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MTSS1: a multifunctional protein and its role in cancer invasion and metastasis

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1. ABSTRACT

MTSS1 (metastasis suppressor-1) was first identified as a metastasis suppressor missing in metastatic bladder carcinoma cell lines. The down-regulation of MTSS1 that may be caused by DNA methylation was also observed in many other types of cancer. While accumlating evidence for the function of MTSS1 support the concept that it is unlikely to be a metastasis suppressor, but actually acts as a scaffold protein that interacts with multiple partners to regulate actin dynamics. It has also been demonstrated that MTSS1 is involved in the Shh signaling pathway in the developing hair follicle and in basal cell carcinomas of the skin. Such evidence indicates that MTSS1 as a multiple functional molecular player and has an important role in development, carcinogenesis and metastasis. However, the biochemical mechanisms by which MTSS1 functions in cells and the physiological role of this protein in animals remain largely unknown. In this review, we will discuss the current knowledge of MTSS1's role in cancer metastasis, carcinogenesis, and development. The clinical significance of MTSS1 will also be discussed .

2. INTRODUCTION

Cancer metastasis is a significant contributor to death in cancer patients. The process of cancer metastasis consists of a long series of sequential, interrelated steps known as the metastatic cascade that are not as yet completely understood. However, it is known that these metastatic events are modulated by many factors, including metastasis activators and suppressors. Metastasis suppressor genes are defined by their ability to suppress *in vivo* development of metastases. To date, only a limited number of metastasis suppressor genes, including *NM23, KAI1, KiSS1, MKK4, BRMS1, RHOGDI2, CRSP3 and VDUP1*, have been identified (1). These metastasis suppressor genes reduce the metastatic propensity of a cancer cell line *in vivo* without affecting its tumorigenicity.

MTSS1 (metastasis suppressor 1), also known as MIM (missing-in-metastasis), MIM-B, BEG4 (Basal cell carcinoma–enriched gene 4) or KIAA0429, was originally identified by Lee *et al.* (2) as a protein down-regulated in

metastatic bladder carcinoma cell lines. Analysis by Northern blotting demonstrated that MTSS1 is widely expressed but most abundant in spleen, thymus, testis, prostate and peripheral blood, with low levels also detected in uterus and colon. Although MTSS1 was proposed to function as a metastatic suppressor protein in both bladder (2, 3, and 4) and prostate cancers (4 and 5), the relevance of such down-regulation to tumor progression has not been confirmed (3 and 6). DNA methylation may be involved in the down-regulation of MTSS1 (4).

Functional analysis revealed that MTSS1 may act as a cytoskeletal scaffold protein to regulate cytoskeletal dynamics through interaction with Rac, actin and actin-associated proteins (7, 8, and 9). Overexpression of MTSS1 often leads to the increase in the formation of lamellipodia, membrane ruffles, and filopodia-like structures and promotes disassembly of actin stress fibres (8, 9, and 10). Several lines of evidence indicate that MTSS1 may be involved in the PDGF signaling pathway that regulates cell shape changes via protein tyrosine kinases (7 and 11). However, the mechanisms by which MTSS1 regulates cell morphogenesis as well as its role in animal tissues have been unknown. MTSS1 was also identified as a new member of the Shh (Sonic Hedgehog) signalling pathway during both development and tumorigenesis (12). Clinical research demonstrates that MTSS1 expression may be of clinical significance in different types of cancer (13 and 14). Thus, MTSS1 may act as a multifunctional molecular in development, tumorigenesis and cancer metastasis. Its role in the regulation of cellular behaviour is still very much an open question.

3. CYTOSKELETAL REGULATION IN CANCER CELL MIGRATION

Cell migration plays a key role in many biological processes, such as embryonic morphogenesis, immune surveillance, and tissue repair and regeneration. Abnormal cell migration drives progression of many diseases, including cancer invasion and metastasis (15, 16 and 17). Cell migration is a highly orchestrated multistep process that is initiated by the protrusion of the cell membrane (18). Protrusive structures formed by migrating and invading cells were termed filopodia, lamellipodia, and invadopodia/podosomes, which have strikingly different designs of the actin polymerization machinery. Formation of these structures is driven by spatially and temporally regulated actin polymerization at the leading edge (19).

3.1 Membrane protrusions formed by migrating cells

Lamellipodia are broad flat protrusions, which contain an actin meshwork (20) and have a major role in driving cell migration by attaching to the substrate and generating force to pull the cell forward. The current model for lamellipodial dynamics (21 and 22) suggests that treadmilling of the branched actin filament array consists of repeated cycles of dendritic nucleation, elongation, capping, and depolymerization of filaments. Members of the WASP family activate the Arp2/3 complex and nucleate formation of actin filaments on pre-existing filaments (23). The branch grows rapidly at its barbed end by addition of actin-profilin complexes. As it grows, it pushes the plasma membrane forward. After a short time, growth of barbed ends is terminated by the binding of capping protein to the barbed end (24). Subsequent network disassembly and actin filament depolymerization is assisted by proteins of the ADF/cofilin family (25).

Filopodia are thin finger-like protrusions composed of parallel F-actin bundles (26 and 27). The main function of filopodia are proposed to sense external cues to set the direction of cell migration and act as sites for signal transduction. A convergent elongation model of filopodia initiation has been proposed that filaments within the lamellipodial dendritic network acquire privileged status by binding a set of molecules (including VASP) to their barbed ends (28). Association of Ena/VASPs with the barbed ends of the filaments could mark these filaments for filopodial elongation by their multiple activities, including inhibition of barbed end capping, enhancement of filament elongation, and F-actin bundling. Initiated filopodia elongate and attain steady-state by the filament treadmilling mechanism (29). According to the treadmilling model, all actin filaments within a bundle elongate at their barbed ends and release subunits from their pointed ends. A small GTPase of the Rho superfamily, Cdc42, has been implicated in the formation of filopodia (30 and 31). Cdc42 interacts with WASP and N-WASP and this, together with PI (4,5)P2 binding, relieves the autoinhibited conformation of WASP leading to the activation of the Arp2/3 complex.

Invadopodia are ventral membrane protrusions with an ECM degradation activity formed by highly invasive cancer cells on thick physiological substrates (32). Carcinoma cells seem to utilize invadopodia type protrusions to migrate and invade through tumor stroma and into blood vessels in the process of metastasis (17 and 33). Podosomes are similar to invadopodia in their appearance and molecular composition. Classic podosomes are formed by cell types of monocytic origin, such as macrophages, dendritic cells, and osteoclasts (34 and 35). Invadopodium/podosome formation is triggered by the N-WASP/WASP, Arp2/3 complex and cortactin, probably by coupled activation of growth factor receptor and integrin signaling. This precursor is stabilized by further recruitment of invadopodium/podosome components and formation of actin network by cofilin. Anchored precursor then gathers matrix-degrading proteinases to degrade ECM and protrude into matrix. The N-WASP/Arp2/3 complex, cortactin, and cofilin continue to induce actin polymerization to maintain the structural core. EGF and CSF-1 stimulate the formation of invadopodia in carcinoma cells and podosomes in macrophages, respectively. Angiogenic chemokines that exist in tumor stroma, such as VEGF, TNFα, and TGFβ, were also shown to induce the formation of podosomes in endothelial cells (36 and 37).

3.2. Signaling pathways involved in cell migration

To date, several important proteins that mediate the signaling pathways have been identified as overexpressed in several types of cancers (16) and in the subpopulation of invasive tumor cells in breast tumors (38).

Among them, the Rho GTPases, WASP family proteins, Arp2/3 complex, cortactin, and LIMK1/cofilin pathways have been studied extensively due to their apparent importance in cell migration and invasion.

3.2.1. Rho GTPases

The Rho-family of small GTPases, including Rho, Rac and Cdc42 control signal transduction pathways that link cell surface receptors to a variety of intracellular responses. Rho proteins generally cycle between an active, GTP-bound, conformation and an inactive GDP-bound conformation. Rho proteins can exchange nucleotide and hydrolyse GTP at slow rates *in vitro*, and these reactions are catalysed by guaninenucleotide-exchange factors (GEFs) and GTPase-activating proteins (GAPs), respectively. Rho GTPases coordinate to regulate multiple aspects of cell migration. Rho regulates the formation of actin stress fibers and focal adhesions, while Rac and Cdc42 regulate the polymerization of actin to form lamellipodial and filopodial protrusions, respectively. In addition, all three GTPases promote the assembly of integrin-based, matrix adhesion complexes (31, 39 and 40).

Rho promotes contractile actin:myosin filament assembly through two effectors, mDia and p160ROCK. Little is known about which molecules lie downstream of mDia, but p160ROCK phosphorylates LIM kinase, leading to cofilin phosphorylation, and myosin light chain (MLC) phosphatase, leading to an increase in MLC phosphorylation. Rac and Cdc42 both regulate actin polymerization through the WAVE family proteins acting on the Arp2/3 complex, and through p65PAK kinase acting on LIM kinase (41).

3.2.2. WASP family proteins

Currently, five mammalian WASP family members are known: WASP, neural WASP (N-WASP), and WAVE (WASP–family verprolin-homologous proteins) 1, 2 and 3, which can be divided into two subgroups, WASP/N-WASP and WAVEs. The WASP and N-WASP proteins are activated by Cdc42, SH3 domain-containing proteins, and phosphoinositides. N-WASP can induce the formation of filopodia downstream of Cdc42 by activating the ARP2/3 complex, while N-WASP may not be involved in the formation of lamellipodia in mammalian cells (42 and 43). It has also been revealed that WASP and N-WASP have a pivotal role in formation of podosomes/invadopodia (44 and 45). WAVEs are predominately regulated by Rac, and are important for lamellipodium and membrane ruffle formation (42). WAVE2 regulates formation of peripheral lamellipodia, which are necessary for general cell migration, while WAVE1 seems to promote formation of dorsal membrane ruffling and stabilization of peripheral lamellipodia. Although WAVE3 seems to be regulated by similar molecular mechanisms as WAVE1 and 2 (46), its physiological role has not been well studied. It has been revealed that WAVE3 regulates cell migration and invasion, and play a role in the progression of tumors (47, 48 and 49).

3.2.3. Arp2/3 complex

The actin-related protein-2/3 (Arp2/3) complex is localized in lamellipodia at the leading edge of migrating cells (42). This complex caps the pointed end of actin filaments and initiates the formation of new filaments that grow at the barbed ends. Since Arp2/3 prefers to bind to newly formed, ATP-rich actin filaments, it amplifies the cofilin-induced burst of actin polymerization (50). The Arp2/3 complex is activated by the Rho–family of GTPases through the WASP family proteins (42). WASP or N-WASP activates the Arp2/3 complex, thereby inducing actin filament nucleation and rearrangement of actin filaments.

3.2.4. Cortactin

Cortactin is a ubiquitously expressed, actin-binding and scaffolding protein that plays crucial roles in the regulation of the actin cytoskeleton. It consists of an amino-terminal Arp2/3 binding site, tandem repeats that bind F-actin, and a COOH-terminal SH3 domain that mediates direct binding to dynamin-2 and N-WASP (51 and 52). Cortactin seems to promote Arp2/3 complex-mediated actin nucleation and stabilizes newly formed branched actin filaments in the dynamic actin cytoskeleton. Cortactin directly activates the actin nucleation activity of Arp2/3 complex through its N-terminal region, although the activity is relatively weak when compared with that of WASP family proteins (51). Cortactin is also able to indirectly promote Arp2/3 complex-mediated actin polymerization by binding to N-WASP and activating it (53). Another study demonstrated that cortactin also stabilizes branched actin filaments produced by Arp2/3 complex (52).

3.2.5. LIMK-1/Cofilin

Cofilin (also called ADF) is localized throughout the lamellipodium but excluded from the leading edge. It is a small ubiquitous protein that is able to bind both monomeric and filamentous actin, and is an essential regulator of actin dynamics at the plasma membrane during cell migration through its ability to sever actin filaments. Upon receptor stimulation, cofilin is activated and severs actin filaments, thus increasing the number of barbed ends, which in turn induces extension of the lamellipoda. Cofilin activity is suppressed by both PIP2 binding and phosphorylation (54). *In vivo* studies suggest that PLC-mediated hydrolysis of PIP2 can release cofilin from this complex thereby activating it (55). LIM-kinase 1 (LIMK-1), a serine/threonine kinase containing LIM and PDZ domains (56, 57 and 58), phosphorylates cofilin at Ser 3, both *in vitro* and *in vivo*. It has been demonstrated that LIMK-1 participates in Rac-mediated actin cytoskeletal reorganization by phosphorylating cofilin (59). Rac stimulates the kinase activity of LIMK-1, which induces phosphorylation and inactivation of cofilin and probably leads to a decrease in the rate of actin depolymerization.

4 PROTEIN STRUCTURE OF MTSS1 AND ITS SPLICING VARIANTS

4.1. Protein structure of MTSS1

MTSS1 gene is located on human chromosome 8p22. Previous studies have reported four possible splicing variants, including MTSS1_v1 or MIM-A (accession no. AB007889.1), MTSS1_v2 or MIM-B (AK027015.1),

MTSS1_v3 or MIM (12del) (AF086645.1), and MTSS1_v4 or MIM-C (AB007889.2) (5 and 60). Full-length cDNA of MTSS1, known as MTSS1_v2 or MIM-B, predicts a protein product of 759 amino acids with a molecular weight of 82.6 kD. It possesses multiple functional motifs including a N-terminal 250-aa IRSp53/MIM homology domain (IMD), a coiled-coil domain, a lysine-rich domain (LRD), a Ser-rich domain (SRD), a Pro-rich domain (PRD), and also a Wiskott-Aldrich syndrome protein homology 2 (WH2) domain at the C-terminus (10 and 50).

The WH2 domain (WASP homology domain-2) is a ~35 residue that is found in multiple regulators of the actin cytoskeleton, including L-thymosins, ciboulots, WASPs, verprolin/WIP (WASP-interacting protein), Srv2/CAP (adenylyl cyclase-associated protein) and several uncharacterized proteins (61). This domain is known to interact and bind actin monomers regulating actin cytoskeletal organization. However, the MTSS1 WH2 domain occurs within a different domain organization than in most cytoskeletal proteins. Contrary to WASP, where Pro-rich sequences and WH2-related sequences border WH2, MTSS1 WH2 domain is found in isolation at the C-terminal end. Therefore, it suggests that WH2 helps recruit MTSS1 and IRSp53, as well as their binding partners, to specific cytoskeletal networks. Consistent with this idea, images of cells overexpressing full-length MTSS1 show a significant loss of stress fibers (9, 10, and 62), but this effect appears diminished for MTSS1 constructs lacking the WH2 region (6 and 62).

IRSp53/MIM homology domain (IMD) that found from both MTSS1 and insulin receptor substrate p53 (IRSp53) induces drastic formation of filopodia upon over-expression in cells (8). Several studies have revealed that IMD cross-links F-actin (6, 8, 62, 63 and 64). The IMD of MTSS1 has been initially described as an actin-binding and bundling domain (6). However, its activity of bundling actin has also been a matter of some discrepancy, as the bundling may be too weak (8) or does not exist (62). It suggests that IMD may act as a partner with other regulators to coordinate actin bundling. Another activity has been demonstrated in the literature for IMD is an interaction with the small GTPase Rac1 (6 and 65) IMD also possesses a PI (4,5)P2-rich membrane binding and deforming activity (63 and 66). Intriguingly, the structure of the IMD resembles that of the BAR (Bin/ amphiphysin/ Rvs) domain, which also binds with small GTPases (67). In contrast to BAR domain, the IMD is not involved in endocytosis or membrane trafficking due to its 'zeppelin-shape' rather than 'banana-shape' of BAR domain (66).

The central region sandwiched in between IMD and WH2 is rich in Pro, Ser and Thr residues. The Pro-rich domain has recently been shown to interact with cortactin SH3 domain to promote the cortactin-mediated actin assembly (7). Colocalization studies indicate that amino acids 408–538 of MTSS1 contain the RPTP delta binding region (62). However, a binding motif for RPTP delta has also been shown in the region close to the Ser-rich domain which contains two major phosphotyrosine residues Tyr-397 and Tyr-398 (11). The coiled-coil domain may be involved in MTSS1 self-association for both actin cross-linking and cellular cytoskeletal changes (62).

4.2. Protein structure of MTSS1's splicing variants

In addition to the full-length MTSS1, other three alternatively splice variants also have been described. MTSS1_v1 gene contains a partial intronic sequence and encodes a protein of 356 amino acids corresponding to the C-terminal part of the full-length MTSS1 including a proline rich region and the WH2 domain. The cDNA of MTSS1_v3 encodes a protein product of 755 amino acids that lacks VDTL sequence as a result of missing part of exon 7. MTSS1_v4 cDNA, which predicts a protein product of 734 amino acids, misses a part of translational region at N-terminal of full-length MTSS1. In addition, the sequence of amino acids from 350 to 413 encoded by exon 11 is replaced with an alternative exon of 39 amino acids (5).

5. FUNCTION OF MTSS1

5.1. MTSS1 ACTS AS A CYTOSKELETAL SCAFFOLD PROTEIN

Overexpression of MTSS1 in various cell lines induces the assembly of actin-rich membrane protrusions, microspikes and also disassembly of stress fibres (9, 10 and 62). Thus, MTSS1 is a good candidate as a scaffold protein involved in actin dynamics.

5.1.1. MTSS1 binding and bundling actin filaments

Both human and mouse MTSS1 contain a WH2 domain in its C-terminal region. This domain is also found in multiple regulators of the actin cytoskeleton, including the β-thymosins, WASP, and verprolin/WIP (WASP-interacting protein). Mattila *et al* reported that the actin monomer-binding site resides in the WH2 domain of MTSS1 (9). Biochemical study revealed that MTSS1 WH2 interacts with actin, preferentially ATP bound G-actin, an active form of actin for polymerization. MTSS1 was also found to have a fivefold higher affinity for ATP than ADP-G-actin and inhibits nucleotide exchange on actin monomers. The high affinity of MTSS1 to ATP-G-actin suggests that the majority of MTSS1 in cells is contained in a complex with actin monomers. There is also evidence that MTSS1 in complex with ATP-G-actin can participate in actin polymerisation at the barded end of filaments. Thus, MTSS1 may have the potential to control the elongation of actin filaments and undesired nucleation. Another report suggests that there may be a competition between N-WASP-VCA, which is a constitutively activated form of N-WASP containing WH2 domain and MTSS1 for G-actin (7). It has been concluded that MTSS1 inhibits N-WASP-mediated actin polymerization in a G-actin binding-dependent manner.

IMD was identified from the N-terminal region of MTSS1, IRSp53, and three uncharacterized proteins (8). It endows MTSS1 with F-actin-binding and bunding activity. Expression of IMDs in cultured mammalian cells induces a dramatic formation of filopodia (8, 62 and 63). F-actin-binding by the IMD of MTSS1 was confirmed by

high-speed co-sedimentation experiment (66), but the affinity of IMDs to F-actin is relatively low compared with most other F-actin–binding proteins. Several lines of evidence confirm that the IMD exists as a dimer in solution (63), and the F-actin-bundling activity is mediated through the bivalent nature of the IMD dimer. Mutational analysis maps the actin-binding sites to the extreme ends of the dimer. However, the ability of MTSS1 IMD to bundle actin *in vitro* has been a matter of some controversy. Mattila *et al* reported that IMDs display only very weak F-actin–bundling activity at physiological ionic conditions that are unlikely to contribute to filopodia formation. The actin-bundling activity was found to be inhibited by phosphoinositol diphosphate (PIP2). The authors suggested that the previously reported actin-bundling activity appears to result from protein aggregation at low salt conditions (68). Lee *et al* found that the IMD of MTSS1 does not bundle F-actin under any of the conditions tested (66). The disagreement between different laboratories concerning the bundling activity of the IMD may have resulted from nonspecific aggregation of the IMD at low ionic strengths, or the use of F-actin preparations that appear to sediment even in the absence of the IMD construct.

5.1.2. MTSS1 interactions with the small GTPase Rac

In addition to interaction with actin, MTSS1 also interacts with the small GTPase Rac through its IMD domain (69). The Rac binding site is located at the ends of the IMD dimer and appears to overlap or be similar to the F-actin binding site, as the two can compete with each other for binding *in vitro*. It was found that Rac binding inhibits the F-actin bundling activity of the IMD of MTSS1 (6 and 69). Studies have also shown that MTSS1 is far more efficient in activating Rac than another IMD containing protein, IRSp53. This suggests that activation of Rac by IMD containing proteins does not seem to be a conserved property. Although the stimulation of Rac activity by MTSS1 can elicit cell morphological changes, it is not sufficient for lamellipodia formation induced by MTSS1. Another report suggested that only the shorter splice variant of MTSS1's IMD interacts with Rac, whereas the longer splice variant containing a four-amino-acid insertion in the loop between helix-2 and -3 does not bind Rac or other Rho-family GTPases with a detectable affinity. They also revealed that interaction with Rac is not necessary for IMD-induced filopodia formation.

5.1.3. MTSS1 interaction with cortactin

As an ARP2/3 complex activator, cortactin facilitates the release of activated WASP proteins from Arp2/3 complex at branching sites. It plays a key role in the formation of lamellipodia and filopodia in motile cell by promoting and stabilizing branched actin filaments. Colocalisation of cortactin with GFP (green fluorescent protein) tagged MTSS1 revealed a direct interaction between cortactin and MTSS1-GFP (4). It was further verified that the interaction between cortactin and MTSS1 occurs between the cortactin SH3 domain and a proline-rich sequence of MTSS1. *In vitro* actin polymerization analysis demonstrates that full-length MTSS1 markedly enhances cortactin and Arp2/3 complex-mediated actin polymerization in an SH3 dependent manner. In contrast, MTSS1-CT, a short splicing variant of MTSS1, binds poorly to cortactin *in vitro* and is unable to promote actin polymerization. This suggests that activation of cortactin-mediated actin polymerization requires full-length MTSS1. One possible function is that full-length MTSS1 has an F-actin binding activity, which may facilitate cortactin-mediated actin polymerization. Under the same condition, however, MTSS1 dramatically inhibits N-WASP-VCA mediated actin polymerization and actin branching as well. It is likely due to a competition for G-actin as VCA and MTSS1 have a very similar affinity for G-actin. Interestingly, overexpression of a MTSS1 mutant that was unable to interfere with VCA enhanced cell migration, whereas a mutant deficient in cortactin binding inhibited further PDGF mediated cell motility. This indicates that MTSS1, together with cortactin may be involved in the signaling pathway stimulated by PDGF.

5.1.4. MTSS1 interaction with the cell membrane

The crystal structure of the IMD domain showed a clear homology to the BAR domains, which possess a well-characterized membrane binding and deforming activity during endocytosis (63 and 66). This suggests that they may have functional similarities as well. Mattila *et al.* (68) have recently shown that the IMD domain from both MTSS1 and IRSp53 directly bind $PI(4,5)P_2$-rich membranes and deform them into tubular structures. Unlike previously characterized membrane-tubulating domains, the IMD appears to bind to the inner surface of the membrane tubule and therefore promote the formation of plasma membrane protrusions rather than invaginations. To map the $PI(4,5)P_2$ binding site on MTSS1 IMD, systematic mutagenesis was performed. The results showed that a relatively large positively charged region at each end of the dimeric IMD is important for $PI(4,5)P_2$ binding. Moreover, the same region was also found to be important for F-actin binding, suggesting that these binding sites overlap on the surface of MTSS1. The authors found that due to the different geometries of the $PI(4,5)P_2$-binding site, IMDs induce a membrane curvature opposite that of BAR domains. It was also shown that the membrane deforming activity of IMD, instead of the previously proposed F-actin bundling and GTPase binding activities, is critical for the induction of the filopodia/microspikes in cultured mammalian cells. However, a somewhat different conclusion made by Suetsugu *et al.*, suggests that both actin and $PI(4,5)P_2$ binding activities make important contributions too (65).

5.1.5. MTSS1 interaction with RPTP delta

RPTP delta (receptor protein tyrosine phosphatase delta) is a membrane bound protein tyrosine phosphatise closely related to DLAR, RPTP alpha, and RPTP kappa, which has been implicated in regulation of cellmatrix and cell–cell interactions (70 and 71). Woodings *et al* first reported that MTSS1 binds to the cytoplasmic domain of RPTP delta (14). They found that the RPTP delta binding site is located within amino acids 404–705 near the proline-rich region. Subsequently, Gonzalez-Quevedo *et al.* (68) showed that MTSS1 binds specifically to the RPTP delta D2 domain. MTSS1-dependent cytoskeletal changes can be inhibited using a soluble

RPTP-D2 domain. A more detailed region within amino acids 400–538 of MTSS1 was considered as the RPTP delta binding site. They also showed that MTSS1 plays a role in the localisation of RPTP delta to specific regions of the plasma membrane, but that the phosphatase activity of RPTP delta did not appear to be required for this localisation. Taken altogether, these studies proposed that MTSS1 links receptor tyrosine phosphatase signalling with actin cytoskeletal reorganization and provides a potential new link between tyrosine phosphorylation signalling and the actin cytoskeleton. It also raises the possibility that MTSS1 proteins may regulate actin assembly in response to extracellular signals.

5.1.6. MTSS1 plays a role in the PDGF signaling pathway

Several lines of evidence have indicated that MTSS1 may be involved in the PDGF signaling pathway that regulates cell shape changes via protein tyrosine kinases. Zhan *et al* reported that overexpression of full-length wild-type MTSS1-GFP inhibited markedly the motility of NIH3T3 cells induced by PDGF and that of human vein umbilical endothelial cells induced by sphingosine 1 phosphate (7). In another report by the same group, tyrosine phosphorylation of MTSS1 was shown to represent an early event in the PDGF signaling cascade. Mutagenesis analysis has revealed that phosphorylation occurs at multiple sites, including tyrosine residues Tyr-397 and Tyr-398 (11). Furthermore, they also found that tyrosine phosphorylation of MTSS1 is mainly mediated by Src, and MTSS1 is a direct substrate of Src. Such evidence indicatesthat MTSS1 represents a novel signaling pathway from PDGF receptor to the actin cytoskeleton via Src-related kinases.

5.2. MTSS1 as a new Shh-responsive gene

Sonic hedgehog (Shh) signaling plays a critical role during development and carcinogenesis. Most hedgehog signaling is controlled through the Ci/Gli family of zinc finger transcription factors. Induction of Shh targets in an uncontrolled fashion can promote cancers, including basal cell carcinoma (72, 73 and 74). It has been shown that MTSS1 behaves as a Shh-responsive gene both *in vivo* and *in vitro* (12). Unlike Ptch1 and Hip, which control pathway activity through Shh binding (75), MTSS1 appears to regulate target gene expression through its association with the Gli complex. It has been proposed that MTSS1, Sufu and Gli form a ternary complex, which enhances transcription of Shh-responsive genes. Mutation analysis revealed that an N-terminal region of MTSS1 which is located between amino acids 160 and 399 is required for both complex associations and transcriptional potentiation. Another report has also shown that actin bundling and transcriptional potentiation are mediated through distinct domains (62). Taken together, is can be said that MTSS1 is a new member of the Shh signalling pathway that modulates Gli responses during both development and tumorigenesis. Futher more, MTSS1's role in regulating the cytoskeleton and transcription indicates, that like β-catenin (76), plakoglobin (77), and p120 (78), it is a member of the growing family of cytoskeletal components that associate with transcription factors to affect nuclear signaling.

6. EXPRESSION OF MTSS1 IN CANCER AND ITS' CLINICAL SIGNIFICANCE

6.1. Expression of MTSS1 in cancer

MTSS1 was first identified and also later confirmed to be down-regulated in metastatic bladder cancer cell lines (2, 3 and 4). It has been shown that there is no clear association between reduced MTSS1 expression and a more invasive phenotype either *in vitro* or *in vivo* (3). Studies on the expression of MTSS1 have also been performed in many other types of cancer. In prostate cancer; expression of MTSS1 has also been shown to be reduced, but contrary to results obtained in bladder cancer, reduction in MTSS1 gene expression in the prostate can contribute to tumor growth and development, as well as metastasis (5). Jiang *et al* also observed a reduction of MTSS1 expression in breast cancer, which may be associated with breast cancer prognosis. Similarly, we have also found a down-regulation of MTSS1 in oesophageal cell line (unpublished data). In contrast, up-regulation of MTSS1 expression has also been observed in other type of cancer. Ma *et al* demonstrated MTSS1 to be overexpressed at both mRNA and protein levels in hepatocellular carcinoma (13). They also found that overexpression of MTSS1 is significantly associated with early pTNM stage, with presence of tumor encapsulation, and absence of venous infiltration. Since the study of MTSS1 has been restricted to a limited of cancer types, the function of this protein has not been established. Further analysis of MTSS1 expression or inactivation in cancer and its association with different human malignancies will help us to achieve better understanding of this protein.

6.2. Methylation may be involved in the regulation of MTSS1 expression

Methylation of a gene promoter at CpG islands is a common mechanism for silencing expression of specific genes in many cancer types. Since down-regulation of MTSS1 occurred in bladder carcinomas and in metastatic bladder cancer cell lines (2 and 60), it is proposed that methylation might contribute to down-regulation of MTSS1. Nixdorf *et al* had been unable to find any regulation of expression by 5-Aza-dC. Treatment of representative cell lines with 5-Aza-dC failed to induce MTSS1 expression (3). They concluded that down-regulation is unlikely to be due to promoter hypermethylation. However, the role of promotor methylation in down-regulation of MTSS1 has been a matter of some discussion. Utikal *et al* demonstrated that DNA methylation of the CpG island in the MTSS1 promoter correlates with silencing of MTSS1 expression (4). Inhibition of DNA methylation by 5-Aza-dC led to an increase of MTSS1 expression in a low expressing cell line. They suggest that MTSS1 expression is inactivated by DNA methylation of the CpG island, which may be reactivated by DNA methylation inhibition. Analysis of DNA methylation using bisulphite sequencing revealed that MTSS1 promoter is methylated in 5′-flanking region in cells and tissue samples with reduced endogenous MTSS1 expression. Another study has also shown that 5-Aza-dC treatment of the gastric cancer cell line AGS caused a more than 4-fold upregulation of MTSS1 expression, which also

confirmed the contribution of methylation in down-regulation of MTSS1 (79). Further studies will help to determine the importance of methylation as a regulator of MTSS1 expression.

6.3. The clinical significance of MTSS1 in cancer

Recent research on the clinical significance of MTSS1 has been reported in different types of cancer. Wang *et al* have shown an inverse relationship between the level of MTSS1 expression and the degree of bladder transitional cell carcinomas (60). By immunohistochemical analysis of 68 human bladder specimens, they found that the level of MTSS1 immunoreactivity is inversely correlated with poor differentiation of bladder transitional cell carcinomas. Statistical analysis indicated a significant difference among normal bladder, high- and low-grade tumor samples ($p = 0.0002$). Similarly, poorer expression of MTSS1 in high-grade tumors as compared to low-grade tumors also is statistically significant ($p = 0.003$). This suggests that the loss of MTSS1 function may be involved in a late stage of the morphological transition from normal urothelium to carcinoma.

Another report has revealed that elevated MTSS1 expression may influence the development of hepatocellular carcinoma and may possibly be a powerful indicator for the disease at an early stage (13). The level of MTSS1 expression was quantified by both real-time qPCR on 40 pairs of matched tumor, adjacent nontumor tissue specimens, and 6 normal liver tissue specimens obtained from healthy donors. The results proved that there is a statistical difference ($P = 0 .028$) in expression between hepatocellular carcinoma tumor and nontumor liver tissues. Significantly higher MTSS1 expression was also observed in tumor tissues when compared with normal liver tissue from healthy donors ($P = 0.0005$). Western blot analysis in 30 randomly selected pairs of hepatocellular carcinoma and their matched nontumor liver tissues showed that up-regulation of MTSS1 expression was found in 70% patient. However, overexpression of MTSS1 in early pTNM stage group (I-II) and encapsulated hepatocellular carcinoma was much more common compared with late pTNM stage group (III-IV) and unencapsulated tumors. This suggests that MTSS1 expression may play a more important role in early hepatocellular carcinoma progression instead of in the advanced stage of the disease.

Jiang *et al* have demonstrated an inverse correlation between MTSS1 and patient prognosis and survival in breast cancer (14). By real-time qPCR and immunohistochemical analysis, MTSS1 expression levels were assessed in a cohort of breast cancer specimens (normal $n = 33$; cancer $n = 127$). The results showed that down-regulation of MTSS1 was related to poorer prognosis ($p = 0.042$), and high levels of MTSS1 correlated with an increased patient overall survival ($p = 0.0108$) and disease-free survival ($p = 0.012$). It has also been demonstrated that MTSS1 expression correlates with early pathologic tumor-node-metastasis stage, venous infiltration, and encapsulation.

To date, studies on the clinical significance of MTSS1 are restricted in a few types of cancer. Whether MTSS1 can be considered as a prognostic indicator in the early stage of cancer, remains to be seen and the relationship between MTSS1 expression and cancer prognosis needs to be investigated further.

7. PERSPECTIVE

To date, there remains several contrasting reviews in the studies on MTSS1. Firstly, whether or not MTSS1 is in fact a metastasis suppressor has not been established. It appears that MTSS1 could be up-regulated in hepatocellular carcinoma (13), but down-regulated in gastric (79), bladder and prostate cancers and benign lesions (2, 3 and 4). Whether the down- or up-regulation of MTSS1 is associated with tumor growth, cancer metastasis and prognosis require further studies. Secondly, the ability of MTSS1 IMD to bundle actin has been a matter of some controversy, as the actin-bindling activity may be to week (68), or does not exit (66). Thirdly, research on the contribution of methylation to the down-regulation of MTSS1, leads to disparate results. One report clearly demonstrated that MTSS1 expression is inactivated by DNA methylation of the CpG island (4), whilst another was unable to find any regulation of expression by methylation (3). All these contrasting results require further investigation.

Furthermore, there also remains open debate about the function of MTSS1 and a numner of unresolved observations. This debate should discuss the following: 1) Since IRSp53 and MTSS1 shared the same IMD domain, do they interact, or compete for binding partners. 2) Although N-WASP was reported as a target of MTSS1 in cells, MTSS1 could also attenuate other WASP-related proteins such as WAVE/Scar proteins, which also contain a WH2 domain (80). Determination of the intrinsic target of MTSS1 in cells will require further efforts to dissect the specific role of MTSS1 in the function of each member of the WASP family. 3) Because MTSS1 is a relatively large, multidomain protein, it may have other activities and regulate actin dynamics in a complex fashion. MTSS1 also probably interacts with other partners, such as Crk and Esp8 (81) to form larger protein complexes that upon a triggering signal recruit the machinery necessary for directed actin polymerization and induction of membrane protrusions. 4) It will be important to reveal the detailed mechanism by which IMDs interact with membranes. Because IMDs have a tendency to form multimers at low salt conditions, it will be interesting to determine the possible role of the IMD oligomerization or cooperativity during membrane deformation. 5) Because the cross-linking activity of many bundling proteins is activated by dephosphorylation (82), it is tempting to speculate that MTSS1 activity could be controlled via a competition between tyrosine phosphatases and tyrosine kinases, such as Abl or Src. 6) Further studies are also required to analyze whether MTSS1 function in the context of other cellular membranes, such as multivesicular bodies or viral spherules, which harbor topologically identical membrane curvature as compared to plasma membrane protrusions (83). 7) Further analysis of MTSS1 expression or inactivation in tissue samples and its association with different human malignancies will define MTSS1 as a

novel candidate to be used as a marker of primary tumours or metastasis (4).

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