Molecular background of the regional lymph node metastasis of gastric cancer (Review)

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Abstract. Gastric cancer (GC) is one of the deadliest types of cancer in the world. Lymph node (LN) metastasis is a complex and malignant behavior of GC, involving a sequence of biological processes, including decreased adherence to adjacent cells, extracellular matrix (ECM) degradation and lymphatic channel permeation. LN metastasis is directly associated with the treatment response, local recurrence and long-term survival of patients with GC. Therefore, the molecular mechanisms of LN metastasis in GC development require further investigation. Recently, a large number of clinical studies have focused on the molecular mechanisms and biological markers of tumor invasion and metastasis. However, few articles have broadly summarized LN metastasis in GC, and the molecular mechanisms of LN metastasis are not yet fully understood. In the present review, the molecular mechanisms of LN metastasis in GC will be discussed, including the following aspects: Cell adhesion and movement, ECM degradation, new vessel formation, and molecular pattern differences between metastatic LNs and the primary tumor. This review may lead to a better understanding of LN metastasis in GC, and the identification of new diagnostic markers.

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1. Introduction

Gastric cancer (GC), one of the most common types of cancer (1), has a high mortality rate, which is predominantly caused by its delayed diagnosis due to the absence of early-stage clinical symptoms (2). A typical malignant behavior of GC is lymph node (LN) metastasis. The majority of epithelial cancers first spread through the lymph vessels into the draining LNs and gain metastatic development (3). The detection of metastasis in the sentinel LNs is often associated with adjuvant therapy decisions and has major prognostic implications for patients (4,5).

Despite their clinical importance, the mechanisms of LN metastasis have yet to be completely characterized. LN metastasis is complicated and involves a sequence of biological processes: The primary tumor cells persistently proliferate, and separate from the adjacent cells and the basement membrane; the extracellular matrix (ECM) is degraded; the tumor cells permeate into the lymphatic channels, and so forth. In recent years, gene expression studies have been performed to detect specific molecular signatures for LN metastases.

At present, a challenge for anti-cancer strategies is the control of metastasis. For this reason, it is essential to explore the regulatory mechanisms of metastasis to aid the identification of therapies to improve the survival rate for cancer patients. In this article, relevant studies regarding the molecular mechanisms of the lymphatic metastasis of GC will be reviewed and summarized (Table I).

2. Degradation of the ECM

Cancer cells invade the surrounding stroma, subsequent to passing through the epithelial basement membrane, during

Table I. Molecules associated with lymph node metastasis in gastric cancer.

Classification	Associated molecules
Degradation of the extracellular matrix	Matrix metalloproteinases, tissue inhibitors of metalloproteinases, ADAM metallopeptidases
Lymphangiogenesis	Vascular endothelial growth factor, platelet-derived growth factor-BB, insulin-like growth factor-1, etc.
Chemokines and cell adhesion molecules	C-C motif chemokine receptor 7, integrins, E-cadherin, etc.
Others	Annexin A1, zinc finger protein 139, c-MET, etc.

metastasis. This process includes two crucial mechanisms: Reduced cell-cell adhesion and ECM degradation (6). ECM degradation is determined by the activity of various proteolytic enzymes, particularly Matrix metalloproteinases (MMPs) (7). In humans, the MMP family, which contains 24 zinc-dependent endopeptidases, participates in tumor cell invasion and metastasis by degrading collagen types IV and V, components of the ECM (8). MMPs are usually relatively inactive, and can be activated when tissues are undergoing repair or remodeling, or in disease or inflammation (9). It has been reported that repressing the expression of certain MMPs is associated with increased survival and favorable prognosis in GC patients (10-12).

MMPs can be specifically regulated by tissue inhibitors of metalloproteinases (TIMPs). The balance between MMPs and TIMPs maintains the integrity of the ECM. During tumor metastasis, the imbalance of MMPs and TIMPs leads to ECM degradation (13,14). TIMP-1 is a major MMP inhibitor; however, it has a complicated role in tumor invasion and metastasis. In some cases, its function seems to be paradoxical: Increased MMP activity promotes tumor progression, while high levels of TIMP-1 are expected to inhibit tumor progression. For example, a previous study indicated that the increased expression of TIMP-1 in GC plasma or tumor tissue was strongly associated with a poor patient outcome (P<0.001) (15). In the study, the authors observed that TIMP-3 protein expression was remarkably downregulated in primary GC tissues, which was associated with the tumor stage and metastasis. Other studies demonstrated that the methylation status of TIMP-3 was significantly associated with LN metastasis, histological differentiation and the clinical stage (16-19) (Table II).

ADAM metallopeptidases share the metalloproteinase domain with MMPs, and are important in ECM degradation (20). A number of clinical studies (21,22) have demonstrated that MMP and ADAM activity are associated with poor cancer outcomes. The upregulation of ADAMs has been observed in GC, and has been demonstrated to produce marked effects on tumor development and metastasis. The MMP family of enzymes has been a focus of GC study, and MMP-specific inhibitors could potentially interrupt tumor progression.

3. Lymphangiogenesis and metastasis

Lymphatic vessel proliferation is frequently observed in GC tissue, allowing tumor cells to permeate into the lymphatic channels. Lymphangiogenesis is the formation of lymphatic vessels within healthy tissues, and the similar process in carcinogenesis (23). Vascular endothelial growth factor (VEGF) is a key factor in tumor lymphangiogenesis and metastasis (24,25). The increased expression of VEGF-C is associated with positive LN status and higher lymphatic vascular density, indicating its potential dual role in lymphatic vessel invasion and lymphangiogenesis (26). It has also been proposed that VEGF-C expressed by tumors interacts with the lymphatic endothelium, leading to its enlargement (27). VEGF-D, which is highly homologous to VEGF-C, induces lymphangiogenesis in tumor tissue and tumor cell diffusion to regional LNs (28). Previous clinical studies have demonstrated that bevacizumab, a humanized monoclonal antibody against VEGF, is clinically effective against GC (29-31). In a phase II study, Shah *et al* (32) evaluated bevacizumab with chemotherapy in 44 eligible cancer patients (GC, n=22; cancer of the esophagogastric junction, n=20; esophageal cancer, n=2). The response rate was 67% for the 39 patients with measurable disease.

Studies on cervical and gastric tumors have revealed a strong association between lymphatic vessel density (LVD), and LN metastasis and prognosis (33,34) (Table II). In addition to the VEGF family, other mediators are also involved in lymphangiogenesis, including platelet-derived growth factor-BB (35), insulin-like growth factor 1 and -2 (36), fibroblast growth factor 2 (37,38), hepatocyte growth factor (39) andangiopoietin-2 (40). The effects of these factors in GC require further investigation.

Chemokines are small secreted proteins that can enter inflammatory sites and secondary lymphoid organs via leukocyte recruitment. Chemokines and chemokine receptors function in a range of physiological processes, including cancer (41,42). In GC, CXC-chemokine receptor (CXCR) CXCR4 and CXCR2 were highly expressed in cancer tissues and predicted advanced tumor stage and poorer overall survival (43,44). C-C motif chemokine receptor 7 (CCR7) is a G protein-coupled receptor preferentially expressed on naive T cells and mature dendritic cells (45). A meta-analysis which included 1,697 participants with GC indicated that increased CCR7 expression was associated with negative clinicopathological prognostic factors including deeper tumor invasion and predicted a worse long-term survival outcome (46). The possible underlying molecular mechanism is that CCR7 contributes to TGF-β1-induced epithelial-mesenchymal transition (EMT), facilitating lymph node metastasis in patients with gastric cancer (47,48). Therefore CCR7 may potentially serve as a novel prognostic indicator and a potential target for gastric cancer therapy. At present, clinical trials of monoclonal

Table II. List of published studies regarding molecular diagnosis in gastric cancer.

Gene symbol	Gene name	Patients, n	Level of expression	Results	(Refs.)
MT1-MMP	Membrane type-1 matrix metalloprotease	810	Upregulated	Associated with distant metastasis and peritoneal dissemination	(12)
MMP-1	Matrix metallopeptidase 1	100	Upregulated	Associated with gastric wall invasion and LN metastasis	(15)
TIMP-1	Tissue inhibitor of metalloproteinases 1	100	Upregulated	Associated with poor prognosis	(15)
TIMP-3	Tissue inhibitor of metalloproteinases 3	92	Upregulated	Associated with a relatively poor disease free survival rate	(19)
VEGF-C	Vascular endothelial growth factor C	56	Upregulated	Associated with high peritumoral lymphovascular density	(33)
PCDH9	Protocadherin9	1,072	Downregulated	Inversely associated with wall invasion and LN metastasis	(55)
ICAM-1	Intercellular adhesion molecule 1	78	Downregulated	Associated with the number of involved LNs	(60)
nm-23	NME/NM23 nucleoside diphosphate kinase 1	101	Upregulated	Negatively associated with lymphatic invasion and distant metastasis	(64)
ANXA1	Annexin A1	1,072	64% reduction	Associated with an advanced T stage and LN metastasis	(65)
MACC-1	Metastasis-associated colon cancer-1	436	Upregulated	Associated with the presence of LN metastasis	(75)
EZR	Ezrin	436	Upregulated	Associated with LN and distant metastasis, and a relatively poor prognosis	(83)
EZR	Ezrin	277	Upregulated	Associated with LN metastasis and advanced clinical stages	(84)

LN, lymph node.

antibodies against CXCR4, CCR2 and CCR4 for cancer treatment are being conducted (49).

As the expression of cell adhesion molecules (CAMs) decreases, metastatic potential increases (50,51). CAMs, glycoproteins on the cell surface, are divided into four families: Lntegrins, selectins, cadherins and immunoglobulin superfamily CAMs. Integrins, the principal adhesion receptors of the ECM, are vital for multicellular organisms (52). A strong association has been identified between serum integrin β1 content and tumor staging, LN metastasis and distant metastasis (53). E-cadherin (E-cad) mediates calcium-dependent cell-cell adhesion (54). A recent study has demonstrated that abnormally expressed protocadherin9 is significantly associated with LN metastasis in patients with GC (55) (Table II). Selectins are adhesion molecules that mediate the initial leukocyte binding to the microvascular endothelium (56). E-Selectin levels were elevated in patients with GC with peritoneal metastasis and associated with poorer prognosis (57). P-selectin and L-selectin act as mediators for the interaction between platelets and leukocytes with circulating tumor cells during tumor dissemination (58). Intercellular adhesion molecule-1 (ICAM-1) can prevent cancer cells from being recognized and attacked by immunocytes, thus accelerating metastasis (59). Yashiro *et al* (60) suggested that ICAM-1 facilitates GC cell adhesion to immune cells, leading to immune tolerance (Table II). However, other studies have demonstrated an association between the serum levels of circulating ICAM-1 and GC tumor progression, with higher levels indicating a worse prognosis (53,61,62). On account of the contradictory results in these studies, ICAM-1 has not yet been established as a prognostic marker in GC.

4. Alterations in gene expression in metastatic LNs

Previous studies have demonstrated that the molecular patterns in metastatic LNs may differ from those in the primary tumor. It was identified in early research that the expression of a metastasis suppressor gene, NME/NM23 nucleoside diphosphate kinase 1, was different between primary and metastatic lesions in GC (63,64) (Table II). In our previous study, Annexin A (ANXA) 1 expression was revealed to be significantly increased in metastatic LNs compared with primary GC tumors or normal gastric tissue in (65) (Table II). Li *et al* (66) demonstrated that zinc finger protein 139 (ZNF139) regulated ANXA1 and ANXA5 expression, and promoted the LN metastasis of GC. ZNF139 is associated with multidrug resistance, and may promote GC invasion and metastasis (67,68).

Another study identified that primary tumors and metastatic LNs expressed a variable level of sialyl-related antigen in GC (69). This may have been caused by: i) Cancer cells producing sialyl-related antigens in the LNs once migrated from the primary tumor lesion; ii) in tumor progression, cancer cells that were unable to produce these antigens in the primary lesion obtaining the ability to produce sialyl-related antigens in the LNs; and iii) during the metastasis to LNs, cancer cells that could produce sialyl-related antigens in the primary lesion losing the ability to produce these antigens (69).

Connexins (Cxs) serve an important role in Gap junctions, which occur in most epithelial tissues (70). Mounting evidence suggests that Cx43 participates in tumor metastasis by

interacting with cell adhesion-associated proteins, including E-cad (71). The expression of Cx43 and E-cadherin was significantly increased in matched metastatic lymph nodes (MLNs) compared with primary gastric tumors. This may contribute to the efficient lymph node metastasis identified in gastric cancer (72).

These studies collectively suggest there is heterogeneity in gene expression between the primary tumor and LN metastases. In conclusion, considering gene status in the primary tumor alone may not be accurate enough to determine a suitable strategy for treatment.

5. Other mechanisms

MET encodes a tyrosine kinase receptor that is associated with cancer progression (73,74). In a recent study, the amplification of MET was observed in patients with GC, and was indicative of the likelihood of tumor invasion and LN metastasis (75). Furthermore, the role of MET gene amplification in c-MET overexpression and MET/hepatocyte growth factor (HGF) pathway activation have been generally recognized (76). The physiological activation of c-MET is induced by its natural ligand, HGF (77). The activation of c-MET by paracrine HGF is pivotal in GC pathogenesis (78). Metastasis-associated colon cancer-1 (MACC-1), a novel key regulator of HGF/MET signaling, can induce MET expression and facilitate tumor invasion and metastasis (75). It has been demonstrated that MACC-1 expression is correlated with c-Met expression in GC (79). Furthermore, silencing MACC-1 can inhibit VEGF-C/VEGF-D expression and thus inhibit lymphangiogenesis (80).

The cytoskeletal protein Ezrin, a member of the Ezrin-Radixin-Moesin family, functions as a molecular cross linker between actin filaments and proteins anchored in the cell membrane. Ezrin participates in various cellular processes relevant to aggressive tumor behavior and various phases of tumor metastasis (81,82). The immunohistochemical results from the analysis of436 GC samples demonstrated that high Ezrin expression in GC lesions was associated with LN and distant metastasis (83,84) (Table II).

6. Conclusions

GC is one of the deadliest types of cancer worldwide, with only a 5-20% 5-year survival rate in China (85). The prognosis for curative resection is unsatisfactory due to a high local recurrence rate, and early LN and systemic metastasis. Hence, it is vital to further investigate the molecular mechanisms in GC progression and identify new diagnostic markers. GC pathogenesis is a complicated process involving diverse alterations to genes and molecules, including the activation of oncogenes, the inactivation of tumor suppressor genes, and the dysregulation of the cell cycle (86). A large number of clinical studies have been performed regarding the molecular mechanisms and biological markers associated with GC cell invasion and metastasis (87,88). However, the molecular mechanisms of GC metastasis have yet to be completely characterized.

In the present review, the molecular mechanisms of GC LN metastasis have been discussed, including the following aspects: Cell adhesion and movement, ECM degradation, new

vessel formation, and molecular pattern differences between metastatic LNs and the primary tumor. In the future, research in this area should consider the molecular markers expressed specifically on metastatic LNs, to allow the detection of whether LNs are involved after surgery. Due to the complexity of the molecular mechanisms, the roles of a number of molecules remain controversial. Therefore, large-scale basic research and a large number of clinical specimens are necessary for developing a further understanding, and the screening of relevant molecules. Novel antitumor drugs targeting the newly identified molecular markers can then be developed to prevent GC metastasis at an early stage.

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