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Diverse molecular functions of aspartate β-hydroxylase in cancer (Review)

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Abstract. Aspartate/asparagine β-hydroxylase (AspH) is a type II transmembrane protein that catalyzes the post-translational hydroxylation of definite aspartyl and asparaginyl residues in epidermal growth factor-like domains of substrates. In the last few decades, accumulating evidence has indicated that AspH expression is upregulated in numerous types of human malignant cancer and is associated with poor survival and prognosis. The AspH protein aggregates on the surface of tumor cells, which contributes to inducing tumor cell migration, infiltration and metastasis. However, small-molecule inhibitors targeting hydroxylase activity can markedly block these processes, both *in vitro* and *in vivo*. Immunization of tumor-bearing mice with a phage vaccine fused with the AspH protein can substantially delay tumor growth and progression. Additionally, AspH antigen-specific $CD4^+$ and $CD8^+$ T cells were identified in the spleen of tumor-bearing mice. Therefore, these agents may be used as novel strategies for cancer treatment. The present review summarizes the current progress on the underlying mechanisms of AspH expression in cancer development.

Contents

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1. Introduction

Human aspartate/asparagine β-hydroxylase (AspH) is a highly conserved enzyme that is widely expressed in proliferating placenta trophoblastic cells and is almost undetectable in normal adult tissues (1). AspH is an ~86 kDa type II transmembrane protein located on the luminal side of the endoplasmic reticulum (ER) that hydroxylates β-carbons of specific aspartyl and asparaginyl residues in consensus sequences of epidermal growth factor-like domains (EGFDs) of target proteins in the presence of ferrous iron (2-6). In contrast to the canonical EGFD disulfide pattern, AspH catalyzes noncanonical EGFD substrates (Cys 1-2, 3-4, 5-6) (7). AspH, which is located at position q12.1 of human chromosome 8, is a member of the α-ketoglutarate (also known as 2-oxoglutarate, 2-OG)-dependent dioxygenase family of prolyl and lysyl hydroxylases, which serve a vital role in collagen biosynthesis (8-10). Via alternative splicing and exon sharing, the gene encodes four functionally distinct proteins: AspH, humbug, junctin and junctate (3,10). Humbug serves a role in calcium homeostasis and belongs to the N-terminal fragment that completely lacks the catalytic activity of AspH (3,11). In contrast, the COOH-terminal region of AspH contains the hydroxylase catalytic domain, which includes dibasic glycine and His2 motifs that are essential for catalytic activity (3). The 26-kDa calsequestrin binding protein junctin and transcript junctate are involved in regulating intracellular transient calcium release from the sarcoplasmic reticulum in cardiac and skeletal muscle (3,10,12,13).

Mutations in the AspH gene can have consequences in lens instability (14). Traboulsi syndrome is an extremely rare ophthalmological disorder that is caused by homozygous variants in the AspH gene, wherein facial dysmorphism, lens dislocation, anterior segment abnormalities, and spontaneous filtering blebs are observed (15-17). Loss of murine hydroxylase activity is associated with increased intestinal tumor incidence and developmental defects similar to those caused by altered Notch signaling (18). In comparison with villous cytotrophoblasts (CTB), extravillous CTB demonstrated stronger AspH immunoreactivity, which led to the clinical condition of impaired embryo implantation (19), suggesting that AspH may serve a role in cell migration and invasion. Additionally, AspH expression is upregulated in breast carcinoma, hepatic carcinoma, cervical cancer and ovarian cancer (20). The AspH

protein is transferred from the endoplasmic reticulum (ER) membrane to the cell surface, which contributes to enhancing cell migration (21,22). Furthermore, the malignant phenotypes of hepatocellular carcinoma were significantly reversed using a selective small-molecule inhibitor (SMI), MO-I-1100, of AspH targeting β-hydroxylase activity (23). These observations suggest that AspH may become a potential biomarker for cancer diagnosis and prognosis.

2. Molecular functions of AspH in cancer

AspH promotes cancer development and metastasis by activating the Notch signaling pathway. The 2-OG-dependent dioxygenase AspH hydroxylates aspartate and asparagine residues in certain EGFDs of its substrates, in particular Notch homologues or Notch ligand homologues (4,6,18). The Notch signaling cascade is a highly conserved pathway that affects cell differentiation, proliferation and apoptosis by mediating cell-cell communication, which is essential for human growth and development (24,25). Mammals have four Notch receptors (Notch1-4) and two ligands [Delta-like and Jagged (JAG)] (26). Both the Notch ligands and the extracellular domain (ECD) of Notch receptors contain tandem EGF-like repeats (27-29). Under the condition of β-hydroxylase activity, AspH binding ligands and receptors in a ligand-dependent manner enhances the stability and interaction between Notch receptors and ligands, leading to conformational changes in Notch (26,30,31). This process makes Notch more sensitive to continuous cleavage by a disintegrin and metalloproteinase (ADAM; S2 cleavage) and by the multiprotein γ-secretase complex (S3 cleavage) (26). On the other hand, AspH promotes the cleavage of the γ-secretase complex by directly interacting with ADAM10/17, releasing the Notch intracellular domain, which enters the nucleus and recruits coactivator proteins from the mastermind-like 1 (MAML1) family, forming a Notch transcription activation complex with recombination signal binding protein Jκ (RBPJ), also known as CSL [CBF1-Su(H)-LAG1] (26,29). Subsequently, downstream Notch-responsive genes are activated, including hairy and enhancer of split-1 (HES1), hairy-related transcription factor-1 (HEY1), CD44, epithelial cell adhesion molecule, c-Myc, MMP2/9, cyclin D3 and proliferating cell nuclear antigen (Fig. 1) (29,32). It has been demonstrated that the Notch signaling pathway serves a role in regulating exosomes, which are transferred from mesenchymal cells to tumors to promote metastasis (33,34). The activation of the AspH-Notch axis induces MMP/ADAM-mediated exosomal synthesis and release, and the latter markedly enhances breast cancer cell extracellular matrix (ECM) degradation/remodeling, infiltration and metastasis (both *in vitro* and *in vivo*) (32). In addition, the structural and functional abnormalities of tumor blood vessels, combined with diffusion deterioration, lead to decreased oxygen levels in regions within solid tumors and induce the expression of stress response proteins, such as hypoxia-inducible factor-1α (HIF-1α) (35). Chen *et al* (36) demonstrated that HIF-1α activates Notch signaling by synergizing with the Notch coactivator MAML1 and subsequently increases both HES1 and HEY1 expression levels under hypoxia. In addition, as the upstream target gene of AspH, HIF-1α enters the nucleus and controls AspH expression at the transcriptional level (37). Upregulated AspH expression stimulates the translocation of Notch to the nucleus by binding to Notch ligands and receptors, consequently governing downstream target genes that mediate cell adhesion, including E-cadherin and tenascin C (30,36-38). This novel molecular mechanism for HIF-1α-AspH-Notch signaling may serve an important role in cancer invasion and metastasis (Fig. 1).

AspH function in MAPK and PI3K signaling pathways. Several studies have indicated that the MAPK and PI3K signaling pathways are the most general events in various types of human cancer (39,40). The abnormal activation of these proteins affects numerous biological processes, including cell proliferation, differentiation, growth, survival, motility and metabolism (39-41). It has been demonstrated that insulin and insulin-like growth factor (IGF-1) stimulate the intrinsic tyrosine kinase activity of the IGF-1 receptor, subsequently activating the PI3K and MAPK signaling pathways and causing the expression of downstream target substrates, including AKT and ERK (42-44). de la Monte *et al* (45) reported that insulin and IGF-1 induce the phosphorylation and activation of the PI3K and MAPK cascades, which stimulate AspH expression and enhance cell motility in hepatocellular carcinoma. Furthermore, GSK3β, which is downstream of both the PI3K and MAPK signaling pathways, is phosphorylated (inhibition) at Ser9 by its upstream kinases AKT and p38 (46). However, high levels of AspH lead to decreased GSK3β phosphorylation, which delays tumor cell senescence and promotes tumor progression by interfering with the communication between GSK3β and upstream kinases (Fig. 2A) (47).

AspH may be used as a novel immunotherapy target. Compared with surgery, radiation and chemotherapy, immunotherapy has provided important benefits to patients with melanoma (48). The purpose of cancer immunotherapy is to promote tumor-specific T-cell responses. In the presence of major histocompatibility and CD28 co-stimulation, the T-cell receptor interacts with antigens to activate T cells, which migrate to tumors, upregulate the expression levels of immune checkpoints, such as cytotoxic T lymphocyte antigen 4 (CTLA-4) and programmed cell death 1, and produce cytokines such as IFN-γ, which leads to the expression of programmed cell death ligand 1 (PD-L1) on tumor cells (48). CTLA-4 and PD-L1 are negative regulators that inhibit T-cell activation and induce tumor cell immune escape (49,50). Therefore, numerous efforts have been devoted to the development of inhibitors targeting immune checkpoints, including ipilimumab and nivolumab; these antibodies promote antitumor $CD8^+$ cytotoxic T lymphocyte (CTL) responses in patients with melanoma (51). In addition, $CD4^+$ T cells promote both the effector and the memory functions of CTLs and enhance their antitumor responses (51). The AspH protein is exposed to the extracellular environment of tumor cells and can be recognized and attacked by the host immune system (52). AspH contains both HLA class I- and class II-limited epitopes, which stimulate AspH antigen-specific $CD4^+$ and $CD8^+$ T-cell responses in human and animal models to elicit antitumor effects (Fig. 3) (52). λ phage nanoparticles expressing human AspH-derived proteins and AspH protein-loaded dendritic cells (DCs) migrate from

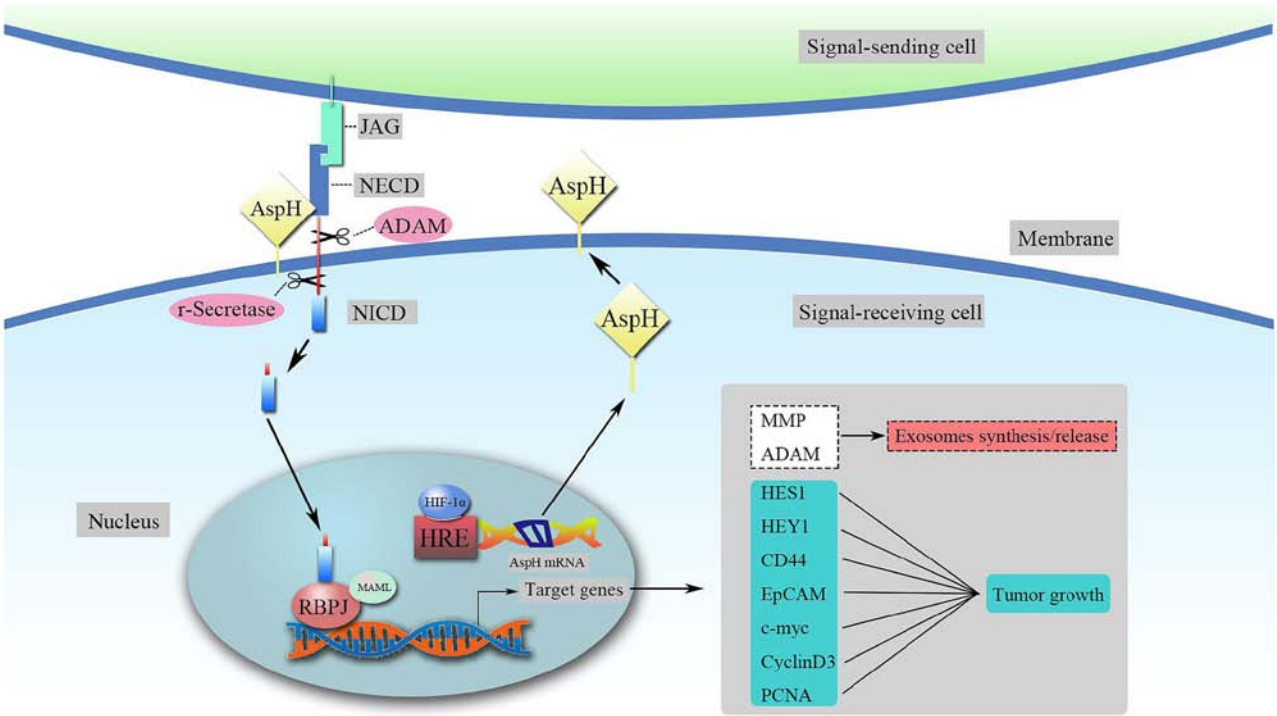

Figure 1. Upregulated AspH activates the Notch signaling pathway. HIF-1α controls AspH expression at the transcriptional level by interacting with the HRE. AspH upregulation promotes the release of the NICD from the Notch receptor. NICD enters the nucleus and forms a Notch transcription activation complex with RBPJ and MAML. Subsequently, the downstream Notch-responsive genes are activated. AspH, aspartate β-hydroxylase; NICD, Notch intracellular domain; NECD, Notch extracellular domain; HRE, hypoxia response element; HIF-1α, hypoxia-inducible factor 1α; MAML, mastermind-like; ADAM, a disintegrin and metalloproteinase; HES1, hairy and enhancer of split-1; HEY1, hairy-related transcription factor-1; EpCAM, epithelial cell adhesion molecule; PCNA, proliferating cell nuclear antigen; JAG, Jagged; RBJP, recombination signal binding protein Jκ; ADAM, a disintegrin and metalloproteinase.

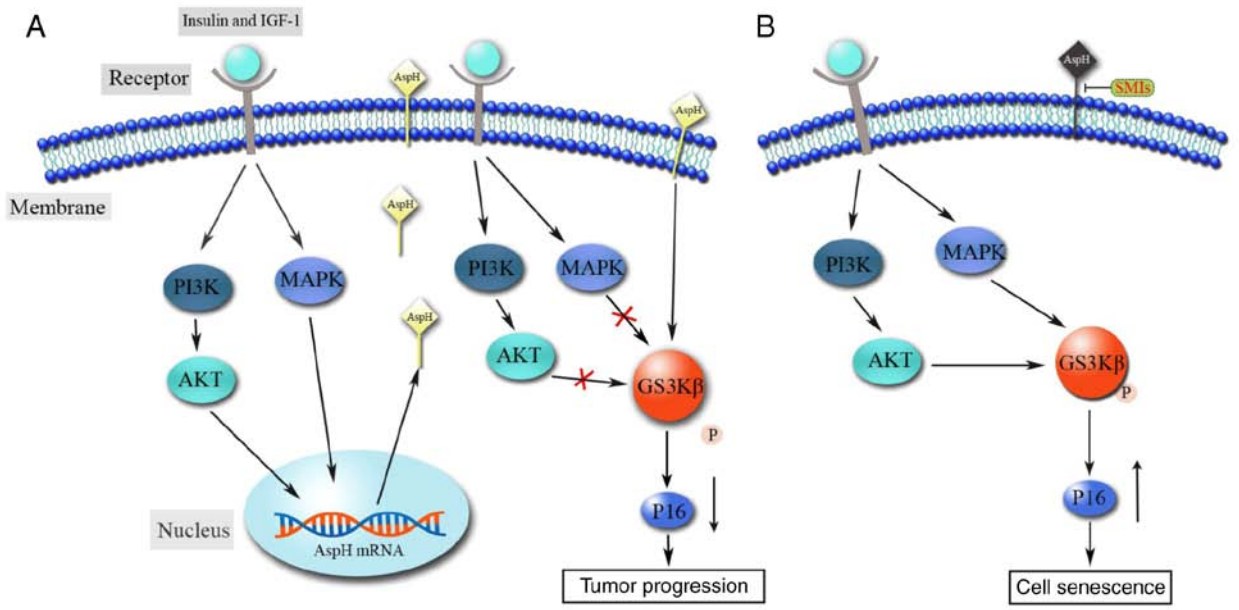

Figure 2. Molecular interpretation of AspH in MAPK and PI3K signaling pathways. (A) Insulin- and IGF-1-stimulated AspH expression is mediated by signals transmitted through MAPK and PI3K. The AspH protein in turn inhibits the phosphorylation of downstream GSK3β, which contributes to tumor progression. (B) Inhibitory effect of AspH on GSK3β phosphorylation can be reversed using SMIs targeting AspH hydroxylase activity. AspH, aspartate β-hydroxylase; SMI, small-molecule inhibitor; IGF-1, insulin-like growth factor 1.

the blood to lymph nodes to activate antigen specific $CD4^+$ and $CD8^+$ T cells; subsequently, T helper (Th)1 and Th2 immune responses are induced to promote lymphocytic infiltration and widespread necrosis in tumors (52,53).

3. AspH expression in various types of cancer

AspH in hepatocellular carcinoma (HCC). HCC is the primary hepatic malignancy, with the highest incidence (~75%) among

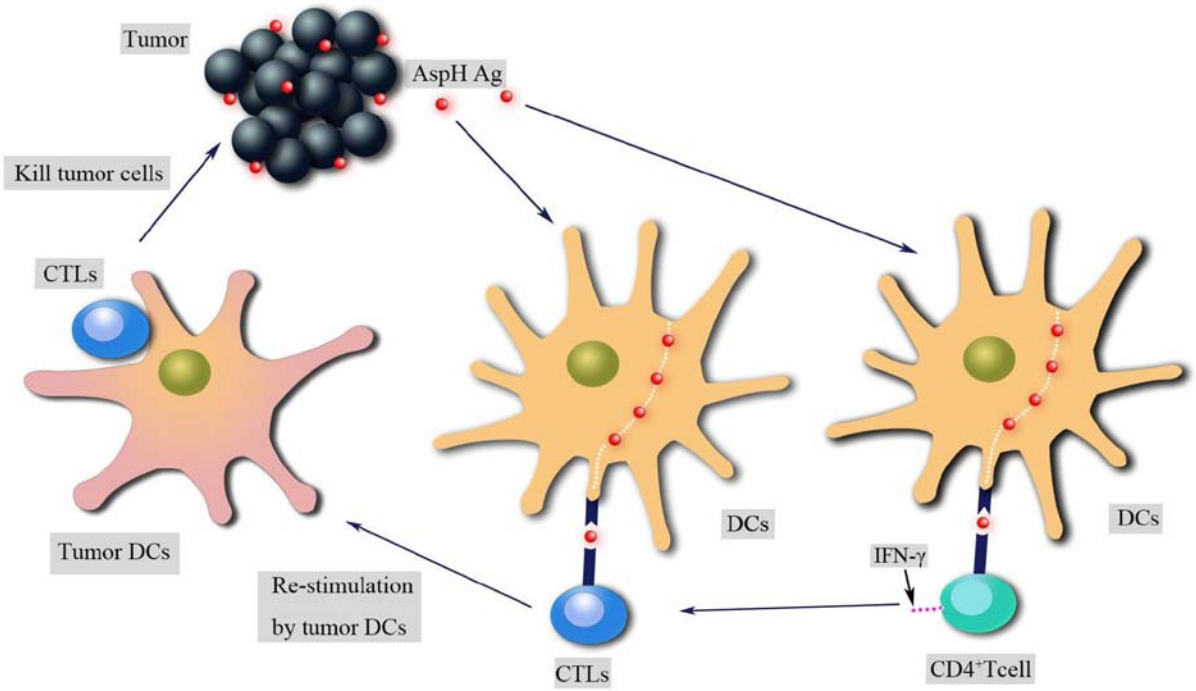

Figure 3. Functions of AspH in the immune system. AspH-specific antigens are taken up by antigen-presenting cells (such as DCs) and migrate from the tumor to draining lymph nodes. During this period, AspH antigens are processed into small peptides, which are then presented on the surface of DCs to stimulate specific $CD4^+$ T-cell and CTL responses. In addition, $CD4^+$ T cells can stimulate CTL activation by secreting IFN-γ. Subsequently, the DCs residing in the tumor restimulate the antigen-specific CTLs, which recognize and kill the tumor cells carrying the antigen. AspH, aspartate β-hydroxylase; Ag, antigen; DC, dendritic cell; CTL, cytotoxic lymphocyte.

liver cancer worldwide in 2019 (54). Although therapeutic efforts have improved over the last few decades, the mortality rate of HCC has increased by 2.8 and 3.4% per year in men and women, respectively (55). Therefore, there is an urgent need for new treatment methods and a deeper understanding of HCC. The relevance of AspH modification in HCC has been extensively studied, and several studies have revealed that AspH is highly expressed in HCC and is associated with cell proliferation, invasion and malignant transformation (1,11,31,56-58). AspH binding to GSK3β inhibits its phosphorylation and inactivation, and blocks the interactions with the upstream kinases AKT and p38 (47). Inhibition of AspH enzymatic activity promotes HCC cell senescence and therefore delays tumor progression by increasing the phosphorylation of GSK3β and p16 expression (Fig. 2B) (47). Additionally, a previous study has revealed that AspH promotes cell proliferation by upregulating cyclin D1 and c-Myc expression (59). MicroRNA (miR)-200a, an upstream target gene of AspH that is rarely detected in liver tumor tissues and cell lines, suppresses cyclin D1 and c-Myc expression by downregulating AspH expression (59). Another study has revealed that AspH expression can be upregulated by insulin and IGF-1 in HCC (45). Insulin and IGF-1 stimulated AspH expression by increasing the phosphorylation of MAPK, ERK and AKT to enhance cell motility and invasiveness (45). Malignant phenotypes, such as tumor cell proliferation, migration, invasion and metastasis of HCC, are partially due to the activation of insulin and IGF-1, which increases AspH expression and subsequently activates the Notch signaling cascade (23,30,31). In addition, in HCC cells treated with an SMI (MO-I-1100) of β-hydroxylase, the activation of Notch signaling was inhibited, and the abilities of cell migration, invasion and metastasis were decreased compared with in untreated counterparts (23). Decreased copy number and dysfunction of mitochondrial DNA (mtDNA) are associated with the malignant phenotypes of HCC (60). AspH upregulation can destroy the integrity of mtDNA by blocking histone H2A member X-mitochondrial transcription factor A signaling, resulting in abnormal mitochondrial membrane potential, decreased ATP generation and increased reactive oxygen species; however, these effects can be reversed using small interfering RNAs against AspH (60). AspH is distributed on the surface of tumor cells, which makes it a target for immunotherapy. AspH-loaded DCs inoculated into HCC tumor-bearing mice can significantly suppress tumor growth, prolong survival and delay recurrence following surgical resection (52). Furthermore, both in healthy donors and patients with HCC, compared with α-fetoprotein-loaded DCs, AspH-loaded DCs can stimulate the activation of antigen-specific $CD4^+$ T cells and $CD8^+$ CTLs, which are important to initiate anti-tumor immune responses (61-63).

AspH in cholangiocarcinoma (CC). CC accounted for 10-25% of primary liver tumors globally in 2011, with a poor prognosis due to a lack of early diagnosis and effective treatment (64,65). It has been demonstrated that AspH is highly expressed in CC, while AspH upregulation is not observed in normal tissues, non-neoplastic epithelial cells and stromal cells (1). Clinicopathologically, AspH upregulation promotes CC invasion, metastasis and poor prognosis (66). Northern blotting suggests that AspH expression is upregulated in CC to promote intrahepatic spread and metastasis, since the AspH protein enhances the sarcomatous change and epithelial-mesenchymal

transition (EMT) of CC (67). Additionally, the activation of the Notch signaling pathway was detected in CC; furthermore, enhanced Notch signaling and upregulation of downstream target genes (such as HEY1 and HES1) were observed when wild-type (wt)-AspH was transfected into HEK293 cells (68). As a cycle regulatory protein, cyclin D1 upregulation is closely associated with the progression and prognosis of CC (69). Knocking down AspH significantly downregulated cyclin D1 expression; however, overexpression of Notch partially rescued cyclin D1 levels, suggesting that AspH promotes CC cell proliferation through Notch-mediated cyclin D1 expression (68). In addition, in *in vitro* experiments, AspH-loaded DCs recruited $CD3^+$ lymphocytes in tumor tissues to inhibit intrahepatic CC development and metastasis (70). In a CC model, a large portion of BDEneu-C24 cells expressed the AspH protein, causing a concentrated collagen matrix reaction during tumor formation; however, $CD3^+$ T cells can penetrate the matrix barrier and reduce or delay the growth of CC (70). Recently, it has been reported that AspH promotes the growth and progression of CC by regulating the phosphorylation (and therefore inactivation) of RB1 (71). As a cancer suppressor gene, RB1 serves a vital role in cell cycle progression from G_0/G_1 to S phase and cell senescence (72,73). AspH upregulation increases the protein-protein interaction between RB1 and cell cycle-associated proteins, which in turn results in enhanced phosphorylation of RB1 (71). In addition, this interaction can be suppressed by inhibitors of hydroxylase activity (71).

AspH in pancreatic carcinoma (PC). PC was the third leading cause of cancer-associated mortality in the USA in 2019, with the lowest 5-year relative survival rate (9%) among all other types of cancer (74). The β-hydroxylase activity of AspH was proven to boost the malignant phenotypes of PC cells, such as cell migration, 2D and 3D invasion, EMT, ECM degradation/remodeling, stemness, microsphere formation and metastasis; these phenotypes were specifically suppressed using an SMI (MO-I-1182) (75). Additionally, it has been revealed that in a patient-derived xenograft (PDX) murine model with spontaneous pulmonary metastasis of human pancreatic ductal adenocarcinoma (PDAC), AspH promotes primary tumor development and pulmonary metastasis; these harmful effects can also be blocked using an SMI (MO-I-1182) (76). On the other hand, the proto-oncogene SRC can be activated by AspH through direct interaction with ADAM12/15 (75). Furthermore, the highly expressed AspH-SRC axis is a marker of poor prognosis in PC due to angiogenesis, invadopodia formation and metastasis (75,77). AspH can promote PC growth by activating Notch signaling cascades (29,78). Mechanistically, the ECD of Notch receptors contains 36 consecutive EGF-like repeats for the β-hydroxylation of aspartate/asparagine (27,29). AspH directly stimulates Notch to upregulate downstream responsive target genes, including HES1 and HEY1 (29). In AspH-overexpressing PDAC cell lines, a human monoclonal antibody against AspH (SNS-622-DM1) exerts significant antitumor effects by facilitating tumor cell G_2/M phase accumulation and increasing cellular cleaved caspase 3 expression (79). Additionally, SNS-622-DM1 can inhibit tumor growth and pulmonary metastasis in a PDX murine model (79).

AspH in colorectal carcinoma (CRC). CRC is the fourth most deadly cancer, with ~900,000 deaths annually worldwide in 2019 (80). A bioinformatics analysis revealed that the mRNA and protein levels of AspH are upregulated in CRC compared with in normal tissues due to gene copy number variations and promoter demethylation (81). AspH accumulates at the invasive tumor margin, which may be associated with cell invasion and infiltration (81). It has been recently reported that Notch signaling recruits TGFβ-dependent neutrophils to drive CRC metastasis; this pathway has an important role in the tumor microenvironment and predicts a poor survival in patients with CRC (82). Notably, knocking down AspH or using specific SMIs (MO-I-1144) decreases Notch expression in CRC, inhibiting tumor development and metastasis (81).

AspH in breast carcinoma. Studies have revealed the presence of AspH gene amplification in invasive/advanced ductal carcinoma and AspH silencing in normal adult breast tissues (32,83). AspH upregulation activates the Notch signaling pathway, increases the synthesis/release of pro-oncogenic exosomes and subsequently enhances EMT, 2D and 3D invasion, stemness, angiogenesis and metastases in breast cancer; these malignant phenotypes are reversed using an SMI (MO-I-1182) (32). AspH stimulates the Notch cascade by directly interacting with Notch receptors, ligands (JAGs) or ADAM10/17 modulators (32). The AspH-Notch axis is essential for the progression and prognosis of breast cancer (32). In mouse models, high levels of AspH induced more aggressive tumors, characterized by rapid growth and extensive metastases (32). Notably, phage vaccination markedly decreased pulmonary metastasis and enhanced survival in the 4T1 breast cancer model (with AspH overexpression) (53). On the other hand, in estrogen receptor-positive breast cancer cells, the activation of MAPK and PI3K cascades upregulates AspH mRNA expression when tamoxifen sensitivity is decreased (84). Furthermore, upregulated AspH expression decreases the progression-free survival of patients with luminal B breast cancer who received adjuvant endocrine therapy (84). Therefore, endocrine sensitivity of endocrine-resistant breast cancer with high AspH expression may be restored by blocking the MAPK and PI3K signaling pathways (84).

AspH in glioblastoma (GBM). GBM was the most common primary malignant brain tumor among adults worldwide in 2016 (85). Via analyzing whole genome alternative splicing events in 498 GBM cases, it was revealed that AspH expression is upregulated in GBM and is associated with the onset and progression of cancer (86). A previous study has demonstrated that protein levels of AspH and of the proliferation-associated protein Ki-67 are upregulated in more aggressive GBM cases compared with well differentiated cases (87). Furthermore, AspH knockdown or SMI (MO-I-1100, MO-I-400, MO-I-500 and MO-I-1151) treatment targeting hydroxylase activity decreases the viability and directional motility of GBM cells (87). Moreover, shorter progression-free survival and overall survival are associated with AspH upregulation and HIF-1α expression in patients with GBM, analyzed using immunohistochemistry (87). The Cancer Genome Atlas gene database revealed that AspH and HIF-1α were significantly upregulated in the mesenchymal subtype of GBM (87). This

Table I. Diverse molecular functions of aspartate β-hydroxylase in cancer.

First author, year	Cancer type	Mechanisms	Molecular targets	(Refs.)
Cantarini *et al*, 2006; Chung *et al*, 2016	Hepatocellular carcinoma	Activating Notch signaling pathway	Notch receptors and ligands	(30,31)
Iwagami *et al*, 2016		Delaying cell senescence	Inhibition of GSK3β phosphorylation	(47)
Tang *et al*, 2017		Destroying mitochondria integrity	Decrease of the interaction between histone H2A member X and mitochondrial transcription factor A	(60)
Yoo *et al*, 2009	Cholangiocarcinoma	Enhancing sarcomatous change and epithelial-mesenchymal transition	Unknown	(67)
Huang *et al*, 2016; Sugimachi *et al*, 2001		Activating Notch signaling pathway	Notch receptors and ligands	(68,69)
Huang *et al*, 2018		Delaying cell growth and senescence	Enhancement of RB1 phosphorylation	(71)
Jove and Hanafusa, 1987	Pancreatic carcinoma	Activating SRC signaling pathway	Interaction with ADAM12/ADAM15	(77)
Dong *et al*, 2015		Activating Notch signaling pathway	Notch receptors and ligands	(29)
Benelli *et al*, 2020	Colorectal carcinoma	Activating Notch signaling pathway	Notch receptors and ligands	(81)
Lin *et al*, 2019	Breast carcinoma	Activating Notch signaling pathway	Notch receptors and ligands	(32)
Shimoda *et al*, 2017		Decreasing endocrine sensitivity	Unknown	(84)
Sturla *et al*, 2016	Glioblastoma	Increasing cell proliferation	Upregulation of Ki-67 protein	(87)
Chen *et al*, 2019	Endometrial carcinoma	Increasing cell proliferation and migration	Unknown	(88)
Sepe *et al*, 2002	Neuroblastoma	Increasing cell motility	p21/Waf1 and p16	(90)
Luu *et al*, 2009	Non-small cell lung cancer	Increasing invasiveness and metastatic	Unknown	(91)
Lee, 2008	Gastric carcinoma	Promoting growth and migration	Unknown	(92)

demonstrates that both AspH and HIF-1α may be involved in mesenchymal transformation and may subsequently induce aggressive and invasive phenotypes (87).

AspH in other types of cancer. Similarly to the aforementioned types of cancer, modulation of AspH function serves a critical role in endometrial cancer (EC), neuroblastoma, non-small cell lung carcinoma (NSCLC) and gastric cancer (88-92). Compared with normal cell lines, AspH expression was upregulated in EC cell lines, while miR-135a expression was downregulated (88). Cell Counting Kit-8 and wound-healing assays revealed that cell proliferation and migration were decreased by miR-135a overexpression. Conversely, high levels of AspH led to increased cell proliferation and migration, and miR-135a overexpression decreased the luciferase activity of EC cells transfected with wt-AspH 3'-untranslated region (UTR) but not mutant-AspH 3'-UTR (88). AspH upregulation restored the inhibitory effects of miR-135a on EC cells (88). These observations suggest that miR-135a affects EC growth and invasion by regulating AspH levels (88). AspH expression was significantly increased in neuroblastoma cells compared with in CNS-derived primitive neuroectodermal tumor cells. Mechanistically, insulin and IGF-1 increased directional motility by inducing AspH expression (89). However, treatment with AKT, ERK or cyclin-dependent kinase 5 (CDK-5) inhibitors significantly decreased insulin- and IGF-1-stimulated AspH mRNA expression and motility (89). These results suggest that ERK, AKT and CDK-5 signaling may mediate insulin and IGF-1 regulation of AspH at the level of transcription (89). In addition, high expression levels of AspH

significantly enhanced neuroblastoma Sy5y cell motility, while the inhibition of AspH by antisense oligodeoxynucleotides decreased the motility of Sy5y cells and enhanced the expression levels of p21/Waf1 and p16, indicating that AspH is involved in tumor invasion and metastasis (90). In NSCLC, FB50 immunohistochemical staining revealed a marked increase in AspH expression, particularly in squamous cell carcinoma (91). High levels of AspH immunoreactivity are associated with poor survival and prognosis in patients with NSCLC, and AspH upregulation may increase the potential for tumor invasiveness and metastatic spread due to alterations in cell shape and adhesion (91). Finally, as a truncated isoform of AspH, humbug expression has been reported to be upregulated in several gastric cancer cell lines, especially in highly aggressive cells (92). High expression levels of humbug increased the anchorage-independent cell proliferation capability according to a colony formation assay; additionally, Transwell migration assays revealed that overexpression of humbug can promote cell migration and invasion compared with control vector-transfected cells (92). Therefore, humbug may be a molecule that affects the development and progression of gastric cancer (92).

4. Conclusion and outlook

An increasing number of studies have revealed that AspH expression is upregulated in several types of human tumor. Its hydroxylase activity serves an essential role in promoting malignant tumor phenotypes, including growth, proliferation, invasion and metastasis. The present review discussed multiple key signaling pathways and mechanisms underlying the function of AspH in cancer. Notably, AspH activates Notch and PI3K-dependent signaling pathways, delays tumor cell senescence, destroys the integrity of mitochondria and subsequently leads to tumor development and a poor prognosis (Table I). Therefore, different specific and selective SMIs targeting hydroxylase activity have been designed and have revealed promising results *in vitro* and *in vivo*. Additionally, the versatile function of AspH in the immune system has been investigated over the last decade. Phage vaccination and DCs fused to the AspH protein yield substantial antitumor effects in animal models. These studies indicate that AspH may become a novel prognostic marker and an immunotarget for antitumor agents. Although there has been some progress with respect to the role of AspH in tumor development, further investigations are required to improve the efficacy of cancer treatment and provide additional benefits to clinical patients.

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Authors' contributions

HZ and WZ designed the study. WZ and XW wrote the manuscript. JH prepared the figures. BB reviewed and edited the manuscript. All authors read and approved the final manuscript and agree to be accountable for all aspects of the research.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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