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Tumor promotion or suppression: Revisiting the role of EXT1 and heparan sulfate

Ayumi Niwa, Hiroyuki Tomita and Akira Hara
Department of Tumor Pathology, Gifu University Graduate School of Medicine, Gifu, Japan

Summary. Heparan sulfate (HS), a linear sulfated polysaccharide attached to proteoglycans, modulates the availability and activity of growth factors and cytokines to regulate cell signaling, adhesion, and migration. Exostosin-1 (EXT1), a key glycosyl-transferase for HS chain elongation, is increasingly implicated in cancer development and progression. Although originally identified as a tumor suppressor in hereditary multiple exostoses, EXT1 exhibits a complex, context-dependent role in cancer. The effects of EXT1 in cancer differ by cell and tumor type, exerting both tumor-suppressing and tumor-promoting effects. Notably, EXT1 also alters the tumor microenvironment via its expression in stromal fibroblasts and endothelial cells, further influencing tumor behavior. This review discusses the functions of HS and EXT1, emphasizing the roles of EXT1 in cancer and its microenvironment. A deeper understanding of these mechanisms may offer novel therapies targeting the HS biosynthetic pathway.

Key words: EXT1, Heparan sulfate, Cancer

Introduction

Heparan sulfate (HS), a linear sulfated polysaccharide attached to HS proteoglycans (HSPGs), is widely distributed on cell surfaces and in the extracellular matrix (ECM) (Lindahl and Höök, 1978; Gallagher et al., 1986). HS binds various extracellular signaling molecules to regulate diverse cellular functions, including development, differentiation, proliferation, cell adhesion, and migration. Its biosynthesis is complex, with exostosin-1 (EXT1) serving as an essential enzyme in the early stages (Lind et al., 1998; McCormick et al., 1998).

EXT1, in complex with exostosin-2 (EXT2), catalyzes HS chain elongation via the alternating polymerization of glucuronic acid (GlcA) and N-acetylglucosamine (McCormick et al., 1998). HS and its biosynthetic molecules contribute to tumorigenesis and progression. HS regulates the binding, storage, and diffusion of many growth factors, chemokines, and morphogens, considerably impacting cell-cell interactions and signal transduction in the tumor microenvironment (Sanderson, 2001; Sasisekharan et al., 2002; Raman and Kuberan, 2010). *EXT1* is recognized as a representative causative gene for hereditary multiple exostoses (HME), and HME with *EXT1* gene mutations is associated with EXT1 protein dysfunction (Cook et al., 1993; Le Merrer et al., 1994; Wu et al., 1994; McCormick et al., 1998). Heterozygous loss or homozygous deletion of the *EXT1* gene has been reported in sporadic osteochondroma (Hameetman et al., 2007), suggesting that somatic gene alterations in EXT1 may be involved in the development of sporadic osteochondroma. In addition to benign tumorigenesis, aberrant EXT1 expression has recently been implicated in tumor malignancy and therapy resistance across diverse cancer types (McCormick et al., 1998; Ropero et al., 2004; Reijmers et al., 2010; Daakour et al., 2016; Ushakov et al., 2017; Ohkawa et al., 2021; Wang et al., 2022; Pfeifer et al., 2023).

Elucidating the role of EXT1 in cancer could enable targeting EXT1 and the HS biosynthetic pathway as novel therapeutic strategies to inhibit cancer progression. Such insights may also improve the efficacy of existing therapies and contribute to overcoming drug resistance. This is critical for advancing our understanding of cancer mechanisms, promoting personalized medicine, and developing more precise diagnostic and therapeutic approaches.

This review aimed to explore the functions of HS

Corresponding Author: Hiroyuki Tomita, MD, PhD, Department of Tumor Pathology, Gifu University Graduate School of Medicine, 1-1 Yanagido, Gifu 501-1194, Japan. e-mail: tomita.hiroyuki.y6@f.gifu-u.ac.jp
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Abbreviations. ALL, acute lymphoblastic leukemia; ECM, extracellular matrix; EGFR, epidermal growth factor receptor; EXT1, exostosin-1; EXT2, exostosin-2; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; Gal, galactose; GBM, glioblastoma; GlcA, glucuronic acid; HME, hereditary multiple exostoses; HS, heparan sulfate; HS6ST, heparan sulfate 6-O-sulfotransferase; HSPG, heparan sulfate proteoglycans; NDST, N-deacetylase/N-sulfotransferase; NSCLC, non-small cell lung cancer; RTK, receptor-type tyrosine kinase; TGF-β, transforming growth factor beta; Xyl, xylose

and EXT1, with a particular focus on the roles of EXT1 in cancer and its microenvironment. We first discuss the basic molecular functions of HS and EXT1, followed by a comprehensive discussion of recent findings on EXT1 in cancer biology.

General characteristics of HS

HS is a ubiquitous glycosaminoglycan found on cell surfaces and in the ECM. HS is one of several glycosaminoglycans, including chondroitin sulfate, dermatan sulfate, hyaluronic acid, and keratan sulfate. HS is crucial for cell growth, adhesion, and signaling (Gallagher and Turnbull, 1992; Gallagher, 1995). Initially identified as a low-sulfated form of heparin (Jorpes and Gardell, 1948) used clinically as an anticoagulant, HS gained interest for its role in regulating numerous processes at the cell-ECM interface.

HS and heparin are structurally similar but differ in localization and biological functions. They are bound to different core proteins and exist in different cellular compartments. HS is ubiquitous on the cell surface and in the extracellular matrix, whereas heparin is mainly stored in mast cell storage vesicles (Straus et al., 1982; Nader et al., 1999; Dreyfuss et al., 2009). HS is a linear polysaccharide that forms a long linear backbone of repeating disaccharide units, structurally similar to heparin. Both HS and heparin are polydisperse linear polymers composed of alternating α-D-glucosamine and uronic acid residues, either β-D-GlcA or α-L-iduronic acid, linked by glycosidic bonds (Dreyfuss et al., 2009; Zhang et al., 2017).

HSPGs are major components widely distributed on cell surfaces and in the ECM, providing structural support for cells and regulating cell signaling (De Pasquale and Pavone, 2020). HSPGs specifically interact with various signaling molecules present in the extracellular environment, including growth factors, chemokines, morphogens, and enzymes. Through these interactions, HSPGs play vital roles in cell proliferation, differentiation, migration, survival, and tissue formation (Dreyfuss et al., 2009).

The structural diversity of HS chains, particularly their sulfation patterns and chain lengths, determines ligand binding specificity and the activation of downstream signaling pathways (Gómez Toledo et al., 2021). Specific proteins bind to specific disaccharide units or sulfated motifs on HS chains and recognize the overall chain conformation (e.g., “kink” structures) (Shriver et al., 2012).

HS biosynthesis

HS synthesis is well-coordinated and involves many enzymes, which catalyze the various steps of HS synthesis with minimal overlap (Nagarajan et al., 2018). HS is biosynthesized in the Golgi apparatus, with enzymes such as HS 6-O-sulfotransferase (HS6ST) and N-deacetylase/N-sulfotransferase (NDST) localized to specific Golgi regions (Humphries et al., 1997; Nagai et al., 2004). The complete set of HS biosynthetic enzymes is found in the endoplasmic reticulum/proximal Golgi, while chondroitin/dermatan sulfate synthesis occurs in the trans-Golgi network (Uhlin-Hansen and Yanagishita, 1993). HS chains are synthesized by binding to specific serine residues of the proteoglycan core protein via a common linkage region (GlcAβ1–3Galβ1–3Galβ1–4Xylβ1–O-Ser). The synthesis of this linkage region begins with the addition of xylose (Xyl) to serine, followed by two galactose (Gal) molecules, and finally, GlcA. HS chains are constructed through the repeated addition of N-acetylglucosamine and GlcA to this linkage region (Sugahara and Kitagawa, 2002). The complex formed by EXT1 and EXT2 is crucial for this elongation (McCormick et al., 2000).

The HS chain structure is complex and undergoes various modifications. These modifications include N-deacetylation/N-sulfation (by NDST), C5-epimerization of uronic acid (by uronic acid C5-epimerase), 2-O-sulfation of uronic acid (by HS 2-O-sulfotransferase), 6-O-sulfation of glucosamine residues (by HS6ST), and 3-O-sulfation (by HS 3-O-sulfotransferase) (Sugahara and Kitagawa, 2002). Modifications to HS chains determine their overall sulfation pattern and structural diversity, contributing to their protein-binding specificity (Walker et al., 1994; Feyzi et al., 1997; Sugaya et al., 2008; Dreyfuss et al., 2009).

HSPG

Heparan sulfate (HS) attaches to core proteins, forming HSPGs (Nagarajan et al., 2018; De Pasquale and Pavone, 2020). HSPGs are classified into several families based on their core protein structure. Major HSPG families include the cell membrane-spanning syndecan family and the glycosyl-phosphatidylinositol-anchored glypican family. ECM-associated HSPGs, such as perlecan, agrin, and collagen XVIII, also serve as key components (Bernfield et al., 1999; Filmus and Selleck, 2001; Iozzo, 2005; Alexopoulou et al., 2007; Ori, 2008; De Pasquale and Pavone, 2020). HSPGs are localized on the cell surface and in the extracellular environment and are involved in various cellular functions.

Interactions of HS with various factors and cell signaling pathways

Owing to its complex structure and negative charge, HS can interact specifically with an array of proteins (Dreyfuss et al., 2009; Raman and Kuberan, 2010; Weiss et al., 2017). These proteins include growth factors (such as fibroblast growth factor (FGF), hepatocyte growth factor, vascular endothelial growth factor, and platelet-derived growth factor subunit B) (Weiss et al., 2017; Gómez Toledo et al., 2021), cytokines, chemokines, enzymes (heparanase) (Matsuda et al., 2001; Nagarajan et al., 2018), and ECM components (Onyeisi et al., 2020). HS often functions as a co-receptor for these proteins, promoting their binding and localization on the

cell surface, alongside stabilizing their interactions with receptors (Weiss et al., 2017).

HS plays a role in cell signaling as a receptor or co-receptor for various ligands. The major intracellular signaling pathways involving HS/HSPGs include Wnt/β-catenin (Valsechi et al., 2014; Yuan et al., 2016), Hedgehog (Capurro et al., 2008; Li et al., 2011), TGF-β/SMAD (Zheng et al., 2013; Ponandai-Srinivasan et al., 2020), FGF (Su et al., 2006; Uchimura et al., 2006; Whipple et al., 2012), epidermal growth factor receptor (EGFR) (Cole et al., 2014; Wang et al., 2014), PI3K/Akt (Lai et al., 2010; Li et al., 2019), ERK (Zhao et al., 2011; Yu et al., 2017), STAT3 (Ibrahim et al., 2013; Solaimuthu et al., 2024), Hippo (Chakraborty et al., 2017; Le et al., 2018), Notch (Ibrahim et al., 2017; Hillemeyer et al., 2022), and integrin signaling (Beauvais et al., 2009; Yang and Friedl, 2016). HSPG levels and structural changes in the HS chain exert complex effects on signaling pathway activity (De Pasquale and Pavone, 2020).

EXT1 is the key enzyme of HS

EXT1 is HS elongation enzyme, originally discovered as a gene responsible for HME. HME is an autosomal dominant skeletal disorder characterized by cartilage-capped tumors, known as osteochondromas or exostoses. These tumors primarily develop in the juxta-epiphyseal regions of long bones from early childhood until puberty (Solomon, 1963; Yamada et al., 2004). Individuals with HME also exhibit a markedly increased risk of developing malignant tumors, including chondrosarcomas, compared with the general population (Schmale et al., 1994; Wuyts and Van Hul, 2000). Genetic linkage analyses in families affected by HME have identified three loci associated with the disease: *EXT1* on chromosome 8q24.1, *EXT2* on the short arm of chromosome 11, and *EXT3* on the short arm of chromosome 19 (Cook et al., 1993; Le Merrer et al., 1994; Wu et al., 1994). In most HME cases, missense or frameshift mutations cause premature translation termination, yielding truncated EXT proteins (Wuyts and Van Hul, 2000; Francannet et al., 2001). Despite extensive genetic analysis, the functional role of EXT proteins was not elucidated until 1998.

Independent studies established a link between the *EXT* gene family and HS-synthesizing glycosyltransferases; for example, work in HS-deficient mouse fibroblast cell line revealed EXT1 involvement in HS biosynthesis (Lind et al., 1998; McCormick et al., 1998). EXT1 and EXT2 are predicted to be type II transmembrane glycoproteins primarily localized to the endoplasmic reticulum. It has been experimentally demonstrated that in the absence of EXT1, EXT2 alone does not exhibit significant glycosyltransferase activity (McCormick et al., 2000). *In vivo*, they form a heterooligomeric complex that accumulates in the Golgi apparatus (McCormick et al., 1998; Kobayashi et al., 2000), where it demonstrates enhanced glycosyltransferase activity compared with EXT1 alone. This suggests the complex is the functionally active form involved in HS polymer modification (McCormick et al., 2000; Senay et al., 2000). EXT1 and EXT2 complexes can add individual D-GlcA and N-acetylglucosamine residues to an artificial substrate molecule (McCormick et al., 1998; Lin et al., 2000). Notably, complete loss of Ext1 causes embryonic lethality, primarily due to failure of gastrulation. Furthermore, it has been reported that Ext1-deficient mice lack HS biosynthesis, indicating that Ext1 is essential for heparan sulfate synthesis (Lin et al., 2000).

Role of EXT1 in cancer

EXT1 was initially identified as the gene responsible for HME and is therefore considered a classic tumor suppressor gene (Liu et al., 2019; Wu et al., 2021; Pfeifer et al., 2023; Jiang et al., 2024). HS binds to many growth factors and cytokines and regulates their signal transduction efficiency. Abnormal EXT1 expression or function affects cancer cell behavior, with either promotive or suppressive effects, depending on cancer type and condition (Fig. 1) (Table 1).

Tumor-suppressing effects

Inhibition of cell proliferation and promotion of apoptosis

In acute lymphoblastic leukemia (ALL), *EXT1* downregulation is associated with poor prognosis (Liu et al., 2019). In ALL-xenograft mouse models, *Ext1* overexpression inhibited tumor growth, whereas its knockdown promoted tumor growth. In ALL cells, EXT1 potentially promotes cell apoptosis via ERK1/2 signaling pathway inactivation (Liu et al., 2019). Additionally, *EXT1* knockdown in melanoma cells induces chemoresistance. Furthermore, it enhances proliferative signaling through EGFR signaling via the JNK and MEK/ERK pathways, which is associated with treatment resistance (Pfeifer et al., 2023).

Suppression of osteosarcoma cell migration

In mouse FBJ osteosarcoma cells, *Ext1* and *Ext2* expression was higher in less metastatic cells, and *Ext1* knockdown enhanced cell motility (Wang et al., 2013). Ext1 knockdown reduces HS chain elongation and cell surface HS levels, contributing to enhanced motility. It also promotes heparanase expression, indicating that EXT1 influences HS metabolism and heparanase.

Epigenetic silencing of EXT1 in cancer cells

EXT1 CpG island hypermethylation is frequently observed in various cancer types, particularly acute leukemia (acute promyelocytic leukemia and ALL) and non-melanoma skin cancer (Ropero et al., 2004). Reintroducing EXT1 into cancer cell lines with epigenetically silenced expression induces tumor suppressor-like properties.

Tumor-promoting effects

Cell proliferation, migration and metastasis promotion

In glioblastoma models, *Ext1* inactivation reduces HS levels, attenuates receptor-type tyrosine kinase (RTK) signaling, and inhibits cell proliferation and tumor growth (Ohkawa et al., 2021). In breast cancer cells, *EXT1* overexpression induces epithelial-mesenchymal transition and enhances cell migration and invasion (Solaimuthu et al., 2024). In xenograft models, *Ext1* knockout slows tumor growth and significantly reduces metastasis (lung metastasis). This suggests that

Table 1. Reported roles of EXT1 in different cancer types.

Cancer type	*EXT1* expression change	Observed function	Associated pathways	Reference
ALL	↑ or ↓	Proliferation, growth, apoptosis	ERK1/2 signaling	Liu et al., 2019
Glioblastoma	↓	↓ Proliferation, ↓ growth, ↑ sensitivity to EGFR inhibitors	RTK signaling	Ohkawa et al., 2021
Breast Cancer	↑ or ↓	EMT, proliferation, migration, growth, metastasis, stemness, chemoresistance	STAT3 signaling	Manandhar et al., 2017; Solaimuthu et al., 2024
NSCLC	↑ or ↓	Proliferation, Migration	WNT signaling	Kong et al., 2021
Osteosarcoma	↓	↑ Migration	HS reduction	Wang et al., 2013
Melanoma	↓	↑ Metastasis, ↑ chemoresistance	JNK and MEK/ERK signaling	Wang et al., 2022; Pfeifer et al., 2023

EXT1, exostosin 1; ALL, acute lymphoblastic leukemia; NSCLC, non-small cell lung cancer; WT, wild type; EGFR, epidermal growth factor receptor; EMT, Epithelial-mesenchymal transition; RTK, receptor-type tyrosine kinase; HS, heparan sulfate.

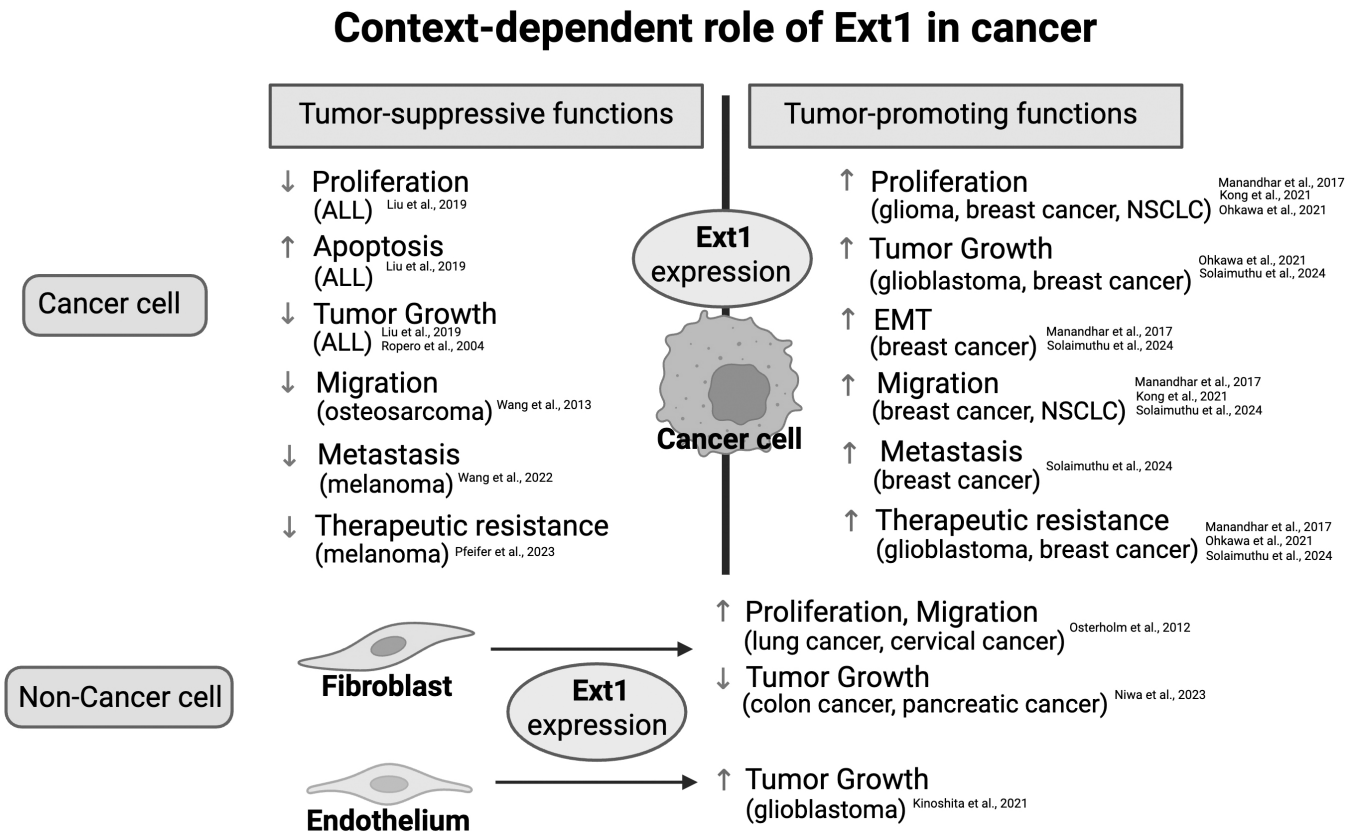

Fig. 1. Context-dependent role of EXT1 in cancer. EXT1 exhibits dual functions in cancer: tumor-suppressive in ALL, melanoma, and osteosarcoma, and tumor-promoting in glioblastoma, breast cancer, and NSCLC. Additionally, EXT1 expression in stromal fibroblasts and endothelial cells influences tumor growth via non-cancer cell-autonomous mechanisms. The context-dependent role of EXT1 depends on cancer type, cellular state, and microenvironmental factors. Created with BioRender.com.

EXT1 is essential for the aggressive phenotype and metastasis of breast cancer. In non-small cell lung cancer (NSCLC), *EXT1* expression influence the WNT signaling pathway and promote cell migration (Kong et al., 2021).

Stem cell induction and treatment resistance

In breast cancer cells, *EXT1* overexpression induces stem cell-like properties and is associated with doxorubicin resistance (Manandhar et al., 2017). In glioblastoma model, EXT1 regulates RTK signaling via HS synthesis, promoting EGFR inhibitor resistance (Ohkawa et al., 2021).

Molecular mechanisms of EXT1

Many EXT1 functions in cancer are related to its role in regulating various signaling pathways mediated by its HS synthase activity.

FGF signaling

EXT1 mutant fibroblasts exhibit reduced FGF2 signaling responses (Katta et al., 2018). *EXT1* knockout in glioblastoma (GBM) weakens RTK signaling, including FGFR. HS synthesized by EXT1 regulates RTK signaling in GBM and promotes resistance to EGFR inhibitors (Ohkawa et al., 2021).

Transforming Growth Factor beta (TGF-β) signaling

In *EXT1* mutant fibroblasts, TGF-β1 expression and secretion are reduced, leading to decreased *P311* expression in co-cultured A549 cells (Katta et al., 2018). This suggests that EXT1 levels in fibroblasts influence TGF-β1 expression via HS and regulate tumor cell gene expression.

STAT3 signaling

In breast cancer cells, loss of EXT1 significantly decreases STAT3 phosphorylation (Y705), indicating that EXT1 regulates STAT3 signaling (Solaimuthu et al., 2024).

Wnt signaling

In NSCLC cells, EXT1 expression promote migration via the WNT signaling pathway (Kong et al., 2021).

Notch signaling

EXT1 was shown to inhibit Notch-1 transcriptional activation. In ALL, EXT1 functions as a tumor suppressor and regulate the Notch pathway in an FBXW7-dependent manner (Daakour et al., 2016).

Function of EXT1 in cancer microenvironment

Effects of EXT1 expression in fibroblasts:

EXT1 expression in fibroblasts and vascular endothelial cells that constitute the tumor stroma may influence cancer cell behavior and tumor proliferation by altering HS synthesis.

Mouse embryonic fibroblasts with Ext1 mutations (Ext1Gt/Gt) produce shorter, sulfated HS chains (Yamada et al., 2004). In 2D co-culture and 3D heterospheroid model experiments with *EXT1* mutant fibroblasts and A549 human lung adenocarcinoma cells, P311 expression was significantly reduced (Katta et al., 2018). *EXT1* mutant fibroblasts had low *TGFB1* mRNA levels and secreted active TGF-β protein. These findings suggest that the HS structure regulated by EXT1 modify tumor-stromal interactions by altering TGF-β1 expression in stromal cells.

The effects of EXT1 mutations in fibroblasts on tumor cell proliferation and migration have been reported to differ between *in vitro* and *in vivo* models. *In vitro*, composite spheroids containing Ext1 mutant fibroblasts (Ext1Gt/Gt) with reduced heparan sulfate (HS) levels showed decreased tumor cell proliferation and impaired cell migration, likely due to looser cell-matrix contacts compared with wild-type fibroblasts (Österholm et al., 2012). In contrast, a conditional knockout mouse model (S100a4-Cre; Ext1f/f) demonstrated that fibroblast-specific deletion of Ext1 promotes the proliferation of colorectal and pancreatic cancer cells by creating an Ext1-deficient tumor microenvironment (Niwa et al., 2023). Together, these findings suggest that fibroblast Ext1 expression plays a complex role in regulating tumor proliferation, migration, and the tumor microenvironment through HS synthesis, and that its effects may vary depending on the experimental model.

Effects of EXT1 expression in vascular endothelial cells

In a mouse model, vascular endothelial cell-specific Ext1 deficiency significantly suppressed GBM tumor growth and improved survival (Kinoshita et al., 2021). This effect is mediated by reduced angiogenesis, as decreased HS in vascular endothelial cells weakened the angiogenic-promoting effect of FGF2. These findings suggest that EXT1 in vascular endothelial cells influences tumor proliferation and progression by regulating angiogenesis via HS synthesis.

Because HS is ubiquitously expressed and participates in diverse cellular processes, the effects of altered EXT1 expression are inherently difficult to isolate and interpret. Moreover, the impact of *EXT1* downregulation in cancer cells may not depend solely on tumor-cell intrinsic properties. High HS levels in the surrounding tumor microenvironment, particularly in

host-derived cells, may influence both EXT1 and HS expression within cancer cells. In some cases, abundant HS in the microenvironment could potentially compensate for cancer cell-intrinsic HS loss owing to decreased EXT1 expression. Experimental evidence indicates that HS present in the tumor microenvironment, i.e., non-cell-autonomous HS, can partially rescue HS-deficient tumor cell growth (Ohkawa et al., 2021). These findings highlight the importance of evaluating both cell-autonomous and non-cell-autonomous factors when assessing the role of EXT1 in cancer biology and emphasize the complexity in fully understanding its function, owing to the dynamic interplay between tumor cells and their microenvironment.

Conclusion and perspectives

This review highlights the multifaceted roles of HS and EXT1, focusing on EXT1 involvement in cancer. By regulating HS biosynthesis, EXT1 influences key signaling pathways and governs a broad spectrum of cancer-related cellular behaviors, including proliferation, apoptosis, migration, invasion, tumorigenesis, and therapy response. Notably, these functions appear to be highly context-dependent, varying by cancer type, cellular state, and microenvironmental cues.

Recent research indicates that both cell-autonomous and non-cell-autonomous factors, including surrounding microenvironment-derived HS, may significantly influence the phenotypic consequences of altered EXT1 expression. This dual influence contributes to functional redundancy or compensation and adds complexity to our understanding of the role of EXT1 in cancer. The current literature has limitations in fully explaining how altered EXT1 expression leads to diverse phenotypic outcomes at the single-cell level. Specifically, the mechanistic basis for cell-to-cell variability in the effects of EXT1 expression remains largely unclear. A deeper understanding of EXT1 function in both tumor cells and their microenvironment is essential for advancing tumor biology, improving prognostic accuracy, and developing new therapeutic strategies.

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