The Role of Integrins in Osteosarcoma

Vi Nguyen, Alan Nguyen, Carolyn A. Meyers, Jia Shen, Michelle A. Scott, Aaron W. James

Integrins are cell adhesion molecules that link the extracellular matrix to the cytoskeleton and play a critical role in diverse cellular processes including gene transcription, signal transduction, proliferation, differentiation, migration, and apoptosis. Integrins are central to cancer biology and have been implicated in the pathogenesis and metastasis of osteosarcoma (OS). Increased αvβ3 integrin expression is directly correlated to the metastatic potential of multiple human OS cell lines. Additionally, αvβ3 integrin interaction with FPRL-1 and TIMP-1 enhances both chemotactic and anti-apoptotic ability in OS cells. Likewise, β1 integrins enhance OS tumor invasiveness and metastasis by mediating cellular migration in response to chemokine stimulation. In summary, integrins hold potential as novel therapeutic targets due to their critical role in regulating cellular adhesion to bone matrix and consequently OS pathogenesis. Although integrins have been thoroughly studied in pre-clinical settings, pharmacological inhibition of integrins has not been successfully translated to clinical practice and is still under evaluation to elucidate its safety and efficacy in human patients.
The 143B-LM4 variant, which displayed an 18-fold higher metastatic rate compared to the 143B parent cell line, when transplanted orthotopically into nude mice tibia, correspondingly exhibited a 6-fold higher level of αvβ3 integrin expression[20]. Likewise, Duan et al.[21] generated LM2-LM7 sublines with increasing lung metastatic potential from the parental SAOS cell line and observed a direct correlation between αvβ3 integrin expression and metastatic rate. Compared to the parental non-metastatic SAOS cells, LM7 cells demonstrated increased chemotactic activity to lung tissue[21]. Furthermore, this migratory ability was specifically enhanced in lung tissue, as migration of LM7 cells through lung endothelial cells was higher than in liver endothelial cells[21]. Significantly, this clinically corresponds to how OS metastasis almost exclusively occurs in the lung. Additionally, LM7 cell adhesion to vitronectin, a glycoprotein found in the extracellular matrix of bone, decreased approximately 14-fold after addition of the αvβ3 integrin antagonist echistatin[21].

### Osteosarcoma (OS)

OS is the most common primary bone malignancy, with approximately 1,000 new diagnoses in the United States annually[16,17]. Although the advent of multimodal therapy has significantly improved survival rates for patients without clinically detectable metastatic OS, prognoses remain bleak for patients with metastatic OS, who exhibit a dismal five-year survival rate of 19-21% and a median survival duration of 17.5 months[18,19]. Thus, there is a need to develop more efficient treatment strategies to combat the detrimental effects of metastasis. Integrins have arisen as a key area of investigation due to their role in a phenomenon known as anchorage-independent growth[1]. Due to altered integrin expression patterns, carcinogenic cells are able to detach from the primary tumor site, contributing to tumor invasion and metastasis[11]. Consequently, it is critical to delve deeper into the specific roles of different integrins in OS as they represent a potential novel therapeutic target.

### Review Criteria

For the current review, NCBI PubMed search terms were used to identify articles of interest, including “osteosarcoma, integrins, pathogenesis and metastasis”. Articles were then filtered by relevance by the corresponding author. An effort was made to include work published within the last 10 years, and include earlier works only when directly relevant.

### αvβ3 Integrin in Osteosarcoma

Normally expressed by endothelial cells, the αvβ3 integrin has been found to be upregulated in tumor vasculature[20]. Specifically, various studies have found that altered expression of αvβ3 in tumor cells is associated with metastatic OS tumorigenesis[20,21]. For example, Tome et al.[20] evaluated αvβ3 integrin expression in LM1-LM4 sublines of the 143B human OScell line with increasing metastatic potential.

### Table 1 The Role of Specific Integrins in Osteosarcoma.

<table>
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<th>Integrin</th>
<th>Role in Osteosarcoma</th>
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<tr>
<td>αvβ3</td>
<td>• Expression levels directly correlate to the metastatic potential of multiple human OS cell lines including 143B, SAOS, U2OS, and MG-63&lt;br&gt;• αvβ3 integrin interaction with FPRL-1 and TIMP-1 enhances both chemotactic and anti-apoptotic ability in OS&lt;br&gt;• Addition of monoclonal antibodies specific to αvβ3 and α5β1 inhibits CXCR4-dependent HOS tumor growth in vivo&lt;br&gt;• Administration of anti-αvβ3 integrin antibody (AIL2B) in mice inhibits metastasis of 143B cells to the lungs and enhances survival rates&lt;br&gt;• Regulates OS tumor cell invasiveness by mediating the attachment of cells to type I collagen&lt;br&gt;• Significantly upregulated expression of αvβ3 integrin found in chemically (HOS-MNNG) and virally (KHOS-NP) transformed HOS subclones&lt;br&gt;• Increased αvβ3 expression is concomitant with increased Mg2+-dependent cell adhesion to type I collagen and accelerated migration and invasion rates&lt;br&gt;• Regulates hematopoiesis by mediating adhesion between U2OS and SAOS cell lines and TSP-1, which confers invasive and metastatic ability&lt;br&gt;</td>
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<td>α2β1</td>
<td>• OS tumor cell lines with increased metastatic potential (U2OS and IOR/OS9) show increased expression of α2β1, α5β1, and αvβ3&lt;br&gt;• Administration of anti-α2β1 integrin antibody (AIIB2) in mice inhibits CXCR4-dependent HOS tumor growth in vivo&lt;br&gt;• Administration of anti-α2β1 integrin antibody (AIIB2) in mice inhibits CXCR4-dependent HOS tumor growth in vivo&lt;br&gt;</td>
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<td>α5β1</td>
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<td>α6β1</td>
<td>• Administration of anti-α6β1 integrin antibody (AIIB2) in mice inhibits metastasis of 143B cells in the lungs and enhances survival rates&lt;br&gt;</td>
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Figure 1 Schematic of Anti-Apoptotic Integrin Signaling Cascade. FAK: focal adhesion kinase; PI3K: phosphatidylinositol 3-kinase; PLA2: phospholipase A2; PKC: protein kinase C.
Other studies have also evaluated the cross-talk between αvβ3 integrin and various pathways that play a role in chemotaxis and apoptosis\(^\text{22-23}\). For example, Ren et al\(^\text{24}\) found that serum amyloid A (SAA), which has been shown to regulate integrin expression and cytoskeleton arrangement, enhances the migratory and invasive ability of U2OS cells in a concentration and time-dependent manner\(^\text{23}\). Furthermore, the inhibition of formyl peptide receptor-1 (FPRL-1), an SAA receptor which regulates chemotaxis, corresponded to down regulation of SAA-induced αvβ3 integrin expression\(^\text{23}\).

In another study, Tsagaraki et al\(^\text{25}\) demonstrated that the interaction between αvβ3 integrin and tissue inhibitor of metalloproteinase-1 (TIMP-1) confers resistance in the MG-63 OS cell line against apoptosis induced by tumor necrosis factor-α (TNF-α). Previous studies have correlated TIMP-1 expression to the inhibition of apoptosis\(^\text{27,28}\), and elevated TIMP-1 levels among cancer patients corresponded to poorer survival rates and increased chemotherapy resistance\(^\text{27}\). In the specific context of OS, treatment of MG-63 cells with TNF-α resulted in a 5-fold increase of TIMP-1 secretion and upregulation of αvβ3 integrin, which was concomitant with apoptotic resistance\(^\text{22}\). Moreover, addition of the integrin inhibitor echistatin disrupted TIMP-1 ligation to αvβ3 and significantly decreased apoptotic resistance\(^\text{27}\).

In summary, increased αvβ3 integrin expression in tumor cells is directly correlated to the metastatic potential of multiple human OS cell lines including 143B, SAOS, U2OS, and MG-63\(^\text{29-31}\). Furthermore, αvβ3 integrin interaction with FPRL-1 and TIMP-1 enhances both chemotactic and anti-apoptotic ability in OS cells\(^\text{22,23}\). Thus, this study elucidated how the αvβ3-FPRL-1 pathway plays a critical role in modulating OS metastasis.

β1 Integrins in Osteosarcoma

β1 integrins have been implicated in tumorigenesis, as they mediate cellular migration in response to chemokine stimulation\(^\text{9}\). For example, Miura et al\(^\text{32}\) used human OS (HOS) transfected cells expressing the CXCR4 receptor for stromal cell-derived factor-1 (SDF-1) to investigate the role of the β1 integrin-chemokine axis in primary tumor development. First, they observed that β1 integrin ligand specificity is dependent on the α-subunit it is attached to. Whereas the α2β1 integrin mediates the chemotactic response to SDF-1 on type I collagen and laminin, the α4β1 and α5β1 integrins mediate SDF-1-stimulated chemotaxis on fibronec tin\(^\text{34}\). Additionally, expression of CXCR4 confers a chemotactic response to SDF-1 and enhances HOS transfected cell growth\(^\text{34}\). Furthermore, both migratory ability and level of tumor growth were dependent on the level of CXCR-4 expression\(^\text{34}\). However, addition of monoclonal antibodies specific to α2β1 and α5β1 inhibited this CXCR4-dependent HOS tumor growth in vivo. Thus, the β1 integrin-SDF-1 axis plays a central role in mediating OS tumor migration and growth.

In another study, Scotlandi et al\(^\text{35}\) compared integrin expression level in different OS cell lines. OS tumor cell lines with increased metastatic potential (U2OS and IOR/OS9) showed increased expression of α2β1, α5β1, and α6β1\(^\text{35}\). In contrast, OS lines with decreased metastatic potential (Saos-2 and SARG) correspondingly showed reduced integrin expression\(^\text{31}\). Of note, Scotlandi et al\(^\text{35}\) also determined that β1 ligand specificity is dependent upon the bound α-subunit, as they observed that α2β1, α5β1, and α6β1 served as receptors for collagens, fibronectin, and laminin respectively\(^\text{34}\).

In a complementary study, Kimura et al\(^\text{36}\) analyzed the effect of integrin inhibition on the in vivo metastatic ability of 143B OS cells. Mice with orthotopically-growing 143B cells in the tibia were injected with either control anti-rat IgG1 antibody or anti-β1 integrin antibody, AIIB2. AIIB2 inhibited seeding of 143B cells on the lung, which served as an experimental assay for metastasis, by approximately 6-fold\(^\text{31}\). Furthermore, the survival rate of the AIIB2 mice was approximately 5-fold of the control group. In summary, β1 integrin plays a critical role in OS lung metastasis and the AIIB2 antibody presents a potential therapeutic mechanism that should be tested in clinical trials.

α2β1 Integrin

The α2β1 integrin regulates OS tumor cell invasiveness by mediating the attachment of cells to type I collagen, the main matrix found in bone tissue\(^\text{29}\). For example, Vihinen et al\(^\text{37}\) proposed that OS progression results from altered interactions with the extracellular matrix, and consequently analyzed the expression of different integrin receptors in 8 HOS cell lines. They found significantly upregulated expression of α2β1 integrin in chemically (HOS-MNNG) and virally (KHOS-NP) transformed HOS subclones, which are both tumorigenic compared to the non-tumorigenic parental HOS cell line\(^\text{29}\). Furthermore, this increased α2β1 expression was concomitant with increased Mg2+-dependent cell adhesion to type I collagen and accelerated migration and invasion rates\(^\text{29}\).

In a similar study, Santala et al\(^\text{38}\) analyzed the expression of matrix proteins and corresponding integrin receptors in the HOS-MNNG and KHOS-NP transformed tumorigenic subclones. Transformation concomitantly decreased type I collagen mRNA levels, induced α2β1 integrin synthesis, and increased cell adhesion to type I collagen, suggesting that downregulated type I collagen protein synthesis correlates to its reduced ability to create its own matrix and leads to new interactions with integrin receptors\(^\text{29}\).

To summarize, through regulating cell adhesion to the type I collagen found in bone tissue, α2β1 integrin enhances OS tumorigenicity, invasiveness, and migration ability.

α4β1 Integrin

The α4β1 integrin can be found in leukocytes, myocytes and tumor cells, where it regulates hematopoiesis\(^\text{31,32}\). Specifically, Decker et al\(^\text{39}\) discovered that α4β1 integrin mediates adhesion between the 70-kDa chymotryptic fragment of thrombospondin-1 (TSP-1) and the U2OS and SAOS cell lines. Expressed in multiple OS cell lines including HOS, U2OS and MG63, TSP-1 is an extracellular glycoprotein that confers invasive and metastatic ability by providing an adhesive matrix for OS cells. Decker et al\(^\text{39}\) found that both U2OS and SAOS demonstrated diffuse immunoreactivity to β1 and α4 subunits respectively. Moreover, dose-dependent adhesion of OS cells to TSP-1 was partially inhibited by the addition of anti-α4 and anti-β1 antibodies respectively\(^\text{31}\). In summary, these experiments show that α4β1 serves as an adhesion receptor for TSP-1 and is expressed in OS cell lines.

CONCLUSION

In summary, integrins are essential in the regulation of diverse cellular processes including: gene transcription, signal transduction, proliferation, differentiation, migration, and apoptosis. In the specific context of OS biology, integrins modulate cell motility, migration, adherence, and invasion, thereby affecting pathogenesis and metastasis. Consequently, integrins represent potential targets for novel therapeutic strategies.
REFERENCES

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