Isthmin Protein Family

Subjects: Developmental Biology

Contributor: Hosen Md Shakhawat, Zaman Hazrat, Zhongjun Zhou

Isthmin (ISM) is a secreted protein family with two members, namely ISM1 and ISM2, both containing a TSR1 domain followed by an AMOP domain. Its broad expression pattern suggests diverse functions in developmental and physiological processes. Multiple studies have focused on the functional analysis of the ISM protein family in several events, including angiogenesis, metabolism, organ homeostasis, immunity, craniofacial development, and cancer.

Keywords: isthmin ; development ; angiogenesis ; immunity ; cancer

1. Gene Organization

Isthmin (ISM) is a secreted protein that was first detected through an unbiased screening for secreted proteins in Xenopus embryos and initially named Xenopus Isthmin (*xIsm*). The ISM protein family has two members, namely ISM1 (~60 kDa) and ISM2 (~63.9 kDa). Both of these proteins contain a hydrophobic signal peptide at the N-terminus along with a centrally positioned thrombospondin type 1 repeat (TSR1) domain. Sequence analysis has revealed that along with the TSR1 domain, ISM also contains another C-terminus domain called the Adhesin-associated domain in MUC4 and other protein (AMOP) domains [1].

2. Expression and Function

ISM1 is expressed in a large number of human tissues, such as lung, liver, breast, brain, stomach, muscle, skin, bone marrow, and colon. Spatiotemporal analysis indicated that in mouse embryos, *Ism1* transcripts were observed in the anterior mesendoderm (AM), paraxial and lateral plate mesoderm (LPM), mid-brain hindbrain boundary (MHB), and trunk neural tube, while in adult stages, the highest expression level of *Ism1* was found in the lung and the brain and a moderate expression level was observed in the heart, kidney, ovary, testis, and bone marrow [2]. On the other hand, a different expression pattern was observed in chicken embryos, such as that vigorous expression of *Ism1* transcripts was observed in the anterior head mesoderm, optic vesicles, LPM, trunk neural tube, and dermomyotome, whereas a strong expression level was observed in the ear, eye, and spinal cord primordia in later stages [2]. Moreover, another previous study identified *ism1* amongst the top 20 genes which are expressed in the site of anterior primitive streak (APS) in the chicken embryo [3]. Interestingly, asymmetric expression of *Ism1* led to the study on the embryonic role of ISM1 on chicken embryos, whereas defective NODAL signalling in the left LPM as well as impaired asymmetric heart morphogenesis were triggered by ISM1, and thus, it was identified as an antagonist of NODAL signalling which plays role in asymmetric organ morphogenesis [4].

Additionally, ISM1 is expressed since the initial stages of embryonic development in both Xenopus and zebrafish. *Ism1* has strong maternal expression in Xenopus eggs and other expression domains in the embryo, including the blastopore lip, paraxial mesoderm, neural folds, cranial neural crest, and ear placode. While the expression pattern is similar to fgf8, ISM1 was demonstrated as part of the FGF8 synexpression group, including the *Spry* and *Sef* genes [1].

In addition, ISM1 expression was also found in the brain, heart, eye, and spleen tissues of zebrafish. In zebrafish embryonic development, *Ism1* was identified at very early developmental stages, including the tail bud stage, shield stage, and early somitogenesis, while the expression of *Ism2* could not be identified up to 24 h post fertilization (hpf). This is despite the fact that *Ism2* expression was most vigorous in two bilateral streams of the mesenchymal cells in the head region and moderate expression was found in trunk [5]. Furthermore, *Ism1* knockdown in zebrafish embryos gave rise to the aberrant formation of intersegmental vessel (ISV) in the trunk [6]. Previously, upregulation of *Ism1* in the zebrafish was identified in relation to the WNT/β-catenin signalling pathway [7], and in another study, *Ism1* was also identified as a NODAL-controlled gene [8]. Additionally, *Ism1* expression in blastoderm required nodal signalling and its subsequent expression, even though the authors observed a compensated function in embryonic development [5] which is opposite to that observed in chicken embryos. Spatiotemporal expression of *Ism1* and being a target gene of multiple signalling pathways as mentioned above indicates there might be other functions of ISM in multiple biological processes. Over the

past few years, multiple studies have focused on the functional analysis of ISM1 in several events, including angiogenesis, metabolism, organ homeostasis, immunity, craniofacial development, and cancer.

3. Angiogenesis

Angiogenesis is a tightly controlled process of forming new capillary blood vessels from pre-existing ones which generates oxygen and nutrients for cells [9]. Following birth, angiogenesis continues to facilitate organ growth; nevertheless, in adulthood, blood vessels mainly persist quiescently, while angiogenesis only occurs in multiple physiological circumstances, such as wound healing, damaged tissue repair, embryonic development, and organogenesis [10]. A plethora of growth factors, cytokines, and secreted proteins participate in the angiogenesis process [11][12]. In addition, angiogenesis could be inhibited by endogenous inhibitors, which are proteins formed and secreted in the body that may inhibit blood vessel formation.

A previous study has demonstrated ISM1 as a novel inhibitor of angiogenesis [13], whereas recombinant mouse ISM1 (rISM1) was expressed in *E. coli* and used to treat human umbilical vein endothelial cells (ECs) in vitro. Here, it was found that through the AMOP domain, ISM1 can inhibit capillary network formation in the initial stages of angiogenesis via its interaction with αVβ5 integrin without affecting EC migration. Additionally, rISM1 is reported to inhibit vascular endothelial growth factor (VEGF)-mediated angiogenesis, while overexpression of ISM1 suppressed tumour angiogenesis in B16 melanoma cells and tumour growth in mice [13].

Furthermore, integrins are cell-surface-signalling receptors involved in the activation of multiple intra-cellular signalling pathways as well as mediation of adhesion to the ECM and adjacent cells [14]. Additionally, integrins also serve as regulators of multiple processes, including cell growth, proliferation, migration, differentiation, apoptosis, and tissue repair [15]. Integrins are also reported to be instantly inhibited by means of antibodies, peptides, and peptidomimetics [16].

Immobilized ISM1 in the ECM promotes EC survival, while conversely, soluble ISM1 induces EC apoptosis via αvβ5 integrin as an antagonist and indicates that the anti-angiogenic functions of ISM1 are diminished when it is present in immobilized form [6]. Contrary to ISM1, other signalling molecules including vitronectin and fibronectin serving as agonists do not exhibit dual function based on their soluble or insoluble state.

Glioma is a common central nervous system (CNS) tumour that accounts for 30% of all intracranial tumour incidences, and angiogenesis is vital to the formation of malignant gliomas. Furthermore, ECM molecules promote cell signalling via activation of particular cell adhesion receptors, access modulation to soluble factors, and modification of mechanical properties of the tissue (Hynes, 2009). Additionally, an earlier study [17] investigated the role of ISM1 in glioma angiogenesis and found that it can hinder HUVEC proliferation by the inhibition of VEGF. Furthermore, it was also demonstrated that ISM1 activated the HUVEC apoptosis through the caspase-3 pathway, and thus, inhibits tumour angiogenesis in vivo [17].

At present, the role of ISM2 in angiogenesis still remains elusive, while it is not surprising that it also contains the TSR1 domain, which has been observed in multiple anti-angiogenic proteins. For instance, the anti-angiogenic function of ADAMTS1 requires the TSR1 domain [18], while it has anti-angiogenic activity in primary gastric cancer [19]. Furthermore, ADAMTS2 is reported to inhibit the formation of the capillary network in EC 3D culture models [20], whereas ADAMTS4 is also reported to suppress angiogenesis and melanoma growth [21]. Additionally, ADAMTS5 was also reported to inhibit angiogenesis through the TSR1 domain [22]. Moreover, SCO-spondin (SCOP) is also reported to function as an angiogenesis inhibitor in glioblastoma [23], while the UNC5B protein is involved in angiogenesis inhibition [24][25].

4. Pathological Processes

In the studies listed above, ISM1 has been demonstrated as an endogenous angiogenesis inhibitor, and it might be interesting to elucidate whether this protein inhibits cancer to generate its own blood vessels. However, over the past few years, increasing evidence has revealed that the atypical expression of ISM1 may affect cancer.

Long non-coding RNAs (lncRNAs) perform a pivotal role in the progression and metastasis of a variety of carcinomas, while lncRNA H19 (H19) is highly prevalent in gastric cancer tissues [26]. Another study demonstrated that ISM1 is a binding protein of lncRNA H19, which mediates ISM1 upregulation and boosts carcinogenesis and metastasis of gastric cancer (Li et al., 2014).

Furthermore, in hepatocellular carcinoma (HCC), cell proliferation and migration are reported to be promoted by ISM1 and regulated by the interaction with circular RNA (circRNA) and micro RNA (miRNA), namely hsa_circ_0091570/miR-1307

hsa_circ_0091570, which competitively binds to miR-1307 by serving as the ceRNA (competing endogenous RNA) that then regulates ISM1 expression [27].

Additionally, colon adenocarcinoma (COAD) is a malignant tumour of the digestive tract that is associated with an extremely high incidence rate and is ranked third amongst all tumours globally, and miRNA is reported to play a major role in this tumour cell proliferation and apoptosis [28][29]. The Wnt/β-catenin signalling pathway serves as a regulatory pathway in tumorigenesis because of its involvement in multiple cellular processes [30], while Wnt3a is substantially enhanced in colon cancer cells in promoting tumour angiogenesis and metastasis [31]. A recent study [32] has shown that miR-1307–3p inhibits the activation of the Wnt3a/β-catenin signalling pathway by targeting and thus downregulation of ISM1, which then inhibits proliferation and promotes the apoptosis of COAD cells. Conversely, ISM1 overexpression promoted activation of the Wnt3a/β-catenin signalling pathway along with proliferation and decelerated cell apoptosis in COAD cells.

Furthermore, a recent study has also reported the elevated expression of ISM1 in the CRC tissue of patients, and on top of that, a positive correlation of ISM1 with cancer-associated signalling pathways including EMT, hypoxia, KRAS, Notch, and Hedgehog were observed [33].

The inner surface of the micro vessels is lined by endothelial cells to generate a semi-permeable barrier that allows exchange of fluids and proteins in blood and tissue, while tight regulation of this endothelial permeability is crucial in the maintenance of organ homeostasis, and thus, dysfunction including endothelial hyperpermeability leads to vascular inflammation related to multiple diseases, such as respiratory distress, acute lung injury, sepsis, diabetes, and cancer [34][35]. Identification of functional signalling molecules involved in vascular permeability (VP) for circulatory homeostasis might have therapeutic potential. ISM1 is demonstrated as a novel VP inducer which acts via cell-surface GRP78-facilitated activation of Src followed by Src-mediated tyrosine phosphorylation of adherens-junction (AJ) proteins and the consequent dissociation of these AJ proteins, therefore resulting in barrier disruption [36].

On the top of that, hypoxia is one of the most common reasons for vascular hyperpermeability and a crucial risk factor of several pathological characteristics in lung disorders [37]. Hyperpermeability of pulmonary microvascular endothelial cell (PMVEC) monolayers induced by hypoxia is vital for vascular leakage and leads to pulmonary diseases including ALI and high-altitude pulmonary oedema (HAPE). AECII are cuboidal cells that comprise about 15% of the overall lung cells, and they account for epithelium reparation and facilitate lung homeostasis through the secretion of several lysozymes and proteins. The elevated ISM1 level in AECII was accountable for hypoxia triggered PMVEC monolayer hyperpermeability in an AECII/PMVEC co-culture system that indicated substantial function of alveolar epithelial cells and a modulatory role of ISM1 in hyperpermeability featured lung diseases [38]. Hypoxia-inducible factor-1α (HIF1α) is a master transcriptional regulator of hypoxia, while the same study demonstrated that elevated HIF1α expression transcriptionally activated *Ism1* gene expression and thus identified *Ism1* as a novel HIF1α target gene [38].

It is already clear that inflammation control and maintenance of homeostasis are of utmost importance for lung health, whereas prolonged lung inflammation leads to chronic obstructive pulmonary disease (COPD), and the severity of disease in COPD patients is directly linked to the accumulation of alveolar macrophages (AMs). It is important to highlight that, by using genetic (*Ism1*) and pathological (COPD) mouse models, one study reported that the secreted protein ISM1 is lung-resident, having a high expression that safeguards lung homeostasis through controlling AM numbers and an efficient phenotype through cell-surface GRP78 (csGRP78)-facilitated apoptosis [39]. Furthermore, the same study revealed that intratracheal delivery of recombinant ISM1 (rISM1) exhibited effective suppression of lung inflammation through the depletion of the pro-inflammatory cs-GRP78^{high} AMs by targeted apoptosis, as well as prevented emphysema development and thus retained pulmonary function in cigarette-smoke-induced COPD mice. Furthermore, *Ism1* knockout in mice exhibited an increase in cs-GRP78^{high} AMs along with upregulation of MMP9, MMP12, and NF-κB p65, in addition to a moderate increase in TGF-β1 and VEGF-A, prolonged lung inflammation, and progressive emphysema [39].

On the other hand, GRP78 is a stress-response protein that belongs to the heat-shock protein family, which has the ability to modulate protein folding and is upregulated in cells under stress. This protein is known to be highly expressed in various human cancers, including melanoma and breast, prostate, lung, and ovarian cancer, and thus it is associated with chemoresistance, increased malignancy, and inadequate patient outcomes [40][41]. Of note is that ISM1 selectively promotes cellular apoptosis, harbouring elevated cell-surface GRP78 in activated ECs as well as in metastatic and aggressive cancer cells, while systemic delivery of the GRP78-specific cyclic peptide BC71 effectively suppressed the growth of subcutaneous tumours in mice [41].

Moreover, orofacial clefts have a complex aetiology and are one of the most prominent birth defects, affecting 1–2 children per 1000 births. Complex diseases have been reported to be linked with copy number variants (CNVs) and ISM1

heterozygous deletions were shown to be enriched in cleft lip and palate cases compared to the controls, therefore indicating that the loss of even one copy predisposes to this disorder (Lansdon, Darbro, Petrin, Hulstrand, Standley, Brouillette, Long, Mansilla, Cornell, Murray, et al., 2018). SM1 is expressed in the same synexpression group as other clefting genes, including spry1, spry2, and fgf8, and knockdown of ISM1 causes craniofacial dysmorphologies in frogs, including cleft-like phenotypes, hence confirming the role of ISM1 in craniofacial development (Lansdon, Darbro, Petrin, Hulstrand, Standley, Brouillette, Long, Mansilla, Cornell, Murray, et al., 2018).

Additionally, trophoblastic cells in placental tissues collected from patients with gestational hypertension and preeclampsia have a strong expression of ISM1, while reduced serum concentrations of ISM2 were observed in preeclampsia patients and, quite the opposite, ISM2 showed prominent expression in choriocarcinoma, thus suggesting a possible contrast in function [42]. Furthermore, strong expression of ISM2 is observed in choriocarcinoma, while moderate expression in lung and prostate adenocarcinoma is observed and mild expression is indicated in colon adenocarcinoma and cohesive gastric carcinoma [42].

In summary, ISM1 upregulation and overexpression are reported in multiple cancers, including gastric cancer, hepatocellular carcinoma (HCC), colon adenocarcinoma, and colorectal cancer, while ISM2 overexpression was reported in choriocarcinoma. However, multiple signalling molecules and pathways are also reported as being involved in the regulation and synexpression. Even though ISM1 is reported as an endogenous angiogenesis inhibitor, it is quite the opposite that overexpression of ISM1 and ISM2 is found in the above-mentioned cancers, while it could be interesting to explore whether tight regulation over the tissue-specific expression of these proteins might play a role in cancer therapy.

5. Metabolism

Endocrine tissues have enhanced expression of ISM1, including the thyroid, pituitary, and adrenal glands, whereas the thyroid gland is reported to have the highest expression of this protein. Furthermore, as discussed earlier, increased expression of ISM1 is found in adipose tissue, pancreas, spleen, liver, and kidney compared to other organs. Secreted by adipose tissue, adipokines participate in systemic metabolism and tissue homeostasis in a paracrine or endocrine manner, while imbalance in adipokines causes metabolic dysfunction. A recent study [43] reported ISM1 as an adipokine secreted by mouse adipocytes that promotes glucose uptake by a signalling cascade which involves an unidentified receptor kinase such as insulin. Furthermore, recombinant ISM1 led to a vigorous rise in GLUT4-dependent glucose uptake in both murine and human adipocytes as well as primary human muscle cells, whereas *Ism1* knockout in adipocytes exhibited a reduction in glucose uptake and insulin-dependent phosphorylation of protein kinase AKT at the serine residue 473 [43].

Furthermore, complete knockout of *Ism1* in mice exhibited a drop in glucose tolerance as well as a decline in glucose uptake in the brown adipose tissue and muscle, while on the other hand, overexpression of *Ism1* in diet-induced mice led to the reduction in adipose tissue mass along with improved glucose tolerance, insulin sensitivity, and boosted suppression of hepatic glucose production in an insulin clamp [43].

On the other hand, even though mouse adipocytes and hepatocytes exhibited ISM1-dependent signalling cascade associated improvised glucose uptake, astonishingly ISM1 suppressed insulin-dependent de novo lipogenesis (DNL). In addition, it was suggested that ISM1 facilitated hepatic DNL inhibition, which prevented lipid accumulation in an NAFLD mouse model, and thus the authors argued that ISM1 signalling indirectly modulates the hepatic lipid accumulation by inhibiting the fatty acid releases from the adipose tissue [43].

Furthermore, a study conducted on Spanish pubertal boys and girls revealed higher ISM1 levels in obese pubertal boys as compared to normal weight and overweight boys, while there were no significant changes observed in pubertal girls, thus indicating a plausible function of ISM1 in male restricted obesity [44].

However, a cohort study conducted on Canadian young adults with childhood obesity history revealed a positive correlation of obesity with risks of T2DM and coronary artery disorders [45]. In contrast and as previously mentioned, placental tissues from patients with gestational hypertension and preeclampsia are associated with a strong expression of ISM1 [42]. However, a cohort study conducted on women with gestational diabetes mellitus (GDM) demonstrated that women who developed preeclampsia did not show more insulin resistance as compared to non-preeclamptic women [46]. However, ISM1 function in glucose homeostasis is enigmatic, no receptor has been identified so far for this adipokine in functional studies, and there is also an absence of studies reporting on ISM2 and its role in metabolism and any other related processes.

6. Immune System

Haematopoiesis is the formation of blood cells in the marrow that involves the replication as well as specialization of hematopoietic stem cells (HSCs) towards the downstream progenitor cells [47]. Multiple classes of secreted proteins, among them the colony stimulating factors (CSFs) or haematopoiesis, are known to function as important regulators during hematopoietic differentiation. Furthermore, these secreted molecules are of clinical significance because of their application in stimulating haematopoiesis in patients with neutropenia and several other haematological diseases [48]. Consequently, identifying the function of novel secreted proteins that show specific spatiotemporal expression patterns within the hematopoietic cells is of considerable interest. ISM1 is reported as required for the normal formation of HSPCs towards their downstream progeny during haematopoiesis in zebrafish because *Ism1* knockout resulted in the drop in frequency of mature blood cell populations, including neutrophil, macrophage, and erythrocyte, while elevated expression of *Ism1* was reported in stromal cell lines [49]. However, the study was conducted on a zebrafish morpholino-based model, and it remains unclear how ISM1 interacts with HSPCs and the underlying mechanism requiring ISM1 during haematopoiesis in zebrafish [49].

Moreover, ISM1 was found to be produced in human and mice barrier tissues, such as mucosa, skin, and some lung lymphocytes, which might be linked to the NK, NKT, and Th17 cell lineages. In addition, the ISM1 expression increases significantly in CD4+ T cells when the cells are polarized to the Th17 lineage in vitro, indicating that ISM1 is a mediator of lymphocyte effector functions and might be involved in both innate and adaptive immune responses [50].

On top of that, a recent study found a role of ISM1 in immunity against viral infections, as its expression was induced by Grass carp reovirus (GCRV) both in vitro and in vivo, while they further demonstrated that rISM1 reduced the cytopathic effects in GRCV-infected cells and promoted the *Ifn* gene and IFN-inducible antiviral protein *Mxa* gene [51]. Additionally, the same study proposed that ISM1 may first induce the activation of the Tbk1–Irf3–Ifn antiviral signalling pathway, which then leads to enhanced expression of *Ifn* and *Mxa*, both of which in turn suppress viral replication [51].

Additionally, as discussed earlier, a study demonstrating ISM1 upregulation in CRC patients also revealed the associations between suppressive immune cells (M2 macrophages, T-regs, and T cell exhaustion), and ISM1 overexpression detected in PD1-resistant patients further indicated that ISM1 upregulation can possibly play a crucial role in the formation of an inhibitory immune microenvironment [33].

References

1. Pera, E.M.; Kim, J.I.; Martinez, S.L.; Brechner, M.; Li, S.-Y.; Wessely, O.; De Robertis, E. Isthmin is a novel secreted pr otein expressed as part of the Fgf-8 synexpression group in the Xenopus midbrain–hindbrain organizer. Mech. Dev. 20 02, 116, 169–172.
2. Osório, L.; Wu, X.; Zhou, Z. Distinct spatiotemporal expression of ISM1 during mouse and chick development. Cell Cycl e 2014, 13, 1571–1582.
3. Alev, C.; Wu, Y.; Kasukawa, T.; Jakt, L.M.; Ueda, H.; Sheng, G. Transcriptomic landscape of the primitive streak. Devel opment 2010, 137, 2863–2874.
4. Osório, L.; Wu, X.; Wang, L.; Jiang, Z.; Neideck, C.; Sheng, G.; Zhou, Z. ISM1 regulates NODAL signaling and asymm etric organ morphogenesis during development. J. Cell Biol. 2019, 218, 2388–2402.
5. Kesavan, G.; Raible, F.; Gupta, M.; Machate, A.; Yilmaz, D.; Brand, M. Isthmin1, a secreted signaling protein, acts dow nstream of diverse embryonic patterning centers in development. Cell Tissue Res. 2020, 383, 987–1002.
6. Zhang, Y.; Chen, M.; Venugopal, S.; Zhou, Y.; Xiang, W.; Li, Y.-H.; Lin, Q.; Kini, M.; Chong, Y.S.; Ge, R. Isthmin exerts p ro-survival and death-promoting effect on endothelial cells through alphavbeta5 integrin depending on its physical stat e. Cell Death Dis. 2011, 2, e153.
7. Weidinger, G.; Thorpe, C.J.; Wuennenberg-Stapleton, K.; Ngai, J.; Moon, R. The Sp1-Related Transcription Factors sp 5 and sp5-like Act Downstream of Wnt/β-Catenin Signaling in Mesoderm and Neuroectoderm Patterning. Curr. Biol. 20 05, 15, 489–500.
8. Bennett, J.; Joubin, K.; Cheng, S.; Aanstad, P.; Herwig, R.; Clark, M.; Lehrach, H.; Schier, A.F. Nodal signaling activates differentiation genes during zebrafish gastrulation. Dev. Biol. 2007, 304, 525–540.
9. Moon, J.J.; West, J.L. Vascularization of engineered tissues: Approaches to promote angio-genesis in biomaterials. Cur r. Top Med. Chem. 2008, 8, 300–310.

10. Reynolds, L.P.; Grazul-Bilska, A.T.; Redmer, D.A. Angiogenesis in the female reproductive organs: Pathological implications. Int. J. Exp. Pathol. 2002, 83, 151–164.
11. Folkman, J.; Shing, Y. Angiogenesis. J. Biol. Chem. 1992, 267, 10931–10934.
12. Huang, S.-D.B.Z. Roles of main pro- and anti-angiogenic factors in tumor angiogenesis. World J. Gastroenterol. 2004, 10, 463–470.
13. Xiang, W.; Ke, Z.; Zhang, Y.; Cheng, G.H.-Y.; Irwan, I.D.; Sulochana, K.N.; Potturi, P.; Wang, Z.; Yang, H.; Wang, J.; et al. Isthmin is a novel secreted angiogenesis inhibitor that inhibits tumour growth in mice. J. Cell. Mol. Med. 2011, 15, 359–374.
14. Van Der Flier, A.; Sonnenberg, A. Function and interactions of integrins. Cell Tissue Res. 2001, 305, 285–298.
15. Mezu-Ndubuisi, O.J.; Maheshwari, A. The role of integrins in inflammation and angiogenesis. Pediatr. Res. 2020, 89, 1619–1626.
16. Hynes, R.O. A reevaluation of integrins as regulators of angiogenesis. Nat. Med. 2002, 8, 918–921.
17. Yuan, B.; Xian, R.; Ma, J.; Chen, Y.; Lin, C.; Song, Y. Isthmin inhibits glioma growth through antiangiogenesis in vivo. J. Neuro-Oncol. 2012, 109, 245–252.
18. Liu, Y.-J.; Xu, Y.; Yu, Q. Full-length ADAMTS-1 and the ADAMTS-1 fragments display pro- and antimetastatic activity, respectively. Oncogene 2005, 25, 2452–2467.
19. Chen, J.; Zhi, Y.; Chang, X.; Zhang, S.; Dai, D. Expression of ADAMTS1 and Its Correlation with Angiogenesis in Primary Gastric Cancer and Lymph Node Metastasis. Am. J. Dig. Dis. 2012, 58, 405–413.
20. Dubail, J.; Kesteloot, F.; Deroanne, C.; Motte, P.; Lambert, V.; Rakic, J.-M.; Lapière, C.; Nusgens, B.; Colige, A. ADAMTS-2 functions as anti-angiogenic and anti-tumoral molecule independently of its catalytic activity. Cell. Mol. Life Sci. 2010, 67, 4213–4232.
21. Rao, N.; Ke, Z.; Liu, H.; Ho, C.-J.; Kumar, S.; Xiang, W.; Zhu, Y.; Ge, R. ADAMTS4 and its proteolytic fragments differentially affect melanoma growth and angiogenesis in mice. Int. J. Cancer 2013, 133, 294–306.
22. Sharghi-Namini, S.; Fan, H.; Sulochana, K.; Potturi, P.; Xiang, W.; Chong, Y.-S.; Wang, Z.; Yang, H.; Ge, R. The first but not the second thrombospondin type 1 repeat of ADAMTS5 functions as an angiogenesis inhibitor. Biochem. Biophys. Res. Commun. 2008, 371, 215–219.
23. Bibes, R.; Gobron, S.; Vincent, F.; Mélin, C.; Vedrenne, N.; Perraud, A.; Labrousse, F.; Jauberteau, M.-O.; Lalloué, F. SCO-spondin oligopeptide inhibits angiogenesis in glioblastoma. Oncotarget 2017, 8, 85969–85983.
24. Larrivée, B.; Freitas, C.; Trombe, M.; Lv, X.; DeLafarge, B.; Yuan, L.; Bouvrée, K.; Bréant, C.; Del Toro, R.; Bréchot, N.; et al. Activation of the UNC5B receptor by Netrin-1 inhibits sprouting angiogenesis. Genes Dev. 2007, 21, 2433–2447.
25. Lejmi, E.; Leconte, L.; Pédron-Mazoyer, S.; Ropert, S.; Raoul, W.; Lavalette, S.; Bouras, I.; Feron, J.-G.; Maitre-Boube, M.; Assayag, F.; et al. Netrin-4 inhibits angiogenesis via binding to neogenin and recruitment of Unc5B. Proc. Natl. Acad. Sci. USA 2008, 105, 12491–12496.
26. Gan, L.; Lv, L.; Liao, S. Long non-coding RNA H19 regulates cell growth and metastasis via the miR-22-3p/Snail1 axis in gastric cancer. Int. J. Oncol. 2019, 54, 2157–2168.
27. Wang, Y.-G.; Wang, T.; Ding, M.; Xiang, S.-H.; Shi, M.; Zhai, B. hsa_circ_0091570 acts as a ceRNA to suppress hepatocellular cancer progression by sponging hsa-miR-1307. Cancer Lett. 2019, 460, 128–138.
28. Koo, K.H.; Kwon, H. MicroRNA miR-4779 suppresses tumor growth by inducing apoptosis and cell cycle arrest through direct targeting of PAK2 and CCND3. Cell Death Dis. 2018, 9, 77.
29. Jovanovic, M.; O Hengartner, M. miRNAs and apoptosis: RNAs to die for. Oncogene 2006, 25, 6176–6187.
30. Wang, Z.; Zhao, T.; Zhang, S.; Wang, J.; Chen, Y.; Zhao, H.; Yang, Y.; Shi, S.; Chen, Q.; Liu, K. The Wnt signaling pathway in tumorigenesis, pharmacological targets, and drug development for cancer therapy. Biomark. Res. 2021, 9, 1–16.
31. Qi, L.; Sun, B.; Liu, Z.; Cheng, R.; Li, Y.; Zhao, X. Wnt3a expression is associated with epithelial-mesenchymal transition and promotes colon cancer progression. J. Exp. Clin. Cancer Res. 2014, 33, 107.
32. Zheng, Y.; Zheng, Y.; Lei, W.; Xiang, L.; Chen, M. miR-1307–3p overexpression inhibits cell proliferation and promotes cell apoptosis by targeting ISM1 in colon cancer. Mol. Cell. Probes 2019, 48, 101445.
33. Wu, Y.; Liang, X.; Ni, J.; Zhao, R.; Shao, S.; Lu, S.; Han, W.; Yu, L. Effect of ISM1 on the Immune Microenvironment and Epithelial-Mesenchymal Transition in Colorectal Cancer. Front. Cell Dev. Biol. 2021, 9, 681240.
34. Kumar, P.; Shen, Q.; Pivetti, C.D.; Lee, E.S.; Wu, M.H.; Yuan, S.Y. Molecular mechanisms of endothelial hyperpermeability: Implications in inflammation. Expert Rev. Mol. Med. 2009, 11, e19.

35. Skaria, T.; Burgener, J.; Bachli, E.; Schoedon, G. IL-4 Causes Hyperpermeability of Vascular Endothelial Cells through Wnt5A Signaling. PLOS ONE 2016, 11, e0156002.
36. Venugopal, S.; Chen, M.; Liao, W.; Er, S.Y.; Wong, W.-S.F.; Ge, R. Isthmin is a novel vascular permeability inducer that functions through cell-surface GRP78-mediated Src activation. Cardiovasc. Res. 2015, 107, 131–142.
37. Gavrilovskaya, I.N.; Gorbunova, E.E.; Mackow, E.R.; Kim, E.T.; White, T.E.; Brandariz-Núñez, A.; Diaz-Griffero, F.; Weitzman, M.D. Hypoxia Induces Permeability and Giant Cell Responses of Andes Virus-Infected Pulmonary Endothelial Cells by Activating the mTOR-S6K Signaling Pathway. J. Virol. 2013, 87, 12999–13008.
38. Li, J.; Xia, Y.; Huang, Z.; Zhao, Y.; Xiong, R.; Li, X.; Huang, Q.; Shan, F. Novel HIF-1-target gene isthmin1 contributes to hypoxia-induced hyperpermeability of pulmonary microvascular endothelial cells monolayers. Am. J. Physiol. Physiol. 2021, 321, C671–C680.
39. Lam, T.Y.W.; Nguyen, N.; Peh, H.Y.; Shanmugasundaram, M.; Chandna, R.; Tee, J.H.; Ong, C.B.; Hossain, Z.; Venugopal, S.; Zhang, T.; et al. ISM1 protects lung homeostasis via cell-surface GRP78-mediated alveolar macrophage apoptosis. Proc. Natl. Acad. Sci. USA 2022, 119, e2019161119.
40. Li, J.; Ni, M.; Lee, B.; Barron, E.; Hinton, D.R.; Lee, A. The unfolded protein response regulator GRP78/BiP is required for endoplasmic reticulum integrity and stress-induced autophagy in mammalian cells. Cell Death Differ. 2008, 15, 1460–1471.
41. Kao, C.; Chandna, R.; Ghode, A.; Dsouza, C.; Chen, M.; Larsson, A.; Lim, S.H.; Wang, M.; Cao, Z.; Zhu, Y.; et al. Proapoptotic Cyclic Peptide BC71 Targets Cell-Surface GRP78 and Functions as an Anticancer Therapeutic in Mice. eBioMedicine 2018, 33, 22–32.
42. Martinez, C.; González-Ramírez, J.; Marín, M.E.; Martínez-Coronilla, G.; Meza-Reyna, V.I.; Mora, R.; Díaz-Molina, R. Isthmin 2 is decreased in preeclampsia and highly expressed in choriocarcinoma. Heliyon 2020, 6, 05096.
43. Jiang, Z.; Zhao, M.; Voilquin, L.; Jung, Y.; Aikio, M.A.; Sahai, T.; Dou, F.Y.; Roche, A.M.; Carcamo-Orive, I.; Knowles, J.W.; et al. Isthmin-1 is an adipokine that promotes glucose uptake and improves glucose tolerance and hepatic steatosis. Cell Metab. 2021, 33, 1836–1852.e11.
44. Ruiz-Ojeda, F.J.; Anguita-Ruiz, A.; Rico, M.C.; Leis, R.; Bueno, G.; Gil-Campos, M.; Gil, A. Serum levels of the novel adipokine isthmin-1 are associated with obesity in pubertal boys. medRxiv 2022.
45. Fang, X.; Zuo, J.; Zhou, J.; Cai, J.; Chen, C.; Xiang, E.; Li, H.; Cheng, X.; Chen, P. Childhood obesity leads to adult type 2 diabetes and coronary artery diseases. Medicine 2019, 98, e16825.
46. Montoro, M.N.; Kjos, S.L.; Chandler, M.; Peters, R.K.; Xiang, A.H.; Buchanan, T.A. Insulin Resistance and Preeclampsia in Gestational Diabetes Mellitus. Diabetes Care 2005, 28, 1995–2000.
47. Antoniani, C.; Romano, O.; Miccio, A. Concise Review: Epigenetic Regulation of Hematopoiesis: Biological Insights and Therapeutic Applications. Stem Cells Transl. Med. 2017, 6, 2106–2114.
48. Nalbant, D.; Youn, H.; Nalbant, S.I.; Sharma, S.; Cobos, E.; Beale, E.G.; Du, Y.; Williams, S.C. FAM20: An evolutionarily conserved family of secreted proteins expressed in hematopoietic cells. BMC Genom. 2005, 6, 11.
49. Berrun, A.; Harris, E.; Stachura, D.L. Isthmin 1 (ism1) is required for normal hematopoiesis in developing zebrafish. PLOS ONE 2018, 13, e0196872.
50. Valle-Rios, R.; Maravillas-Montero, J.L.; Burkhardt, A.M.; Martinez, C.; Buhren, B.A.; Homey, B.; Gerber, P.A.; Robinson, O.; Hevezi, P.; Zlotnik, A. Isthmin 1 Is a Secreted Protein Expressed in Skin, Mucosal Tissues, and NK, NKT, and Th17 Cells. J. Interferon Cytokine Res. 2014, 34, 795–801.
51. Li, C.; Zhong, S.; Ni, S.; Liu, Z.; Zhang, S.; Ji, G. Zebrafish Ism1 is a novel antiviral factor that positively regulates antiviral immune responses. Dev. Comp. Immunol. 2021, 125, 104210.