Hepatocyte growth factor activator inhibitor type-1 in cancer: Advances and perspectives (Review)

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Abstract. Cancer is one of the most common diseases, with high morbidity and mortality rates. Large-scale efforts have been made to understand the pathogenesis of the disease, particularly in the advanced stages, in order to develop effective therapeutic approaches. Hepatocyte growth factor activator inhibitor type-1 (HAI-1), also known as serine protease inhibitor Kunitz type 1, inhibits the activity of several trypsin-like serine proteases. In particular, HAI-1 suppresses hepatocyte growth factor (HGF) activator and matriptase, resulting in subsequent inhibition of HGF/scatter factor and macrophage-stimulating protein (MSP). HGF and MSP are involved in cancer development and progression, via the receptors Met receptor tyrosine kinase (RTK) and Ron RTK, respectively. Therefore, HAI-1-mediated downregulation of HGF and MSP signaling may suppress tumorigenesis and progression in certain types of cancers. Abnormal HAI-1 expression levels have been observed in various types of human cancer. The exact function of HAI-1 in cancer pathogenesis, however, has not been fully elucidated. In this review, the focus is on the potential impact of aberrant HAI-1 expression levels on tumorigenesis and progression, the underlying mechanisms, and areas that require further investigation to clarify the precise role of HAI-1 in cancer.

Contents

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1. Introduction

Hepatocyte growth factor activator inhibitor type-1 (HAI-1), encoded by the *serine protease inhibitor, Kunitz type 1* gene, is a membrane-bound Kunitz-type serine protease inhibitor (1). HAI-1 was firstly purified from the conditioned medium of the MKN45 human stomach carcinoma cell line and identified as an inhibitor of hepatocyte growth factor activator (HGFA) (2). HAI-1 has also been demonstrated to inhibit a number of type-II transmembrane serine proteases (TTSPs), including matriptase, hepsin, transmembrane protease serine 13 (TMPRSS13) and human airway trypsin-like protease (HAT) (3-6). As a protein predominantly expressed in epithelial cells, HAI-1 is vital for cell growth, survival and mobility (1).

Increasing evidence has demonstrated that HAI-1 suppresses tumorigenesis and progression via regulation of the activity of a range of serine proteases in the tumor microenvironment. HGFA, a target trypsin-like serine protease of HAI-1, is secreted as a single-chain zymogen precursor and is activated by thrombin during blood coagulation. The activated HGFA induces the activation of two known macromolecular substrates, namely hepatocyte growth factor (HGF) and macrophage-stimulating protein (MSP), which are critical proteins involved in cancer pathogenesis (7,8). Downregulation of the activity of these two substrates through HAI-1-mediated HGFA inhibition, therefore, suppresses tumorigenesis and progression. In addition, TTSPs, another subtype of target HAI-1 proteases, facilitate epithelial carcinogenesis and progression (9). Therefore, HAI-1 is an important and promising therapeutic target in tumor treatment. This review focuses on recent advances in the understanding of HAI-1 with regard to the development and progression of cancer, and future studies concerning HAI-1 are proposed.

2. HAI-1 functional domains and proteases inhibited by HAI-1

HAI-1 is composed of an N-terminal extracellular region with two Kunitz domains (KD1 and KD2) separated by a

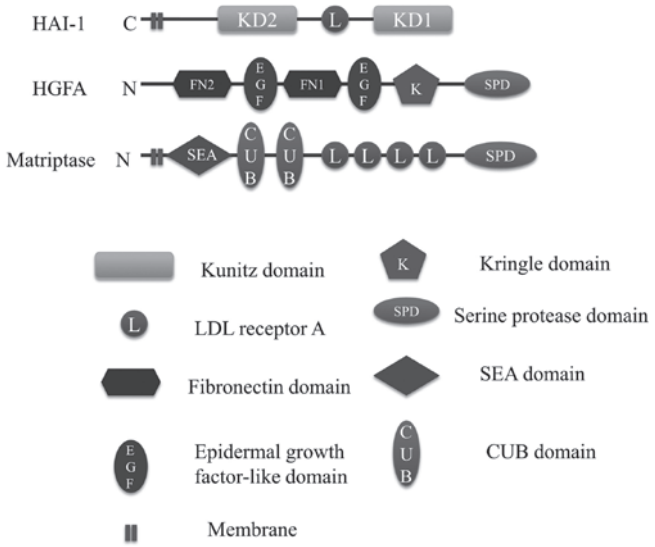

Figure 1. Domain structures of HAI-1, HGFA and matriptase. HAI-1, hepatocyte growth factor activator inhibitor type-1; HGFA, hepatocyte growth factor activator; LDL, low-density lipoprotein; SEA, sea urchin sperm protein/enteropeptidase/agrin; CUB, Cls/Clr, urchin embryonic growth factor, bone morphogenetic protein-1.

low-density lipoprotein receptor (LDLR)-like domain, a transmembrane region and a short cytoplasmic region (Fig. 1) (10). The primary transmembrane form (66 kDa) of HAI-1 is released as several soluble proteins (58, 48, 40 and 39 kDa) into the extracellular milieu by proteolytic cleavage (11). The transmembrane and soluble forms of HAI-1 exhibit inhibitory activity against serine proteases in the pericellular microenvironment (11,12). Among these HAI-1 molecules, the 58 kDa and 40 kDa HAI-1 proteins are the predominant soluble forms in the conditioned medium from cancer cell cultures. The 40 kDa HAI-1, which lacks KD2, exhibits higher inhibitory activity against HGFA than the 58 kDa band (11). However, studies have demonstrated that KD1 is also responsible for protease inhibition via interaction with target proteins (12-15). Furthermore, KD1-protease complex formation is enhanced by the LDLR-like domain but attenuated by KD2 (12).

HAI-1 exerts marked inhibitory activity against a variety of serine proteases, including HGFA, matriptase, hepsin, plasmin, trypsin, prostasin, TMPRSS13 and HAT (1,3-6,16,17). Studies have demonstrated that the proteases inhibited by HAI-1 clearly promote carcinogenesis and progression. For instance, HGFA expression is upregulated in breast, colorectal and renal cell carcinomas accompanied by downregulation of HAI-1 (18-20). Matriptase, another protease inhibited by HAI-1, is overexpressed in a variety of malignant tumors, and possesses the ability to promote oncogenesis and progression (1). Hepsin-encoding gene, *Hpn*, is among the most consistently and quantitatively overexpressed genes in human prostate cancer, as detected by cDNA microarray and tissue array assays, and hepsin is the most reliable single marker to distinguish prostatic neoplasia from benign prostate hyperplasia (21-23). Prostasin, one glycosylphosphatidylinositol-anchored serine protease, has been reported to be upregulated in ovarian cancer but downregulated in high-grade prostate cancer (17,24,25). Therefore, HAI-1 may contribute to the prevention of cancer growth and progression via inhibition of these serine protease activities.

3. Aberrant HAI-1 expression levels in cancer correlate with malignant phenotypes and clinicopathological parameters

Aberrant HAI-1 expression levels have been demonstrated in various types of cancer and have diagnostic and prognostic implications. The expression profiles and functions of HAI-1 have been investigated extensively in pre-clinical and clinical studies (Table I).

Abnormal HAI-1 gene expression levels have been detected in a wide variety of human cancer cell lines; certain cell lines with a highly invasive nature exhibited low HAI-1 mRNA expression levels (26). In addition, as determined by *in vitro* and *in vivo* models, HAI-1 exerts a potential inhibitory effect on cancer cell invasion and migration, important hallmarks of cancer (27). Forced expression of HAI-1 significantly inhibited the invasion and migration of cervical, endometrial and uterine cancer cells *in vitro* (28-30). Furthermore, breast, pancreatic, prostate and oral carcinoma cells exhibited enhanced invasive properties *in vitro* in response to HAI-1 knockdown (31-34), a result also validated in nude mice bearing xenografts (33,35,36). Such findings are important for the analysis of the pathogenic role of HAI-1 in cancer cells. However, whether HAI-1 suppresses or promotes proliferation of cancer cells remains elusive (28-34,37).

Immunohistochemical (IHC) staining has detected reduced HAI-1 expression levels in endometrial, cervical and colorectal carcinomas and uterine leiomyosarcoma, compared with adjacent normal tissues (19,28-30). In addition, reduced HAI-1 mRNA levels have been detected by polymerase chain reaction in breast, gastric, colorectal and renal cell carcinoma (RCC) tissues (18-20,38). Further detailed analysis revealed that reduced HAI-1 expression levels were associated with worse clinicopathological parameters (advanced stage, lymph node metastasis and distant metastasis) and/or poor prognosis (reduced disease-free survival and overall survival times) in ovarian, gastric, cervical, endometrial, renal cell

Table I. Expressional and functional studies of HAI-1 in cancer.

Cancer type	Model	HAI-1 expression	Consequence/cancer association	Reference
Breast	MCF-7 cell line	High expression levels	Low invasion	Parr and Jiang(26)
	MDA MB-231 cell line	Low expression levels	High invasion	Parr and Jiang (26)
	MDA-MB-231 cell line	Knockdown	Enhanced migration, proliferation, invasion	Parr and Jiang (31)
	Breast cancer specimens	Lower levels in grade 3	Decreased in poorly differentiated tumors	Parr *et al* (18)
Colorectal	Primary colorectal carcinoma specimens	Lower levels in carcinoma tissue	Associated with disease progression	Kataoka *et al* (19)
Pancreatic	SUIT-2 cell line	Knockdown	Reduced cell growth, but enhance invasion	Cheng *et al* (34)
	SUIT-2 cell line, nude mice	Knockdown	Enhanced pulmonary metastasis	Fukushima *et al* (35)
Ovarian	Ovarian cancer specimen	Lower levels in stage III/IV	Loss of expression associated with advanced stage	Oberst *et al* (38)
Gastric	Gastric cancer specimens	Low expression levels	Associated with invasion and lymph node metastasis	Zeng *et al* (39)
Cervical	SiHa and HeLa cell lines	Overexpression	Inhibited growth, invasion, lead to apoptosis	Nakamura *et al* (28)
	Cervical cancer specimens	Low expression levels	Poor prognosis	Nakamura *et al* (28)
Endometrial	KLE and HEC-251 cell lines	Overexpression	Inhibited growth, invasion and migration	Nakamura *et al* (30)
	Endometrial cancer specimens	Low expression levels	Poor prognosis	Nakamura *et al* (30)
Uterine	SK-LMS-1 and SKN cell lines	Overexpression	Inhibited growth, invasion and migration	Nakamura *et al* (29)
	Uterine leiomyosarcoma specimens	Low expression levels	Poor prognosis	Nakamura *et al* (29)
Prostate	PC-3 and DU-145 cell lines	Knockdown	Inhibited growth, enhance invasion and migration	Sanders *et al* (32)
	Prostate cancer specimens	Low expression levels	Associated with increasing aggressiveness	Saleem *et al* (40)
	Prostate cancer samples	High mean serum level	Distant metastasis and hormone resistance	Nagakawa *et al* (47)
Kidney	Renal cell carcinoma specimen	Low expression levels	Involved in cancer progression	Yamauchi *et al* (20)
Oral cavity	HSC-3 and SAS cell lines	Knockdown	Reduced growth, but enhanced migration	Baba *et al* (33)
	SAS cell line, nude mice	Knockdown	Enhanced tumorigenicity	Baba *et al* (33)
	Oral squamous cell carcinoma specimens	Reduced expression levels at the invasion front	Associated with invasion, lymph node metastasis	Baba *et al* (33)
Liver	Hep3B cell line	Knockdown	Inhibited growth	Nagata *et al* (37)
	Hepatocellular carcinoma specimens	Positive in 35% cancer tissues	Involved in cancer progression	Nagata *et al* (37)
	Hepatocellular carcinoma specimens	Positive in 31% cancer tissues	Associated with poor prognosis	Funagayama *et al* (41)

HAI-1, hepatocyte growth factor activator inhibitor type-1.

and oral squamous cell carcinomas, and uterine leiomyosarcoma (19,20,28-30,33,39-40), but not in hepatocellular carcinoma (HCC) (37,41). Notably, HAI-1 was only marginally detectable in normal hepatocytes (42), while >30% HCC tissues were identified as HAI-1-positive by IHC (41), thus increased HAI-1 expression levels appear to be associated with advanced tumor stage and poor prognosis in HCC (37,41).

However, the exact role of HAI-1 in several types of cancer, including breast, colorectal and prostate cancer, remains controversial. Although reduced HAI-1 expression levels were associated with poorly differentiated breast cancer (18), high-level expression of HAI-I was found to be associated with poor patient outcome in a breast cancer tissue microarray analysis (43). HAI-1 downregulation in colorectal

cancer has been observed in a number of studies, but enhanced immunoreactivity of HAI-1 was detected in colorectal cancer cells at the invasion front, which may be involved in distant metastasis, although this trend was not statistically significant (44). In human prostate cancer tissues, the HAI-1 protein levels were elevated compared with those of benign prostate tissues (45,46). The mean serum levels of HAI-1 in 118 patients with prostate cancer were reported to be significantly higher than those in 27 patients with benign prostatic hyperplasia. Furthermore, increased HAI-1 levels in serum were associated with distant metastasis and the development of hormone-resistance in prostate cancer (47). However, another study observed using immunohistochemistry indicated that HAI-1 expression levels were reduced in all grades of prostate cancer specimens (40).

According to current research, HAI-1 may exhibit different functions in different types of cancer or even at different stages/sites in the same type of cancer (26,32,37,40,47). However, the differences in measuring HAI-1 expression levels, the lack of standardized methods (including antibody) among studies create difficulties in reaching a conclusion regarding HAI-1 expression in cancer and its association with clinicopathological parameters. Further studies with large samples and standardized criteria are warranted to elucidate the role of HAI-1 in tumor pathology, and to determine the diagnostic and prognostic value of HAI-1 expression.

4. Molecular mechanisms of HAI-1 in cancer

As described above, HAI-1 exerts a suppressive effect on cancer invasion and metastasis, processes which result in a poor prognosis for cancer patients (48); however the molecular basis of HAI-1-mediated cancer inhibition remains poorly understood. In the present review, advances in the understanding of the diverse molecular mechanisms regulating HAI-1-mediated effects via target serine proteases, particularly HGFA and matriptase, are summarized. Studies have shown that increased expression levels of HGFA and/or matriptase were accompanied by significantly downregulated HAI-1 expression. Thus, the net balance between HGFA/matriptase and HAI-1 was shifted in favor of HGFA/matriptase in various types of carcinoma, including breast, ovarian, renal, prostate and colorectal carcinoma (18-20,38,40,43,49,50). In addition, *in vitro* studies have validated the finding that HAI-1 knockdown-induced enhanced migration is partially reversed by silencing of matriptase or other serine protease expression (33,34,36).

The two best-characterized HAI-1-inhibited proteases (HGFA and matriptase) activate pro-HGF and pro-MSP, and are responsible for the subsequent activation of Met receptor tyrosine kinase (RTK) and Ron RTK, respectively (1,7,51). Dysregulation of the HGF-Met signaling pathway has been implicated in the development and metastasis of human cancer (52,53). Tumor xenografts with overexpressed HGF or Met exhibit high metastatic ability in mouse models (54-58). In addition, angiogenesis and lymphangiogenesis are promoted in tumors due to the induction of endothelial cell growth by HGF-Met cascade, as revealed by *in vitro* and *in vivo* studies (59-61). The downstream effectors of Met RTK activate several distinct signaling cascades, among which the RAS-mitogen activated protein kinase (MAPK) and phosphatidylinositol 3 kinase (PI3K)-AKT signaling pathways are predominant. The RAS-MAPK cascade eventually activates the extracellular signal-regulated kinases (ERKs), which transmit signals downstream, and results in the transcription of genes controlling cell proliferation, differentiation, adhesion, migration and apoptosis (62). Activation of the PI3K-AKT-mammalian target of rapamycin signaling pathway results in cancer cell proliferation and invasion (63). Met activation may also enhance the function of Rap1 and modulate the adhesion molecules cadherin and integrin, and therefore promote cell migration (64,65). As with the signaling activation pattern activated by HGF-Met, MSP-Ron signaling is also mediated by the RAS-MAPK and PI3K-AKT signaling pathways (Fig. 2) (7). Therefore, HAI-1 inhibits tumor development and progression via suppression of protease-mediated downstream signaling pathways.

In addition, the activation of the RAS-MAPK and PI3K-AKT signaling pathways is crucial for RTK-mediated epithelial-to-mesenchymal transition (EMT) in cancer cells (7). EMT is recognized as a potential mechanism for carcinoma metastasis and the loss of E-cadherin is a hallmark of EMT (66). The predominant transcriptional repressors of E-cadherin are zinc finger transcription factors, including Snail (Snai1), Slug, smad-interacting protein 1 (SIP1) and a basic helix-loop-helix transcription factor, Twist (67). An increasing amount of evidence has demonstrated that the interactions among HAI-1 and target serine proteases contribute to EMT in certain carcinoma cells. Support for this concept includes the finding that human pancreatic cancer cells with stable knockdown of HAI-1 exhibited an elongated spindle-like morphology and an enhanced migratory ability. Vimentin, SIP1 and matrix metalloproteinase (MMP)-9 expression was upregulated in these cells but E-cadherin expression was downregulated. The subsequent silencing of matriptase in these HAI-1 knockdown cells resulted in reversal in the expression levels of MMP-9 accompanied by a recovery of E-cadherin expression levels (34). In another study, HAI-1 overexpression resulted in a significant increase in E-cadherin expression levels but a reduction in Vimentin, SIP1, Snail and Twist expression levels in human endometrial cancer cell lines (30). The involvement of HAI-1 in EMT was further confirmed by other studies: Reduced E-cadherin expression levels in HAI-1-knockdown pancreatic cancer cells was reversed by recombinant KD1 (35) and HAI-1 knockdown oral squamous cell carcinoma cell lines exhibited more elongated morphology and reduced E-cadherin expression levels (33). All evidence reveals that HAI-1 inhibits tumor metastasis, partly by inhibiting EMT.

HAI-1 may also suppress the invasion and metastasis of tumor cells by inhibiting the activity of certain cognate serine proteases that activate fibrinolytic enzymes, MMPs and single-chain urokinase-type plasminogen activator (4,68-70). These enzymes are responsible for the degradation of extracellular matrix components and further potentiate local tumor invasion and metastasis (71).

Recently, a transgenic mouse model revealed that HAI-1 suppressed intestinal tumorigenesis. Enhanced tumor formation was observed in mice with deficient intestinal HAI-1 expression. Notably, a total of 22 genes (including those

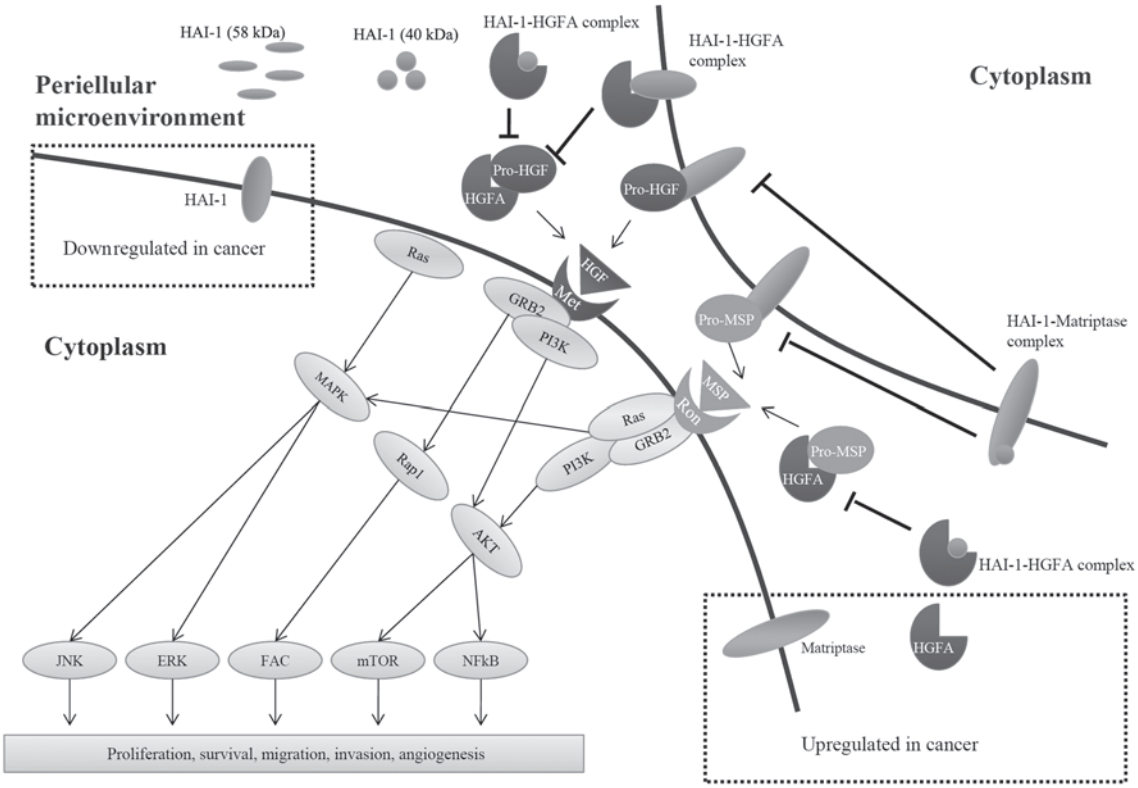

Figure 2. Signaling pathways mediated by HAI-1. HAI-1 is downregulated in cancer cells, whereas the HAI-inhibited proteases (HGFA and matriptase) are upregulated. Thus, the balance between HGFA (or matriptase) and HAI-1 is shifted in favor of HGFA (or matriptase) in cancer cells. HGFA and matriptase convert pro-HGF and pro-MSP into active HGF and MSP, which further bind to Met RTK and Ron RTK, respectively. The RAS-MAPK and PI3K-AKT signaling pathways are activated downstream of the HGF-Met and MSP-Ron complexes. These signaling pathways promote cell proliferation, survival, migration, invasion and angiogenesis. HAI-1, hepatocyte growth factor activator inhibitor type-1; HGF, hepatocyte growth factor; HGFA, HGF activator; MSP, macrophage-stimulating protein; RTK, receptor tyrosine kinase; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3 kinase; AKT, protein kinase B; GRB2, growth factor receptor-bound protein 2; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase; mTOR,. mammalian target of rapamycin; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells.

encoding ligands, receptors, transcription factors and downstream genes) associated with the Wnt signaling pathway were identified by microarray analysis to be augmented in the tumors. Furthermore, the expression of several other genes involved in mucosal permeability and angiogenesis, including *cldn2*, *lt1*, *cdh13*, *cdh5* and *tnfrsf12a*, was also upregulated (72). As barrier dysfunction may contribute to epithelial malignancy (73,74), HAI-1 may suppress tumorigenesis resulting from inhibition of the expression of these associated genes.

HAI-1 may exhibit different functions depending on the presence of cognate serine proteases in the intra- or extra-cellular milieu. The membrane-form HAI-1 acts not only as an inhibitor to HGFA, but also as an acceptor molecule, generating a reservoir of active HGFA on the cell surface; the HAI-1-HGFA complex on cell membrane may be dissociated and release the active HGFA into the surrounding microenvironment (75). Another study demonstrated that HAI-1 acted as an essential cofactor in the activation of pro-matriptase (76). Therefore, abnormal HAI-1 function may potentially contribute to tumor development and progression under specific conditions.

In conclusion, these findings established that HAI-1 is key in the development and progression of cancer; however, identification of the acute mechanism remains incomplete and requires further investigation.

5. Conclusion and future perspectives

HAI-1 is a vital protein involved in a number of biological and pathological processes due to its ability to inhibit cognate serine proteases in the extracellular milieu. The majority of these serine proteases are involved in the development and progression of cancer; therefore, HAI-1 exerts a suppressive function in cancer through regulation of these proteases.

Thus far, considerable achievements have been gained in the understanding of the pathological role of HAI-1 in tumors, particularly in the impact of aberrant HAI-1 expression levels on tumor growth, invasion, angiogenesis and metastasis. Existing studies have identified several of the molecular mechanisms mediated by HAI-1 and the target serine proteases. As determined by these findings, the prognostic and pharmaceutical properties of HAI-1 render the molecule a promising factor in cancer diagnosis and treatment.

However, paradoxical results have been obtained regarding HAI-1 expression patterns in certain types of cancer. The regulatory mechanisms that result in aberrant HAI-1 expression levels under different circumstances remain elusive. Further investigation into HAI-1 is important not only for providing greater insight into the molecular aspects of HAI-1 in cancer, but also for the possible development of novel diagnostic and therapeutic approaches. Even at the early stages of HAI-1 clinical investigation, understanding the acute roles of HAI-1

in cancer no doubt contributes, at least partly, to the eventual control of human cancer.

Acknowledgements

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