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Review article

Molecular mechanisms of chondrosarcoma metastasis

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ARTICLE INFO

Article history:
Received 15 November 2011
Received in revised form
30 December 2011
Accepted 12 January 2012
Available online 10 March 2012

Keywords:
chondrosarcoma
ICAM-1
integrin
migration
MMP

ABSTRACT

Chondrosarcoma is highly malignant, with a strong capacity for local invasion as well as distant metastasis. Surgical resection remains the primary mode of therapy. This cancer shows a predilection for metastasis to the lungs. This article will highlight numerous molecular mechanisms mediating cell motility, as described in such cases. Numerous experiments have demonstrated that upregulation of integrin and matrix metalloproteinases (MMPs) and intercellular adhesion molecule-1 (ICAM-1) expression lead to increased tumor cell migration and invasion. Data from these experiments suggest that targeting these pathways and molecules may enhance control of chondrosarcoma and decrease metastasis ratio.

1. Introduction

Chondrosarcoma is the second most common primary malignant bone tumor after osteosarcoma and most common in patients age 20 years or older [1]. It is a malignant bone tumor of chondrogenic origin, typically occurring in the fourth and fifth decades of life [2]. Chondrosarcoma can be divided into primary subtypes, based on histopathology: conventional, dedifferentiated, clear cell, and mesenchymal [3]. Local invasiveness and metastatic potential primarily depend on tumor grade and are primary predictors of clinical outcome [4]. Most diagnosed tumors (>90%) are of the conventional subtype, with approximately 90% of these showing pathology of low to intermediate grade (grades 1 and 2).

Chondrosarcomas primarily develop in the skull base in cartilaginous rests within the synchondroses of basilar skull bones [5]. These tumors are most commonly observed in the petrous portion of the temporal bone as well as in petro-occipital, spheno-occipital, and sphenopetrosal synchondroses areas. Location of these tumors highlights differences in bone development between the cranial vault and skull base. Bones of the cranial vault chiefly develop by intramembranous ossification; those in the skull base form by endochondral ossification and contain remaining chondrocytes, even in the mature skull [6]. Chondrosarcomas account for 6% of skull base tumors but for only 0.15% of all intracranial tumors. Most chondrosarcoma is characterized by low- and intermediate-grade conventional-type lesions with indolent growth and low metastatic potential. Only 12% of all skull base chondrosarcomas show mesenchymal type histopathology, associated with a higher grade and worse clinical prognosis [7]. Compared to chondrosarcomas with

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2211-8020/$ – see front matter
doi:10.1016/j.biomed.2012.01.001

conventional histopathologic traits, those with mesenchymal characteristics are associated with an approximately tenfold increase in 5-year mortality.

Chondrosarcoma is relatively resistant to radiotherapy and chemotherapeutic regimens [8,9], making surgical resection the primary treatment, with an average 5-year survival rate of 50% [8,10]. Because chondrosarcoma is highly malignant, with a strong capacity of local invasion and distant metastasis [11,12], an approach in which ability of a tumor to invade and metastasize is decreased may facilitate development of effective adjuvant therapy. Numerous studies have investigated signaling pathways involved in chondrosarcoma growth and invasion. This article summarizes molecular mechanisms involved in chondrosarcoma metastasis.

2. Metastasis of chondrosarcoma

Carcinomas metastasize following a complex succession of cell-biologic events, collectively termed invasion-metastasis cascade, whereby epithelial cells in primary tumors invade locally through surrounding extracellular matrix (ECM) and stromal cell layers; intravasate into lumina of blood vessels; survive transport through the vasculature; arrest at distant organ sites; extravasate into parenchyma of distant tissue; initially survive in foreign microenvironments for micrometastases; and reinitiate their proliferative programs at these metastatic sites [13]. Many such complex cell-biologic events are orchestrated by molecular pathways operating within carcinoma cells. Notably, nonautonomous cell interactions between carcinoma and nonneoplastic stromal cells play vital roles throughout the invasion-metastasis cascade. Deregulating these intrinsic and extrinsic signaling cascades allows incipient metastatic carcinoma cells to generate high-grade, life-threatening malignancy [13].

Chondrosarcoma is a highly malignant tumor with the potent capacity to cause local invasion and distant metastasis, preferentially in the lungs [14]. Recurrence typically occurs as pulmonary metastases or, less frequently, metastases to distant bones or as a local recurrence. Initial steps in pulmonary metastases resemble those of metastases to any other site. Primary tumor cells invade the surrounding normal tissue by producing proteolytic enzymes that traverse walls of small blood vessels in normal tissue or by enzymes induced by a tumor that then enters the circulation [15]. These tumors cells then travel to distant organ sites. These events have been described as inefficient because many cancer cells do not survive action of normal protective host-surveillance mechanisms during these initial stages [16,17]. Surviving cancer cells can enter the lungs and cause pulmonary metastases. Malignant cells must possess specific properties for this to occur: e.g., capacity for migrating to lungs and generating their own blood supply (Fig. 1). Each step entails important molecular interaction between tumor cells and normal host cells, each a potential target for development of drugs designed to abrogate the metastatic process. This article highlights several molecular mechanisms mediating cell motility described for human chondrosarcoma. Numerous experiments have demonstrated that upregulation of integrin and matrix metalloproteinases (MMPs), and intercellular adhesion molecule-1 (ICAM-1) and cyclooxygenase-2 (COX-2) expression lead to increased tumor cell migration and invasion.

2.1. *Integrin and metastasis of chondrosarcoma*

Integrins are transmembrane receptors that connect cells with their surrounding environments. This superfamily of cell adhesion receptors recognizes and primarily binds ligands of ECM, including fibronectin, laminin, collagen, and vitronectin [18]. Integrins, as primary receptors for cellular adhesion to ECM molecules, act as crucial transducers for bidirectional cell signaling, regulating cell survival, differentiation, proliferation, migration, and tissue remodeling [19]. Generally, α and β subunits noncovalently bind to form an $\alpha\beta$ heterodimer, with two subunits involved in ligand recognition. Ligand binding head present in extracellular domain is connected by two arms, each linked to intracellular domain of integrin by a single transmembrane helix. All α and β subunits consist of numerous extracellular domains; α subunits of most integrins possess a domain of approximately 200 amino acids known as inserted (I) domain or von Willebrand factor A domain [20]. Cytoplasmic tails of human integrins typically contain fewer than 75 amino acids. However, the β4 tail contains more than 100 amino acids. Commonly, length of the extracellular portion is $\leq$788 amino acids for β subunits and 1,104 amino acids for α subunits [20]. In humans, integrins consist of 18 α and 8 β subunits that covalently attach to form 24 unique $\alpha\beta$

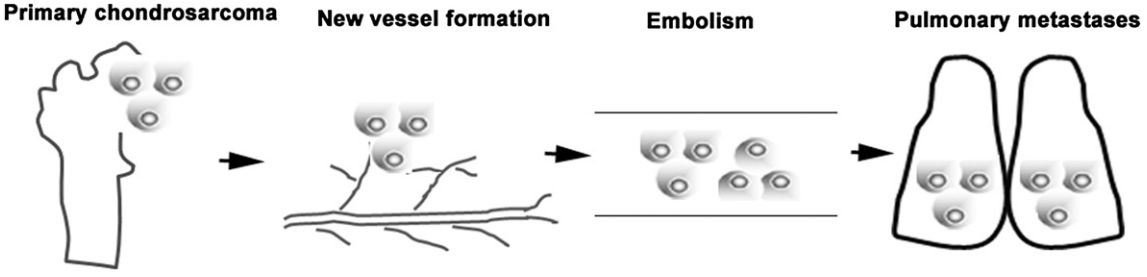

Fig. 1 – Metastasis of chondrosarcoma. Primary chondrosarcoma promotes new blood vessel formation. These blood vessels carry cancer cells to capillary beds in the lungs. Aggregates of tumor cells and other blood cells eventually form embolisms in distant lung capillaries. These cancer cells can then adhere to vascular endothelial cells to escape blood vessels. While entering the lung, these cells are exposed to factors in the microenvironment that support metastases.

transmembrane heterodimers, depending on cell type and cellular functions [21]. After ligand binding, integrin clustering occurs due to conformational changes, activating a signaling cascade and recruiting multiprotein complexes to focal adhesions.

Progression of several diseases is also related to modulation of integrin function and abnormal integrin expression: deleterious embryonic development, autoimmune diseases, cardiovascular diseases, and cancer [21]. Several studies reveal integrin signaling as altered in cancer cells to facilitate cancer progression by mediating metastasis, tumor invasiveness, tumor-induced angiogenesis, lymphangiogenesis, desmoplasia, and inflammation [22]. Integrins provide an appropriate tumor microenvironment during tumorigenesis via crosstalk with growth factor receptors and oncogenes. Modification in epigenetic regulation of integrin and integrin-linked kinase gene expression is directly related to carcinogenesis and cancer stem cell formation [22]. Activation and elevated expression of integrin-coupled signaling effectors have been implicated in a wide variety of human cancers, such as in the breast, colon, prostate, and ovaries [22]. Additionally, integrin has been implicated in metastasis of the lung, breast, bladder, and colon [23–25].

Integrins α2, α5, αv, β1, and β3, all highly expressed in chondrocytes, are the focus of multiple functional studies examining effect of integrin upregulation in chondrosarcoma cell migration [26–31]. Most data were acquired via Transwell cell migration and wound healing assays to examine migration and invasion activity of chondrosarcoma cells (JJ012 and SW1353 cell lines). Integrin αvβ3 expression is thought to be regulated by activation of transforming growth factor-β (TGF-β), stromal cell-derived factor-1 (SDF-1), leptin, glial cell-derived neurotropic factor, tumor necrosis factor-α (TNF-α), and interleukin-8 (IL-8) [26–31] (Table 1). Stimulation of cells with these factors induced cell migration and αvβ3 integrin expression in human chondrosarcoma cell lines. Pretreatment of cells with αvβ3 antibody or inhibitor (RGD) reduced cell motility of human chondrosarcoma cells; transfection of cells with αv or β3 integrin small interfering RNA (siRNA) also reduced cell migration of human chondrosarcoma cells. Yet αvβ3 integrin as a functional receptor induces expression of cysteine-rich protein 61 (Cyr61), connective tissue growth factor (CTGF) and osteopontin, along with nephroblastoma overexpressed (NOV)-mediated cell migration and invasion [32–35]. Hence, inside-out and outside-in integrin signaling is involved in metastasis of chondrosarcoma. Integrin α2β1 is a key factor in regulating migratory activity of chondrosarcoma cells. Prior results linked α2β1 integrin with cyclooxygenase-2 (COX-2)-, bradykinin-, and adiponectin-mediated cell migration of chondrosarcoma cells [36–38] (Table 1). Stimulation of cells with these factors increased cell surface and protein levels of α2β1 integrin in human chondrosarcoma cells. Cell migratory rates were reduced to control levels by addition of a monoclonal antibody against integrin α2β1. Furthermore, α5β1 integrin mediated insulin-like growth factor-I (IGF-I) cell motility of chondrosarcoma cells [39] (Table 1). Therefore, αvβ3, α2β1, and α5β1 are major integrin components in chondrosarcoma metastasis.

Table 1 – Integrin and metastasis of chondrosarcoma.

Integrin	Activators
αvβ3	TGF-β, SDF-1, leptin, GDNF, TNF-α, IL-8
α2β1	COX-2, bradykinin, adiponectin
α5β1	IGF-I

COX-2 = cyclooxygenase-2; IGF-I = insulin growth factor-I; IL-8 = interleukin-8; SDF-1 = stromal cell-derived factor-1; TGF = transforming growth factor-β; TNF = tumor necrosis factor-α.

2.2. *MMPs and metastasis of chondrosarcoma*

MMPs, or matrixins, a subfamily of metalloproteases, comprise 23 distinct proteases in humans. MMP was first identified in 1962 as responsible for degradation of fibrillar collagen in tadpole tails during metamorphosis and thus termed interstitial collagenase [40]. After identification of a similar collagenase in human skin, this protease was renamed MMP-1. MMPs have since been identified as major enzymes responsible for turnover of the ECM by proteolytic degradation of virtually all proteinaceous ECM components [41]. MMPs are primarily excreted proteins with several conserved domains. All contain a catalytic domain shielded by prodomain in an inactive form of the enzyme; this propeptide interacts with a catalytic region through conserved cysteine residue and Zn^{2+} ion in the catalytic pocket. Except for MMP-7, MMP-23, and MMP-26, all MMPs contain a C-terminal hemopexin-like domain that functions primarily as a substrate recognition sequence [42]. Although MMPs retain catalytic activity toward a wide range of substrates when this domain is absent, the hemopexin domain, which has a four-blade propeller structure with each blade consisting of four antiparallel β-sheets and one α-helix, is essential for degradation of triple-helical collagens [43]. Gelatinases (MMP-2 and -9) contain a series of three fibronectin type II inserts in the catalytic domain, which facilitate binding of gelatin and collagen [43].

MMP function is regulated at several levels. First, induction of gene expression is controlled by numerous growth factors and cytokines and may be suppressed by TGF-β and glucocorticoids. Recent studies indicate a pivotal modulatory role for epigenetic processes in MMP expression [44]. In addition to regulation by soluble factors, MMP expression may also be regulated by cell-cell contact or interaction of cells with ECM components such as ECM metalloprotease inducer or CD147 (EMMPRIN). Expressed MMPs are largely excreted as inactive proenzymes, with propeptide effectively limiting substrate entrance into and catalysis in the catalytic pocket by blocking the catalytic zinc (II) ion via a cysteine switch mechanism. Activation of proMMPs occurs through several mechanisms, all of which disrupt cysteine switch. The most important mechanism may be proteolytic removal of the prodomain by other endopeptidases such as furin [45]. Removal of the MMP prodomain, which contains a furin-like proprotein convertase recognition site, has been described for nine MMPs, including all membrane-type MMPs. Alternatively, the prodomain can be proteolytically removed by plasmin and other serine proteases or even other MMPs. This mechanism is well described for MMP-2 for which proMMP-2 binds the

endogenous MMP inhibitor tissue inhibitor of metalloproteases 2 (TIMP-2). This complex in turn serves as ligand for membrane-bound MMP-14 (or membrane type 1 MMP), leading to activation of MMP-2 [45].

MMPs play vital roles in many processes—cell proliferation, differentiation, apoptosis, migration—through degradation of both matrix and nonmatrix substrates [46–48]. These processing enzymes exhibit linkage to cancer and tumor progression, during which proteolysis of the ECM is required to accommodate increased growth, migration, and invasion of tumor cells. Expression of MMP-1, MMP-2, MMP-3, MMP-9, and MMP-13 has been demonstrated in human chondrosarcoma cells [49]. MMP-1 is particularly important in chondrosarcoma, as it is upregulated in locally invasive tumors and is responsible for degradation of collagen in cartilaginous tissue. Transfection of cells with MMP-1 siRNA reduced chondrosarcoma cell motility [50]. In contrast, thrombin and Wnt-induced secreted protein-1 (WISP-1) induced cell migration via upregulation of MMP-2 [51,52]. Stimulation of cells with thrombin and WISP-1 increased MMP-2 enzyme and protein expression. An MMP-2 siRNA and inhibitor also reduced motility of chondrosarcoma cells. MMP-3 is secreted as an inactive soluble proform, activated by a variety of proteases [49] (Table 2). Tang et al. [53] demonstrated that C-C motif ligand 5 (CCL5) enhances MMP-3 expression and cell migration. MMP-3 inhibitor or siRNA blocked CCL5-mediated cell migration and invasion. By contrast, MMP-9 upregulation is involved in osteopontin-mediated cell motility [34] (Table 2). Most effective for degrading type II collagen is MMP-13, thought to participate actively in situations that require rapid and effective remodeling of collagenous ECM is [49]. Previous studies show MMP-13 involved migration of human chondrosarcoma cells induced by bone morphogenetic protein-2 (BMP-2), Cyr61, CTGF, NOV, and IL-6 expression [32,33,35,54,55] (Table 2). MMP-1, MMP-2, MMP-3, MMP-9, and MMP-13 are major MMPs contributing to metastasis of chondrosarcoma cells.

2.3. *Intercellular adhesion molecule-1 and metastasis of chondrosarcoma*

ICAM-1 (also known as CD54), a member of the immunoglobulin (Ig) supergene family, is an inducible surface glycoprotein, primarily expressed in leukocytes, endothelial cells, and fibroblasts, which mediates adhesion-dependent, cell-cell interactions [56,57]. ICAM-1 possesses five extracellular Ig-like domains, a transmembrane segment, and a short cytoplasmic tail. Human ICAM-1 has five extracellular Ig-like domains, each presenting differences in ligand specificity: e.g., lymphocyte function-associated antigen-1 (LFA-1) antigen shows a binding preference for the first Ig domain, whereas Mac-1 binds to the third domain of extracellular regions of a molecule. The first Ig domain also harbors a binding site for major group human rhinoviruses and *Plasmodium falciparum*-infected erythrocytes [56,57]. The extracellular domain of ICAM-1 is crucial for transendothelial migration of leukocytes from the capillary bed into the tissue [58], and ICAM-1 may facilitate movement (or retention) of cells through the ECM [58].

The variable binding ability of ICAM-1 is directly related to the multifunctional physiologic and biologic roles of this molecule. Participation of ICAM-1 in inflammatory processes and in migration of activated leukocytes in inflammatory foci, first studied in the skin, is well established [59]. This complex process is mediated by several adhesive molecules expressed on both leukocyte and endothelial membrane. ICAM-1 is also a potent costimulatory molecule in T cell-mediated cytotoxicity; contribution of this adhesive molecule to host immune response led to a hypothesis that inhibition of ICAM-1 expression may correlate with cancer, because cancer is in fact a cascade of reactions closely connected to loss of normal immune surveillance. Conformational change in ICAM-1 and/or significantly altered concentration of its soluble form will positively correlate with breast cancer, hematologic malignancies, gastrointestinal cancer, and melanoma [59]. ICAM-1 plays a key role in lung cancer cell invasion [60]. ICAM-1 antibody or antisense ICAM-1 complementary DNA is likewise reported to reduce invasiveness of breast cancer cells [61]. ICAM-1 regulates motility of human chondrosarcoma cells [62]. Additionally, protein levels of ICAM-1 in human chondrosarcoma patients were significantly higher than in healthy patients. Treatment of chondrosarcoma with CCN6 demonstrably induces ICAM-1 mRNA and protein expression. Pretreatment of cells with ICAM-1 Ab or transfection of cells with ICAM-1 siRNA reduced CCN6-induced cell migration [62]; ICAM-1 thus plays an important role in motility of chondrosarcoma cells.

2.4. *COX-2 and metastasis of chondrosarcoma*

COXs are rate-limiting enzymes that catalyze conversion of arachidonic acid to prostaglandins (PGs). Two COX isoforms with distinct tissue distributions and physiologic functions have been identified [63]. COX-1 is constitutively expressed in many tissues and important for control of homeostasis [64]. Conversely, COX-2 is an inducible enzyme activated by extracellular stimuli such as growth factors and proinflammatory cytokines [65]. Recent investigations indicate overexpression of COX-2 frequently observed in many types of cancer (colon, lung, breast, pancreas, head, and neck) [66–68], typically associated with poor prognosis and short survival time. Four prostaglandin E (PGE) receptor subtypes have been identified (EP1-EP4) and their effects on human cancer cells analyzed [69]. Studies show EP1 as coupled to Ca^{2+} mobilization; EP2 and EP4 activate adenylate cyclase, whereas EP3 inhibits adenylate cyclase [69,70]. These studies indicate cancer cells expressing multiple subtypes of PGE receptor;

Table 2 – MMPs and metastasis of chondrosarcoma.

MMPs	Activators
MMP-2	Thrombin, WISP-1
MMP-3	CCL5
MMP-9	Osteopontin
MMP-13	BMP-2, Cyr61, CTGF, NOV, IL-6

BMP-2 = bone morphogenetic protein-2; CCL5 = C-C motif ligand 5; CTGF = connective tissue growth factor; Cyr61 = cysteine-rich protein 61; IL-6 = interleukin-6; MMP = matrix metalloproteinase; NOV = nephroblastoma overexpressed; WISP-1 = Wnt-induced secreted protein-1.

each subtype may link to diverse actions of PGE_2. Liu et al. [36] reported overexpression of COX-2 or exogenous PGE_2, increasing migration of human chondrosarcoma cells. Also, human chondrosarcoma tissues and chondrosarcoma cell lines significantly expressed COX-2 at levels above those in normal cartilage. Using pharmacologic inhibitors, activators, or genetic inhibition of EP receptors, Liu et al. found the EP1 receptor, not other PGE receptors, involved in PGE_2-mediated cell migration [36]. COX-2/PEG2/EP1 axis thus plays a vital role in metastasis of chondrosarcoma cells.

3. Discussion

Unlike in cases of mesenchymal malignancies such as osteosarcoma and Ewing sarcoma in which dramatic increases in long-term survival are reported with the advent of systemic chemotherapy, cases of chondrosarcoma continue to show poor prognosis because of lack of effective adjuvant therapy [12]. The metastatic potential of conventional chondrosarcomas correlates well with histologic grade of the tumor. Because of the relatively indolent growth rates of many low- and moderate-grade chondrosarcomas, approximately 15% of patients die of metastatic disease more than 5 years after initial diagnosis [12]. It thus is important to develop an effective adjuvant therapy for preventing chondrosarcoma metastasis. This article summarized recent studies examining metastasis of chondrosarcoma. Identifying signal pathways increases understanding of human chondrosarcoma metastasis, which may yield effective therapy.

Acknowledgments

This study was supported by grants from the National Science Council of Taiwan (NSC99-2320-B-039-003-MY3; NSC 100-2320-B-039-028-MY3).

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