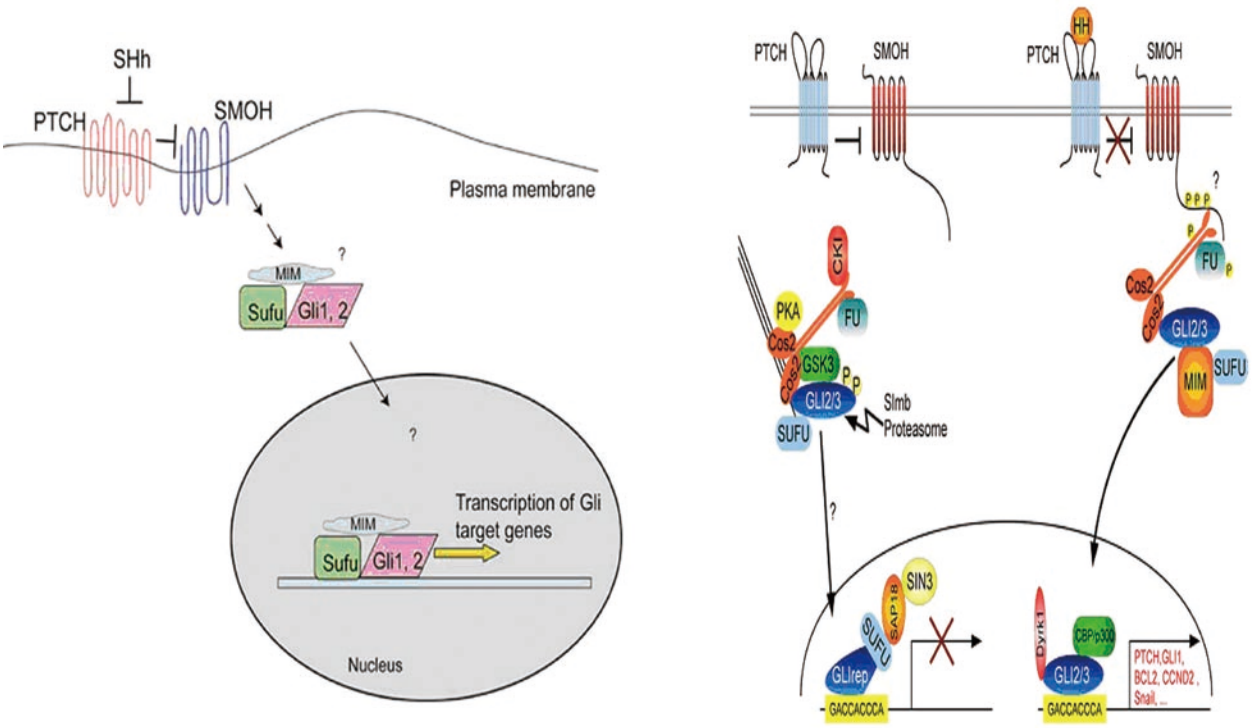

Figure 1 Involvement of MIM in sonic hedgehog signalling. The drawing shows a simplified version of sonic hedgehog signalling as it may relate to the role of MIM in transcriptional regulation see also (7).

Shh is the most broadly expressed mammalian Hh signaling molecule. It controls cell growth, proliferation, and differenciation; it also plays role in tumor cell proliferation and invasion (22). MIM is a Shh–responsive gene. It has been demonstrated that MIM, Sufu and Gli form a ternary complex, which enhances transcription of Shh–responsive genes. Most of the studies focused on the effect of Hh–Gli signaling on tumorigenesis and tumor growth (5). Recently, researchers found that overactivation of Hh–Gli signaling pathway played an important role in biological behavior of invasion and metastasis of pancreatic cancer, ovary cancer, and liver cancer (23-25). Nagai Shuntaro is the first to demonstrate that Gli1 improved invasive potential of pancreatic cancer by up–regulation of Matrix metalloproteinase 9 (MMP9) (23). However, mechanism of overexpressed MIM activating Hh signaling and controlling development and tomorigenesis is still unclear (5, 7). Ma Stephanie first demonstrated MIM–B was over–expressed at both mRNA and protein levels in hepatocellular carcinoma (20). Over–expression of MIM–B is associated with early pTNM stage, presence of tumor encapsulation, and absence of venous infiltration. A high level of MIM–B expression was observed at the early stages of the disease, suggesting that MIM–B may play an important role in promoting the early development of hepatocellular carcinoma and is unlikely to be involved in tumor invasion and metastasis. Clinic pathological features of patients with hepatocellular carcinoma (HCC), including age, sex, tumor size, liver cirrhosis, AFP (alpha fetoprotein) level, HBsAg (hepatitis B virus surface antigen) is not correlated with tumor MIM transcript expression level.

The progress in the study of MIM/MIM–B

We have been established a nude mice model bearing HCC xenograft with high metastatic potential for in vivo study, increased lung metastases were found in the palliative resection group as compared to the sham operation group and the black control group (26). Briefly, orthotopic HCC models were established by implantation of human HCC cell line MHCC97H xenografts with high metastatic potential. Thirty–six nude mice bearing HCC were randomized into three groups 14 days post–operation, including palliative resection group, control group1 (sham operation), and control group2 (black control group). Six mice in each group were sacrificed by cervical dislocation 14 days following palliative resection. Oligo Tumor Metastasis Microarray and GEArray Expression Analysis Suite soft ware were adopted for gene analysis. The methods of support vector machine (SVM), gene significance analysis and gene correlation degree analysis were used to find the markers that could differentiate the metastatic potential of HCC. Gene function net was constructed based on the special gene clustering analysis and multi–dimensional scale. Real–time PCR was applied to detect mRNA expression. Remaining mice were killed 35 days after palliative resection for the examination of pulmonary metastasis. We found that the value of pulmonary metastatic nodules was 14.3 ± 4.7, 8.7 ± 3.6 and 8.4 ± 5.1 in three groups, respectively (both $p < 0.05$). Different gene expression was shown among groups via gene analysis. Results of gene clustering analysis showed that MTSS1 was situated in the central position of the gene function net of residual HCC tissues. The condensation degree of gene function net in residual HCC was higher than that in its own control group (0.1940 Vs 0.0098). The constructive density of the tumor gene function net in residual HCC was higher than those in controls (0.0670 Vs 0.0145, 0.0210 and 0.0146). MIM–B mRNA and protein expression was significantly up–regulated in residual HCC tissues, accorded with snail and MMP2 (to be published).

MIM is an important regulator in cell growth and development. There has been accumulating evidence suggesting a role of MIM–B, in carcinogenesis, yet its role in the development of hepatocellular carcinoma has not been examined thus far (27). We discovered similar results as Ma reported about the association between Clinicopathological features and expression level of MIM–B mRNA in 37 cancer residue cases (28). In our study, the expression level of MIM–B mRNA in cancer residue was higher in cases sign of metastasis showed earlier; the expression level of MIM–B mRNA in cancer residue correlated with the number of metastatic nodules in the lungs; HCC with high metastatic potential expressed a high level of MIM–B mRNA. It has not been reported that MIM controls HCC progress via activating Hh–Gli signalling pathway. We found the elevation of MIM–B protein level as well as over–activation of Hh–Gli pathway in HCC, implying MIM–B may activate Hh–Gli pathway in certain behavior.

Conclusion

MIM/MIM–B appears to be a newly discovered protein that may be involved in tumor invasion and metastasis. Further studies is required to characterize exact role of MIM/MIM–B protein in regulation of tumor invasion and metastasis, to further describe distribution of MIM/MIM–B protein in normal tissue as well as primary and metastatic tumors, to illustrate changes of downstream proteins in Hh–Gli signaling pathway, to discuss safety problems and effectiveness of RNAi (RNA interference) in depressing MIM/MIM–B expression. To the best of our knowledge, there is no report about the effect of down–regulation of MIM/MIM–B expression on tumorigenesis and metastasis. Based on former literatures and our preliminary researches, it is necessary to explore the molecular regulation mechanism of tumor metastatic potential. Our in vitro and in vivo experiments suggested knockdown MTSS1 gene in HCC with high metastatic potential significantly decreased its metastatic potential (to be published). MIM–B expression may serve as a biomarker for the prediction of early tumor development of hepatocellular carcinoma. However, it's still unknown whether depression of MIM/MIM–B prevents tumor progression

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