THE EFFECT OF INTEGRIN (α5β1, αvβ3) BLOCKING ANTIBODY ON HUMAN OSTEOSARCOMA

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**INTRODUCTION**
Integrin receptors are transmembrane heterodimeric proteins (α, β) that mediate cell adhesion to several ECM proteins. Cell interaction with the extracellular matrix (ECM) is a mandatory event for tumor growth, invasion and metastasis (1).

Integrin αβ1 is known to be related with angiogenesis, and its expression is up-regulated in tumor-associated blood vessels in invasive carcinoma (2). Hence, αβ1 was proposed as a diagnostic marker for a variety of solid tumors (3). Integrin αβ1 and its ligand fibronectin are expressed at significantly increased levels in neovessels induced by growth factors of solid tumors. Moreover antagonists of integrin α5β1 and fibronectin block tumor induced angiogenesis (4).

Previously we had studied the effect of integrin αβ1 and αvβ3 on the proliferation of osteosarcoma cells using blocking antibodies, and reported that the both integrin αβ1 and αvβ3 influenced proliferation of osteosarcoma cells with stronger correlation in case of αvβ3.

In the current study, we examined the invasion and migration ability of osteosarcoma cells using antagonists of integrin αβ1 and αvβ3 respectively.

**METHODS**
Four standard osteosarcoma cell lines (HOS, U2OS, MG-63, Saos-2) and seven cell lines established from patients were used in this study.

Murine monoclonal anti-α5β1 and anti-αvβ3 (Chemicon International Inc. Temecula, CA) were used for growth inhibition assays. Adhesion and proliferation assay were performed in osteosarcoma cell lines according to the protocols we used before. Cell invasion and motility assay were carried out by using the boyden chamber. After treating HOS osteosarcoma cell line with anti-α5β1 antibody and anti-αvβ3 antibody, migration status was observed. Mouse IgG1 antibody was used as negative control. The boyden chamber which have filter of gelatin coating used for examination of migration status of HOS cells (Neuro Probe Inc., MD, USA).

**RESULTS**
Previously, we demonstrated the tumor cells proliferation in osteosarcoma was inhibited by treating them with blocking antibodies against integrin αvβ3 and α5β1. More decrease of tumor cells proliferation was observed when treating cells with anti-αvβ3 antibody than with anti-α5β1 antibody.

In the current study, the invasion and migration of tumor cells was significantly decreased after treating with either anti-αvβ3 antibody or anti-α5β1 antibody. The decrease of migration ability of osteosarcoma cells seemed similar when blocking either anti-αvβ3 antibody or anti-α5β1 antibody (Fig. 1).

**DISCUSSION AND SUMMARY**
Integrin plays a central role in many processes such as anchorage dependent growth, apoptosis, differentiation, cellular migration and metastasis, most of which processes are characteristically dysregulated in malignancy. In many cancers, altered expression of αβ1-integrin and αβ3 integrin receptors was reported. Integrin αβ1 is up-regulated on endothelial cells in response to angiogenic growth factors and has been established as a target for antiangiogenic therapy. In human melanoma cell lines, the differential expression of αβ1 integrin and αvβ3 integrin modulates release of MMP-2 and subsequent invasive behavior and melanoma cells selected for lack of the αβ1 integrin have significantly reduced proliferation and tumorigenicity (5). Furthermore, α5β1 or αvβ3 integrins are directly involved in cell survival and apoptosis via several mechanisms (6).

Based on the data of this study, inhibition of invasion and migration as well as cellular proliferation (previously presented) by blocking with neutralizing monoclonal anti-αβ1 antibody and anti-αvβ3 antibody was confirmed in osteosarcoma.

αvβ3 integrin seemed to be more significant as a receptor involved in osteosarcoma cellular proliferation than αβ1. However, in terms of invasion and migration ability, it seemed that both αvβ3 and αβ1 integrins had similar significances as the major receptor proteins involved in cell invasion and motility in osteosarcoma.

**CONCLUSION**
Our data suggest that αvβ3 and αβ1 integrin engagement may be of importance in the spreading of human osteosarcoma, and inhibition of these integrin activities might have implications from the therapeutic viewpoint in the treatment of osteosarcoma.

**REFERENCES**

**Figure 1**. Migration of HOS cells: effects of different integrins. The number of cells indicated is the mean ± SD counted per well. *, p < 0.1; **, p < 0.001