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# Cyclooxygeanse-2 promotes metastasis in osteosarcoma

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## Abstract

Cyclooxygenase-2 (COX-2), an inducible form of the enzyme that catalyzes the first step in the synthesis of prostanoids, is associated with carcinogenesis, which is suspected to promote angiogenesis and tissue invasion of tumors and resistance to apoptosis. COX-2 is also involved in metastasis and poor prognosis of cancer. Osteosarcoma with COX-2 positivity is from 67 to 92 %. COX-2-positive rate in metastatic lesions was greater than that of biopsy and/or resected samples of the primary site in osteosarcoma. And, what role does COX-2 play in osteosarcoma metastasis? Genetic studies support a cause-effect connection between COX-2 and tumorigenesis. COX-2 expression had a poor prognosis with regard to metastasis, and patients with increased COX-2 expression in lung metastases died of the disease. COX-2 expression has also been established as a marker in human osteosarcoma, and COX-2 inhibition has been suggested as a possible way of improving therapeutic outcome. In addition, COX-inhibitors inhibit the tumor initiation, matrix metalloproteinases (MMPs), cell differentiation and T cell proliferation and suppression of the antitumor activity of natural killer cells and macrophages, angiogenic mechanism. Therefore, we can exert the COX-inhibitors to potentialize the effects of chemotherapeutic agents, and reverse the metastasis in osteosarcoma to facilitate the patient who may benefit from addition of COX-inhibitors to standard cytotoxic therapy.

**Keywords:** COX-2, COX-inhibitors, Metastasis, Osteosarcoma

## Introduction

Cyclooxygeanse-2 (COX-2) is overexpressed in most solid tumors, such as colorectal, liver, pancreatic, breast, lung cancer as well as osteosarcoma [1–6]. The activity of COX-2 is suspected to promote angiogenesis, tissue invasion of tumors [7], metastasis [8, 9], and resistance to apoptosis [10, 11]. Genetic studies support a cause-effect connection between COX-2 and tumorigenesis. Therefore, we can exert the drugs to affect COX-2 and achieve the therapies of human malignancies. Both non-selective non-steroidal anti-inflammatory drugs (NSAIDs), and selective COX-2 inhibitors can inhibit proliferation, invasiveness of tumors.

Osteosarcoma is the most common primary bone tumor generally affecting children and young adults which has been reported to express COX-2 constitutively. Approximately 20 % of patients present with lung metastases at initial diagnosis, additionally, in 40 % of patients metastases occur at a later stage. As we know, osteosarcoma with COX-2 positivity is from 67 to 92 % [9, 12, 13]. Dickens et al. [12] reported the COX-2-positive rate in metastatic lesions was greater than that of biopsy and/or resected samples of the primary site in osteosarcoma. And, what role does COX-2 play in osteosarcoma metastasis?

## Cyclooxygenase

The cyclooxygenases (COX) are enzymes, known as prostaglandin (PG) rate-limiting synthase, catalyze the metabolism of arachidonic acid (AA) to PGs. Finally, a series of biologically active prostaglandins (PGD2, PGE2, PGF2α, and PGI2) and thromboxane A2 (TXA2) are formed. There are three isoforms of the enzyme that have been identified: COX-1, COX-2, and COX-3 [14]. COX-1 is considered a “housekeeping enzyme”, constitutively expressed in human cells. COX-3, an alternate splice variant of COX-1, is most abundant in the canine cerebral cortex. COX-2 is an inducible enzyme and is

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associated with inflammatory diseases and carcinogenesis, which is suspected to promote angiogenesis and tissue invasion of tumors [7, 15].

## Molecular factors in metastatic osteosarcoma

The metastatic cancer cells subsequently complete the following steps: Invasion through the extracellular host matrix and entrance into the circulation (I), survival in the circulation (II) and evasion of the host immune system (III), arrest and extravasation at a target organ site (IV), adherence and survival in the target organ microenvironment (V, VI) and finally formation of neovasculature to allow growth at the target organ site (VII) [16–21]. PosthumaDe-Boer J [16] reported that there are many molecular alterations as target for therapy in metastatic osteosarcoma: (I) Migration and invasion MMPs, m-Calpain, Wnt, Src, Notch; (II) a Anoikis resistance PI3K/Akt, Src/PI3k/Akt, Src/Ras/MAPK, NF-κB, Wnt/β-catenin, BcL family, (II) b Apoptosis resistance Src, NF-κB, Wnt/β-catenin, Fas/FasL; (III) Evasion of immune system HLA-1, IL-10, Fas; (IV) Arrest and extravasationCXCR4-CXCL12,CXCR3-CXCL9-11, CXCR4/MMPs, CXCR3-4/Erk/NF-κB; (V) Adherence Ezrin/MAPK/Akt, Ezrin/β4-Integrin/PI3K, CD44/Akt/mTOR, (VI) Dormancy Integrin-α5β1, Integrin-α5β1/Erk/p38, Bcl-XL, IGF/PI3K, ECM; (VII) Angiogenesis and proliferationEGFR. PDGFR, VEGF, IGFR, TGF-β, MMPs, VEGF/Erk/NF-κB, VEGF/PI3K, EGFR/Src/Ras/MAPK/STAT3, Src, Integrin/PI3K/Erk1-2, Wnt/β-catenin/CyclinD-Survivin.

## COX-2 promotes metastasis in osteosarcoma

COX-2 overexpression in osteosarcoma increases cell mobility and invasiveness, which correlates with the occurrence of distant metastasis in patients with osteosarcoma and also may affect post-metastatic survival [8].

The cancer stem cells (CSCs) share several characteristics with embryonic and somatic stem cells including self-renewal and differentiation abilities, and represent a small fraction of the cellular population of the tumor. Osteosarcoma CSCs have been identified in humans and dogs suggesting that these cells may be responsible for treatment failure in this disease [22, 23]. Pang LY [24] reported that global transcriptional analysis and comparison with parental cells identified COX-2 expression to be significantly increased in this population. They identified that COX-2 was expressed 141-fold more in CSC spheres than daughter adherent cells. Meanwhile, COX-2 expression is elevated in cancer stem cells, which is required for tumoursphere formation, and tumourspheres increased invasiveness and tumourigenicity. They found that COX-2 inhibition had no effect on CSC growth, or resistance to chemotherapy. However inhibition of COX-2 in daughter cells prevented sphere formation, indicating a potential significant role for COX-2 in tumor initiation.

Invasion is mediated mainly by matrix metalloproteinases (MMPs). Among them, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are known to be strongly correlated with metastatic potential of cancer cells [25]. Lee EJ [26] reported that COX-2 overexpression enhanced mobility and invasiveness of U2OS cells, which was accompanied by increases of matrix metalloproteinase-2 and -9 (MMP-2 and -9) activities. Selective COX-2 inhibitors, NS-398 and celecoxib, inhibited cell proliferation and abrogated the enhanced mobility, invasiveness and MMP activities induced by COX-2 overexpression.

In our study, we reported that Celecoxib, a cyclooxygenase-2 inhibitor, induces apoptosis in human osteosarcoma cell line MG-63 via down-regulation of PI3K/Akt [27]. PI3K/Akt plays an essential role in the cell/extracellar matrix (ECM) and cell/cell adhesion. Lack of the correct adhesion, the adhesion-dependent signals will be interrupted, which will result in adhesion-related apoptosis: anoikis [28]. Activation of PI3K/Akt signaling has been shown to mediate survival signals triggered by the engagement of E-cadherin [29] and other classical cadherins [30, 31]. One possible connection between integrins and β-catenin is the integrin-activated, antiapoptotic kinase PKB/Akt. PKB is known to inhibit the activity of glycogen synthase kinase 3-β, a serine kinase that functions directly to reduce β-catenin protein and signaling [32, 33]. It has been established that brain-derived neurotrophic factor (BDNF) binding to TrkB receptors results in a highly specific receptor autophosphorylation [34] and in turn activates the PI3K/Akt pathway [35]. Another target of the PI3K/Akt pathway may be survivin [36]. Survivin is a member of the inhibitor of apoptosis protein (IAP) family, the regulation of survivin by adhesion was examined.

PGE2, downstream of COX-2, can regulate immune function through inhibition of dendritic cell differentiation and T cell proliferation and suppression of the antitumor activity of natural killer cells and macrophages [37].

COX-2 inhibitors blocked tumor growth via an antiangiogenic mechanism [38]. VEGF plays a key role in angiogenesis since it stimulates almost every step in the angiogenic process [39, 40]. Other factors can stimulate angiogenesis include EGF, bFGF, hepatocyte growth factor, interleukin-8, and placental growth factor [41, 42]. Immunohistochemical analysis for VEGF revealed that COX-2 inhibitor prominently suppressed the expression of VEGF in lung metastatic lesion compared with control treatment. In addition, it also reduced VEGF, EGF and bFGF mRNA and protein expression [43]. In vivo, high doses of meloxicam suppressed LM-8 tumor growth and lung metastasis [6].

## Conclusion

In this review, we have tried to encompass the role of COX-2 in the regulation metastasis in osteosarcoma. COX-2 expression and the abundance of its enzymatic product PGE2 play key roles in the development of cancer. Genetic studies support a cause-effect connection between COX-2 and tumorigenesis. COX-2 expression had a poor prognosis with regard to metastasis, and patients with increased COX-2 expression in lung metastases died of the disease. COX-2 expression has also been established as a marker in human osteosarcoma, and COX-2 inhibition has been suggested as a possible way of improving therapeutic outcome [8]. Therefore, COX-2 inhibitors may prove to have a therapeutic role in counteracting the metastasis of osteosarcoma.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

Both LQ and BL carried out reading the literature and drafting the manuscript. Both authors read and approved the final manuscript.

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