

The Role of Integrins in Osteosarcoma

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

Vi Nguyen, Alan Nguyen, Carolyn A. Meyers, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, the United States
Alan Nguyen, Jia Shen, School of Dentistry, University of California, Los Angeles, the United States

Michelle A. Scott, Nationwide Children's Hospital, Columbus, Ohio, the United States

Correspondence to: Aaron W. James, MD, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, 675 Charles E. Young Dr. South, Rm 2641A, Los Angeles, CA 90095, the United States.

Email: Awjames@mednet.ucla.edu

Telephone: +1-310-206-6754

Received: December 16, 2016

Revised: April 20, 2016

Accepted: April 21, 2016

Published online: June 23, 2016

Key words: Integrins; Osteosarcoma; Pathogenesis; Metastasis

© 2016 The Authors. Published by ACT Publishing Group Ltd.

Nguyen V, Nguyen A, Meyers CA, Shen J, Scott MA, James AW. The Role of Integrins in Osteosarcoma. *International Journal of Orthopaedics* 2016; 3(3): 544-548 Available from: URL: <http://www.ghrnet.org/index.php/ijo/article/view/1605>

INTRODUCTION

Integrin Function

Integrins are members of a glycoprotein family that form heterodimeric cell surface receptors which connect the extracellular matrix to the cytoskeleton^[1]. Integrins act as cell adhesion molecules that modulate cell motility by providing traction^[1,2]. In the human genome, a total of 18 α -subunits and 8 β -subunits bind each other in a non-covalent fashion to form 24 different integrins, and this specific combination determines the ligand specificity of the integrin^[1]. In general, integrins with the $\alpha 4$, $\alpha 5$, $\alpha 8$, $\alpha 11b$, or αv subunits bind fibronectin and vitronectin, which contain the RGD (Arg-Gly-Asp) sequence and are found in the extracellular matrix^[1]. Conversely, laminins and collagen are recognized by integrins with $\alpha 3$, $\alpha 6$, or $\alpha 7$ subunits and $\alpha 1$, $\alpha 2$, $\alpha 10$, or $\alpha 11$ subunits respectively^[1]. Depending on the α -subunit it is bound to, the $\beta 1$ integrin family binds fibronectin, laminin, or collagens^[3,4].

Integrins play an active role in controlling signal transduction by activating kinases that phosphorylate regulatory cytoskeletal proteins, which affects a multitude of cellular processes including gene transcription, proliferation, differentiation, migration, and apoptosis^[1,2,5,6]. For example, integrins mediate cell cycle progression through activation of growth-promoting signaling pathways, including the FAK-Src and the Ras-ERK/MAPK cascades^[6,7]. Additionally, FAK activation has been shown to enhance integrin-induced cell migration and survival, and exhibits higher expression levels in invasive tumors^[8]. Integrins also prevent apoptosis by suppressing the p53 pathway or inducing the expression of anti-apoptotic proteins such as Bcl-2^[6]. Furthermore, integrins regulate cell migration via activation of the Rho-family of small guanine nucleotide-binding proteins that control the actin cytoskeleton^[6].

ABSTRACT

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 $\alpha v \beta 3$ integrin expression is directly correlated to the metastatic potential of multiple human OS cell lines. Additionally, $\alpha v \beta 3$ integrin interaction with FPRL-1 and TIMP-1 enhances both chemotactic and anti-apoptotic ability in OS cells. Likewise, $\beta 1$ 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.

(Please see Figure 1).

In addition, integrins modulate matrix remodeling by regulating the activity levels of proteolytic enzymes which degrade the basement membrane^[8]. Thus, the disruption of this barrier to surrounding tissue provides a mechanism for cancer cell invasiveness. Examples of these enzymes include the matrix metalloproteinases MMP-2 and MMP-9, which degrade extracellular collagens^[8].

Thus, due to their multifaceted involvement in cell survival, proliferation, and motility, integrins have been implicated in the disease progression and metastatic potential of various cancers. For example, integrins have been implicated in chondrosarcoma (CS) pathogenesis and metastasis. Specifically, the upregulation of human CS cell motility, migration, and invasion occurs via $\alpha\beta3$ and $\alpha5\beta1$ integrin-dependent pathways^[9-14]. For a more detailed review of integrins in CS, please refer to a recent review^[15].

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^[1]. 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.

$\alpha\beta3$ Integrin in Osteosarcoma

Normally expressed by endothelial cells, the $\alpha\beta3$ integrin has been found to be upregulated in tumor vasculature^[20]. Specifically, various studies have found that altered expression of $\alpha\beta3$ in tumor cells is associated with metastatic OS tumorigenesis^[20,21]. For example, Tome *et al.*^[20] evaluated $\alpha\beta3$ integrin expression in LM1-LM4 sublines of the 143B human OS cell line with increasing metastatic potential.

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 6-fold higher levels of $\alpha\beta3$ 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 $\alpha\beta3$ 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 $\alpha\beta3$ integrin antagonist echistatin^[21].

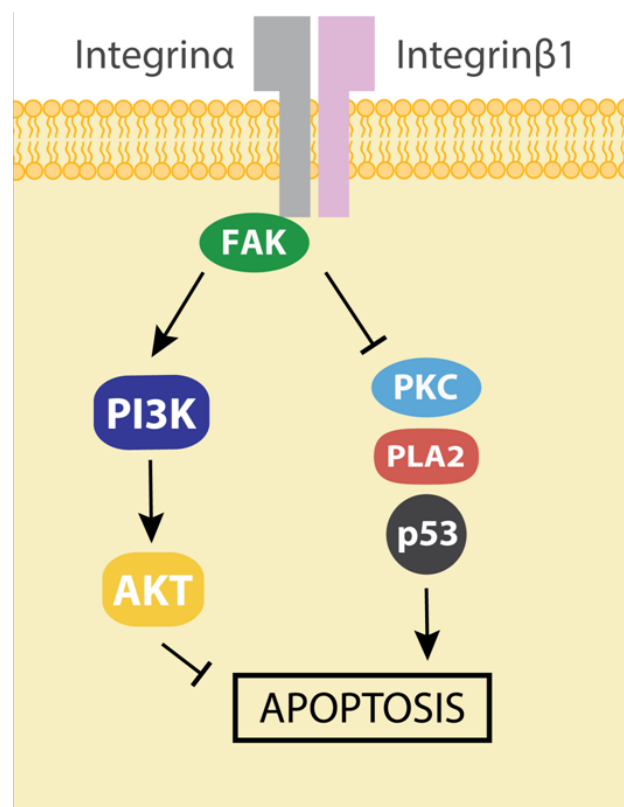


Figure 1 Schematic of Anti-Apoptotic Integrin Signaling Cascade. FAK: focal adhesion kinase; PI3K: phosphatidylinositol 3-kinase; PLA2: phospholipase A2; PKC: protein kinase C.

Table 1 The Role of Specific Integrins in Osteosarcoma.

Integrin	Role in Osteosarcoma
$\alpha\beta3$	<ul style="list-style-type: none"> Upregulated expression in tumor vasculature Expression levels directly correlates to the metastatic potential of multiple human OS cell lines including 143B, SAOS, U2OS, and MG-63 $\alpha\beta3$ integrin interaction with FPRL-1 and TIMP-1 enhances both chemotactic and anti-apoptotic ability in OS
$\beta1$	<ul style="list-style-type: none"> Addition of monoclonal antibodies specific to $\alpha2\beta1$ and $\alpha5\beta1$ inhibits CXCR4-dependent HOS tumor growth <i>in vivo</i> OS tumor cell lines with increased metastatic potential (U2OS and IOR/OS9) show increased expression of $\alpha2\beta1$, $\alpha5\beta1$, and $\alpha6\beta1$ Administration of anti-$\beta1$ integrin antibody (AIB2) in mice inhibits metastasis of 143B cells to the lungs and enhances survival rates
$\alpha2\beta1$	<ul style="list-style-type: none"> Regulates OS tumor cell invasiveness by mediating the attachment of cells to type I collagen
$\alpha4\beta1$	<ul style="list-style-type: none"> Significantly upregulated expression of $\alpha2\beta1$ integrin found in chemically (HOS-MNNG) and virally (KHOS-NP) transformed HOS subclones Increased $\alpha2\beta1$ expression is concomitant with increased Mg²⁺-dependent cell adhesion to type I collagen and accelerated migration and invasion rates
	<ul style="list-style-type: none"> Regulates hematopoiesis by mediating adhesion between U2OS and SAOS cell lines and TSP-1, which confers invasive and metastatic ability

Other studies have also evaluated the cross-talk between $\alpha\beta3$ integrin and various pathways that play a role in chemotaxis and apoptosis^[22,23]. For example, Ren *et al*^[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^[23]. Furthermore, the inhibition of formyl peptide receptor-1 (FPRL-1), an SAA receptor which regulates chemotaxis, corresponded to down regulation of SAA-induced $\alpha\beta3$ integrin expression^[23].

In another study, Tsagaraki *et al*^[22] demonstrated that the interaction between $\alpha\beta3$ 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^[25,26], and elevated TIMP-1 levels among cancer patients corresponded to poorer survival rates and increased chemotherapy resistance^[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 $\alpha\beta3$ integrin, which was concomitant with apoptotic resistance^[22]. Moreover, addition of the integrin inhibitor echistatin disrupted TIMP-1 ligation to $\alpha\beta3$ and significantly decreased apoptotic resistance^[22].

In summary, increased $\alpha\beta3$ 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^[20-23]. Furthermore, $\alpha\beta3$ integrin interaction with FPRL-1 and TIMP-1 enhances both chemotactic and anti-apoptotic ability in OS cells^[22,23]. Thus, this study elucidated how the $\alpha\beta3$ /FPRL-1 pathway plays a critical role in modulating OS metastasis.

$\beta1$ Integrins in Osteosarcoma

$\beta1$ integrins have been implicated in tumorigenesis, as they mediate cellular migration in response to chemokine stimulation^[4]. For example, Miura *et al*^[4] used human OS (HOS) transfected cells expressing the CXCR4 receptor for stromal cell-derived factor-1 (SDF-1) to investigate the role of the $\beta1$ integrin-chemokine axis in primary tumor development. First, they observed that $\beta1$ integrin ligand specificity is dependent on the α -subunit it is attached to. Whereas the $\alpha2\beta1$ integrin mediates the chemotactic response to SDF-1 on type I collagen and laminin, the $\alpha4\beta1$ and $\alpha5\beta1$ integrins mediate SDF-1-stimulated chemotaxis on fibronectin^[4]. Additionally, expression of CXCR4 confers a chemotactic response to SDF-1 and enhances HOS transfectant cell growth^[4]. Furthermore, both migratory ability and level of tumor growth were dependent on the level of CXCR-4 expression^[4]. However, addition of monoclonal antibodies specific to $\alpha2\beta1$ and $\alpha5\beta1$ inhibited this CXCR4-dependent HOS tumor growth *in vivo*. Thus, the $\beta1$ integrin-SDF-1 axis plays a central role in mediating OS tumor migration and growth.

In another study, Scotlandi *et al*^[3] 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 $\alpha2\beta1$, $\alpha5\beta1$, and $\alpha6\beta1$ ^[3]. In contrast, OS lines with decreased metastatic potential (Saos-2 and SARG) correspondingly showed reduced integrin expression^[3]. Of note, Scotlandi *et al*^[3] also determined that $\beta1$ ligand specificity is dependent upon the bound α -subunit, as they observed that $\alpha2\beta1$, $\alpha5\beta1$, and $\alpha6\beta1$ served as receptors for collagens, fibronectin, and laminin respectively^[3].

In a complementary study, Kimura *et al*^[28] 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- $\beta1$ integrin antibody, AIIB2. AIIB2 inhibited seeding of 143B cells on the lung, which served as an experimental assay for metastasis, by approximately 6-fold^[28]. Furthermore, the survival rate of the AIIB2 mice was approximately 5-fold of the control group. In summary, $\beta1$ 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.

$\alpha2\beta1$ Integrin

The $\alpha2\beta1$ integrin regulates OS tumor cell invasiveness by mediating the attachment of cells to type I collagen, the main matrix found in bone tissue^[29]. For example, Vihinen *et al*^[29] 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 $\alpha2\beta1$ 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^[29]. Furthermore, this increased $\alpha2\beta1$ expression was concomitant with increased Mg²⁺-dependent cell adhesion to type I collagen and accelerated migration and invasion rates^[29].

In a similar study, Santala *et al*^[30] 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 $\alpha2\beta1$ 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^[30].

To summarize, through regulating cell adhesion to the type I collagen found in bone tissue, $\alpha2\beta1$ integrin enhances OS tumorigenicity, invasiveness, and migration ability.

$\alpha4\beta1$ Integrin

The $\alpha4\beta1$ integrin can be found in leukocytes, myocytes and tumor cells, where it regulates hematopoiesis^[31,32]. Specifically, Decker *et al*^[33] discovered that $\alpha4\beta1$ 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*^[33] found that both U2OS and SAOS demonstrated diffuse immunoreactivity to $\beta1$ and $\alpha4$ subunits respectively. Moreover, dose-dependent adhesion of OS cells to TSP-1 was partially inhibited by the addition of anti- $\alpha4$ and anti- $\beta1$ antibodies respectively^[33]. In summary, these experiments show that $\alpha4\beta1$ 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.

ACKNOWLEDGEMENTS

The present work was supported by the UCLA Department of Pathology and Laboratory Medicine, the Orthopaedic Research and Education Foundation with funding provided by the Musculoskeletal Transplant Foundation, and NIH/NIAMS K08 AR068316-01.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

REFERENCES

- van der Flier A, Sonnenberg A. Function and interactions of integrins. *Cell Tissue Res.* Sep 2001; **305**(3): 285-298.
- Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell.* Apr 3 1992; **69**(1): 11-25.
- Scotlandi K, Serra M, Manara M, Baldini N. Human osteosarcoma cells, tumorigenic in nude-mice, express beta(1)-integrins and low-levels of alkaline-phosphatase activity. *Int J Oncol.* Nov 1993; **3**(5): 963-969.
- Miura K, Uniyal S, Leabu M, Oravec T, Chakrabarti S, Morris VL, Chan BMC. Chemokine receptor CXCR4-beta1 integrin axis mediates tumorigenesis of osteosarcoma HOS cells. *Biochem Cell Biol.* Feb 2005; **83**(1): 36-48.
- Aplin AE, Howe AK, Juliano RL. Cell adhesion molecules, signal transduction and cell growth. *Curr Opin Cell Biol.* Dec 1999; **11**(6): 737-744.
- Giancotti FG, Ruoslahti E. Integrin signaling. *Science.* Aug 13 1999; **285**(5430): 1028-1032.
- Shishido S, Bonig H, Kim YM. Role of integrin alpha4 in drug resistance of leukemia. *Front Oncol.* 2014; **4**: 99.
- Hood JD, Cheresh DA. Role of integrins in cell invasion and migration. *Nat Rev Cancer.* Feb 2002; **2**(2): 91-100.
- Hou CH, Yang RS, Hou SM, Tang CH. TNF-alpha increases alphavbeta3 integrin expression and migration in human chondrosarcoma cells. *J Cell Physiol.* Mar 2011; **226**(3): 792-799.
- Lai TH, Fong YC, Fu WM, Yang RS, Tang CH. Stromal cell-derived factor-1 increase alphavbeta3 integrin expression and invasion in human chondrosarcoma cells. *J Cell Physiol.* Feb 2009; **218**(2): 334-342.
- Chen YJ, Wei YY, Chen HT, Fong YC, Hsu CJ, Tsai CH, Hsu HC, Liu SH, Tang CH. Osteopontin increases migration and MMP-9 up-regulation via alphavbeta3 integrin, FAK, ERK, and NF-kappaB-dependent pathway in human chondrosarcoma cells. *J Cell Physiol.* Oct 2009; **221**(1): 98-108.
- Tan TW, Lai CH, Huang CY, Yang WH, Chen HT, Hsu HC, Fong YC, Tang CH. CTGF enhances migration and MMP-13 up-regulation via alphavbeta3 integrin, FAK, ERK, and NF-kappaB-dependent pathway in human chondrosarcoma cells. *J Cell Biochem.* May 15 2009; **107**(2): 345-356.
- Tzeng HE, Chen JC, Tsai CH, Kuo CC, Hsu HC, Hwang WL, Fong YC, Tang CH. CCN3 increases cell motility and MMP-13 expression in human chondrosarcoma through integrin-dependent pathway. *J Cell Physiol.* Dec 2011; **226**(12): 3181-3189.
- Wu CM, Li TM, Hsu SF, Su YC, Kao ST, Fong YC, Tang CH. IGF-I enhances alpha5beta1 integrin expression and cell motility in human chondrosarcoma cells. *J Cell Physiol.* Dec 2011; **226**(12): 3270-3277.
- Chen JC, Fong YC, Tang CH. Novel strategies for the treatment of chondrosarcomas: targeting integrins. *Biomed Res Int.* 2013; **2013**: 396839.
- Mirabello L, Troisi RJ, Savage SA. Osteosarcoma incidence and survival rates from 1973 to 2004: data from the Surveillance, Epidemiology, and End Results Program. *Cancer.* Apr 1 2009; **115**(7): 1531-1543.
- Ottaviani G, Jaffe N. The epidemiology of osteosarcoma. *Cancer Treat Res.* 2009; **152**: 3-13.
- Mialou V, Philip T, Kalifa C, Perol D, Gentet JC, Marec-Berard P, Pacquement H, Chastagner P, Defascheltes AS, Hartmann O. Metastatic osteosarcoma at diagnosis: prognostic factors and long-term outcome--the French pediatric experience. *Cancer.* Sep 1 2005; **104**(5): 1100-1109.
- Aljubran AH, Griffin A, Pintilie M, Blackstein M. Osteosarcoma in adolescents and adults: survival analysis with and without lung metastases. *Ann Oncol.* Jun 2009; **20**(6): 1136-1141.
- Tome Y, Kimura H, Maehara H, Sugimoto N, Bouvet M, Tsuchiya H, Kanaya F, Hoffman RM. High lung-metastatic variant of human osteosarcoma cells, selected by passage of lung metastasis in nude mice, is associated with increased expression of alpha(v) beta(3) integrin. *Anticancer Res.* Sep 2013; **33**(9): 3623-3627.
- Duan X, Jia SF, Zhou Z, Langley RR, Bolontrade MF, Kleinerman ES. Association of alphavbeta3 integrin expression with the metastatic potential and migratory and chemotactic ability of human osteosarcoma cells. *Clin Exp Metastasis.* 2004; **21**(8): 747-753.
- Tsagaraki I, Tsilibary EC, Tzinia AK. TIMP-1 interaction with alphavbeta3 integrin confers resistance to human osteosarcoma cell line MG-63 against TNF-alpha-induced apoptosis. *Cell Tissue Res.* Oct 2010; **342**(1): 87-96.
- Ren P, Sun D, Xin D, Ma W, Chen P, Gao H, Zhang S, Gong M. Serum amyloid A promotes osteosarcoma invasion via upregulating alphavbeta3 integrin. *Mol Med Rep.* Dec 2014; **10**(6): 3106-3112.
- Connolly M, Veale DJ, Fearon U. Acute serum amyloid A regulates cytoskeletal rearrangement, cell matrix interactions and promotes cell migration in rheumatoid arthritis. *Ann Rheum Dis.* Jul 2011; **70**(7): 1296-1303.
- Liu XW, Bernardo MM, Fridman R, Kim HR. Tissue inhibitor of metalloproteinase-1 protects human breast epithelial cells against intrinsic apoptotic cell death via the focal adhesion kinase/phosphatidylinositol 3-kinase and MAPK signaling pathway. *J Biol Chem.* Oct 10 2003; **278**(41): 40364-40372.
- Boulday G, Fitau J, Coupel S, Soullillou JP, Charreau B. Exogenous tissue inhibitor of metalloproteinase-1 promotes endothelial cell survival through activation of the phosphatidylinositol 3-kinase/Akt pathway. *Ann N Y Acad Sci.* Dec 2004; **1030**: 28-36.
- Schrohl AS, Meijer-van Gelder ME, Holten-Andersen MN, Christensen IJ, Look MP, Mouridsen HT, Brüner N, Foekens JA. Primary tumor levels of tissue inhibitor of metalloproteinases-1 are predictive of resistance to chemotherapy in patients with metastatic breast cancer. *Clin Cancer Res.* Dec 1 2006; **12**(23): 7054-7058.
- Kimura H, Tome Y, Momiyama M, Hayashi K, Tsuchiya H, Bouvet M, Hoffman RM. Imaging the inhibition by anti-beta1 integrin antibody of lung seeding of single osteosarcoma cells in live mice. *Int J Cancer.* Nov 1 2012; **131**(9): 2027-2033.
- Vihinen P, Riikonen T, Laine A, Heino J. Integrin alpha 2 beta 1 in tumorigenic human osteosarcoma cell lines regulates cell adhesion, migration, and invasion by interaction with type I collagen. *Cell Growth Differ.* Apr 1996; **7**(4): 439-447.
- Santala P, Larjava H, Nissinen L, Riikonen T, Maatta A, Heino J. Suppressed collagen gene expression and induction of alpha 2 beta 1 integrin-type collagen receptor in tumorigenic derivatives of human osteogenic sarcoma (HOS) cell line. *J Biol Chem.* Jan 14 1994; **269**(2): 1276-1283.
- Kilger G, Holzmann B. Molecular analysis of the physiological and pathophysiological role of alpha 4-integrins. *J Mol Med (Berl).* Jul 1995; **73**(7): 347-354.
- Lobb RR, Hemler ME. The pathophysiologic role of alpha 4 integrins *in vivo*. *J Clin Invest.* Nov 1994; **94**(5): 1722-1728.

- 33 Decker S, van Valen F, Vischer P. Adhesion of osteosarcoma cells to the 70-kDa core region of thrombospondin-1 is mediated by the alpha 4 beta 1 integrin. *Biochem Biophys Res Commun.* Apr 26 2002; **293**(1): 86-92.

Peer reviewers: Laura Scaramuzzo, I.R.C.C.S. Galeazzi Orthopaedic Institute, Spine Division 1, Via Riccardo Galeazzi 4, 00161 Milan, Italy; Georgios Tsoulfas, MD, PhD, FICS, FACS, 66 Tsimiski Street, 54622 Thessaloniki, Greece.