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Potential signaling pathway involved in sphingosine-1-phosphate-induced epithelial-mesenchymal transition in cancer (Review)

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Abstract. The developmental process of epithelial-mesenchymal transition (EMT) occurs when epithelial cells acquire invasive mesenchymal cell characteristics, and the activation of this process has been indicated to be involved in tumor progression. EMT could be induced by growth factors, cytokines and matrix metalloproteinases (MMPs). sphingosine-1-phosphate (S1P) is a biologically-active lipid that plays an important role in cancer metastasis. S1P also contributes to the activation of EMT. However, the mechanism underlying S1P-induced EMT is unclear. Increased evidence has demonstrated that the cell surface glycocalyx is closed associated with S1P and plays an important role in tumor progression, suggesting that S1P-induced EMT could be Snail-MMP signaling-dependent. Thus, we hypothesize that an S1P-glycocalyx-Snail-MMP signaling axis mediates S1P-induced EMT. This is an essential step towards improved understanding of the underlying mechanism involved in S1P-regulted EMT, and the development of novel diagnostic and anticancer therapeutic strategies.

Contents

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1. Introduction

The developmental process of epithelial-mesenchymal transition (EMT) occurs when epithelial cells acquire invasive mesenchymal cell characteristics, and the activation of this process has been indicated to be involved in tumor progression (1-3). EMT is associated with decreased expression of epithelial-specific genes, such as E-cadherin, and an increase in the expression of mesenchymal-specific genes, including N-cadherin and vimentin (4,5). EMT is believed to ultimately promote tumor metastasis by promoting the migration of tumor cells across the basement membrane and their invasion into the surrounding microenvironment (2,6). Understanding the mechanism underlying EMT has profound results with regard to the responsiveness of a tumor to a range of available treatments.

Numerous studies have demonstrated that the interaction of tumor cells with their microenvironment can induce the expression of growth factors, cytokines and matrix metalloproteinases (MMPs), further leading to EMT (7-9). The transforming growth factor-β (TGF-β), Wnt, Notch and nuclear factor-κB (NF-κB) signaling pathways have been found to be critical for EMT induction (10,11). Sphingosine-1-phosphate (S1P), a biologically-active lipid, has been found to play a vital role in inflammatory diseases and cancer. It has been shown that S1P contributes to tumor metastasis by modifying the extracellular environment and via the induction of the invasion, motility and migration of cells to other locations, as well as by EMT (12,13).

The tumor extracellular environment provides various stimuli (such as interstitial fluid shear stress and traction force) and the matrix for adjacent cells to contact. An early and decisive event during tumor development is hypoxia, which triggers a metabolic shift and induces processes such as coagulation, angiogenesis and extracellular matrix (ECM) remodeling (14,15). The glycocalyx localizes at the surface of stromal and malignant tumor cells, and regulates a diverse range of molecular activities involved in cell-cell and cell-matrix interactions, as well as ECM remodeling. Recently, the glycosaminoglycans (GAGs) of the glycocalyx were indicated to play important roles in the mechanotransduction pathways involved in flow-regulated tumor invasion and metastasis (16). In the

present review, we postulate that the glycocalyx takes center stage in S1P-induced EMT.

2. Hypothesis

We hypothesize that S1P promotes EMT in cancer by remodelling the glycocalyx and inhibiting the Snail-MMP signaling pathway. S1P plays a vital role in EMT, and the identification of an S1P-glycocalyx-Snail-MMP signaling axis could provide insight into novel anticancer therapeutic strategies.

3. Evaluation and rationale of the hypothesis

Cell surface glycocalyx is closely associated with tumor progression. The glycocalyx is a complex layer of numerous membrane-bound macromolecules that covers the mammalian cell surface (17). The glycocalyx is mainly formed from glycoproteins that bear acidic oligosaccharides and terminal sialic acids, as well as proteoglycans with their associated GAG side chains (17). Distinct disaccharide unit repeats characterize the GAGs and give rise to a variety of components, including chondroitin sulfate (CS), heparan sulfate (HS) and hyaluronic acid (HA). Sulfated GAGs covalently attach themselves to specific sites within proteoglycans (18). The syndecan family and glypican family are two major protein core families of HS proteoglycans that occur in almost all mammal cells. In syndecan-1, two extra sites positioned closer to the membrane are reserved for CS (18). HA is a disaccharide polymer of greater length (1,000-10,000 kDa), which is synthesized on the cell surface and interacts with HA receptors, such as the transmembrane glycoprotein CD44, and CS chains (19).

The changes in the structure and function of the glycocalyx are associated with disease occurrence. The components of the glycocalyx, including syndecans, glypicans and HA, serve as potential prognostic markers. Under specific pathophysiological conditions, including tumor onset, progression and metastasis, the expression and shedding of the glycocalyx components can be changed (20). As the most well-characterized syndecan family member, syndecan-1 is mainly expressed by epithelial cells (21). In breast carcinoma, the loss and overexpression of syndecan-1 correlates with a poor prognosis and an aggressive phenotype (22-24). In *in vitro* models of breast cancer, syndecan-1 is able to promote tumorigenesis via the regulation of tumor cell spreading and adhesion, proliferation and angiogenesis (25,26). Syndecan-4 is widely expressed in the normal human mammary epithelium, albeit typically at low levels, and it is the second most prolific HS proteoglycan that is produced by the majority of breast carcinoma cell lines (27). Excess focal adhesion formation is promoted by the overexpression of syndecan-4, resulting in a reduced level of cell migration (28). Additionally, syndecan-4-deficient mice and cells exhibit impaired wound repair and mesenchymal cell migration (29,30). Glypicans that are localized on the cell surface via a glycosylphosphatidylinositol moiety may regulate the cell responses to cell adhesion molecules and the ECM. Human breast and pancreatic cancer cells strongly express glypican-1 (31,32), and it is required by pancreatic cancer cells for efficient TGF-β signaling (33). HA is also

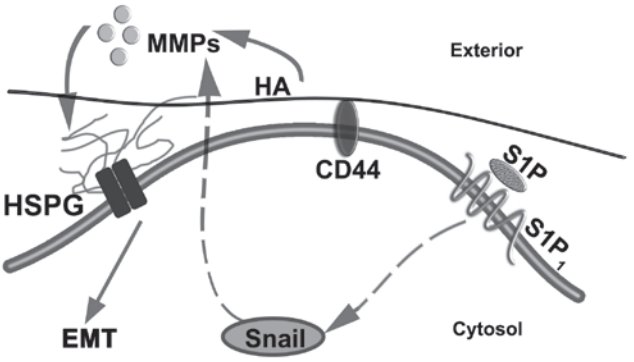

Figure 1. Glycocalyx-Snail-MMP signaling axis potentially involved in S1P-induced EMT in cancer. MMPs, matrix metalloproteinases; HSPG, heparan sulfate proteoglycans; HA, hyaluronic acid; CD, cluster of differentiation; S1P, sphingosine-1-phosphate; EMT, epithelial-mesenchymal transition.

closely correlated with tumor cell growth, proliferation and metastasis. Recent studies have shown elevated serum HA levels in breast cancer patients (34).

Close association between the glycocalyx and S1P and MMPs. S1P maintains the integrity of the endothelial glycocalyx structure and inhibits MMP activity. Recent studies showed that the release of MMPs degrades the syndecan-1 ectodomain and its associated GAGs when S1P levels fall below a critical range of 100-300 nM and S1P1 is vacated (35), and that S1P induces the recovery of the glycocalyx via the phosphoinositide 3-kinase (PI3K) signaling pathway (36). In another study, HA increased the secretion of MMP-2 and MMP-9 in multidrug-resistant MCF-7 cells, and such an effect was blocked by the NF-κB inhibitor BMS-345541 (37). Furthermore, HA has been shown to activate the secretion of MMP-2 in time- and focal adhesion kinase-dependent manners in a QG90 cell line derived from human small cell lung carcinoma (38).

Tumor invasion and metastasis is inhibited after shedding of the glycocalyx. A study showed that the physiological levels of interstitial flow shear stress upregulated MMP levels and enhanced the motility of metastatic cells (16). The degradation of the glycocalyx on the tumor cell surface by hyaluronidase and heparinase blocked the flow-induced cell invasion. This study suggested that HA and HS play important roles in tumor invasion and metastasis.

S1P-induced EMT can be Snail-MMPs signaling pathway-dependent. S1P is formed by phosphorylation of sphingosine, catalyzed by sphingosine kinases 1 and 2 (39). S1P is a ligand for the S1P-specific G-protein coupled receptors, termed $S1P_{1-5}$. The effects of S1P on cell invasion, motility and migration are mediated via receptor-dependent pathways (12,39-41). In general, $S1P_1$ is exclusively coupled with G_i protein to activate cell migration through extracellular signal-regulated kinase, PI3K, Akt, phospholipase C and Rac signaling (42-44). The $S1P_2$ and $S1P_3$ receptors could couple with the G_i, G_q and $G_{12/13}$ proteins to inhibit cell migration via ρ signaling (41,45).

S1P modulates the levels of MMPs, such as MMP-2 and MMP-9, regulating cell invasion (46-48). Recent findings have suggested that EMT-associated MMPs are involved

with the progression of cancer via three distinct mechanisms: i) Elevated MMPs levels in the tumor microenvironment are able to directly induce EMT in epithelial cells; ii) cancer cells that undergo EMT are able to generate more MMPs, facilitating cell invasion and metastasis; and iii) EMT is able to produce activated stromal-like cells that induce cancer progression through further production of MMPs (49). Previously, transcriptional profiling studies of Ras-transformed mouse mammary epithelial cells that were induced to undergo EMT by TGF-β treatments demonstrated the upregulation of MMP-2, -12 and -13 (50,51). The TGF-β-induced EMT of MCF10A cells stimulated the expression of MMP-2 (52). Additionally, the expression of Snail in MCF-7 cells induced a MT1-MMP- and MT2-MMP-dependent invasion program (53). In SCp2 cells, Snail expression was MMP-3-dependent, as Snail levels decreased rapidly after MMP-3 withdrawal (54). The TGF-β signaling pathway is the most extensively studied (55). TGF-β-induced EMT involves smad and non-smad pathway activation and is mediated by the transcriptional repressors and master regulators of EMT, such as Snail (56). Snail-knockdown inhibits cell migration and invasion induced by NF-κB and causes the suppression of inflammation-mediated breast cancer metastasis (10). Thus, Snail plays a vital role in TGF-β and NF-κB signaling, as well as in MMP-induced EMT. Overall, it is possible that S1P-induced EMT is Snail-MMP signaling pathway-dependent.

4. Conclusion

EMT is a central process in tumor metastasis. S1P plays important roles in cell migration, motility and invasion. Numerous studies have shown the close association among S1P, the glycocalyx and the Snail-MMP signaling pathway, suggesting that a glycocalyx-Snail-MMP signaling axis mediates the S1P-regulated EMT in tumor progression and malignancy (Fig. 1). Once validated, the identification of this novel S1P-glycocalyx-Snail-MMP signaling axis may provide insight into novel diagnostic and anticancer therapeutic strategies.

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