**Introduction:** The Notch signaling pathway functions as an organizer in embryonic development. Notch proteins belong to a family of conserved transmembrane receptors that play a fundamental role in cell fate decisions including cell proliferation, differentiation, and apoptosis. In mammalian cells, there are four Notch receptors (termed Notch-1, -2, -3, and -4) and five ligands (termed Jagged-1, -2, and Delta-like (Dll)-1, -3, and -4). Although similar, the receptors show differences in structure that are likely responsible for their different expression patterns and unique functions. Notch signaling is initiated by receptor-ligand interactions between neighboring cells resulting in a successive proteolytic cleavages by γ-secretase. This processing results in the release of the intracellular domain (NIC, the functionally active form of Notch), which translocates to the nucleus and binds RBP-Jk. RBP-Jk/Notch interactions result in the expression of various target genes including Hes (Hairy/Enhancer of Split), Hey (Hairy/Enhancer of Split), NF-kB, and PPAR families of transcription factors, and cell cycle regulators such as p21CIP1/WAF1 and cyclin D. Recent studies have shown constitutive activation of the Notch pathway in various types of malignancies. However, it remains unclear whether this pathway is activated in human osteosarcoma. In an attempt to better understand osteosarcoma pathogenesis, we investigated the expression and activation of Notch proteins in osteosarcoma and examined the effect of pharmacologically unique γ-secretase inhibitor on osteosarcoma cell survival. The results demonstrated elevated levels of notch signaling molecules in vitro and in vivo compared to normal osteoblast. NIC was accumulated in osteosarcoma cell nucleus. And also, γ-secretase inhibitor treatment prevented osteosarcoma cells proliferation in vitro and in vivo.

**Materials and Methods:** RNA was purified by TRIzol (Invitrogen). RT-PCR was performed with Notch 1-4, Jagged 1-2, Dll1,3,4, Hes1,7, Hey1,2, and HeyL specific primers. Immunohistochemistry for NIC, Jagged, Hes, and Dll was performed using human osteosarcoma cell lines and human osteosarcoma samples. Inhibition of Notch activation: Cells were treated with increasing concentrations of various γ-secretase inhibitors to inhibit Notch activation. γ-secretase inhibitors are considered nonselective or pan-Notch inhibitors. Cell survival was quantitated using a MTT assay. Nude mice were injected with 143B osteosarcoma cells intradermally, and palpable tumors formed in 7 days. On the seventh day, nude mice were intraperitoneally injected with γ-secretase inhibitor. Injections continued every each day and tumor dimensions were measured.

**Results:** RT-PCR revealed high expression of Notch-1, 2, Jagged1 Hes, and Hey in all of osteosarcoma cell lines. And also, 7 osteosarcoma human samples also showed high expression of Notch-1, 2, 3, 4, Jagged1 Hes, and Hey mRNAs. In addition, immunohistochemistry showed high expression of Notch-IC in nucleus of osteosarcoma cell lines and human osteosarcoma samples. And also, expression of Jagged and Hes was observed in osteosarcoma cell lines and human osteosarcoma samples. These data suggest that notch signaling pathways are activated in osteosarcomas. Next, we investigated the effects of Notch pathway inhibition on osteosarcoma growth using pharmacologic inhibitors of γ-secretase in vitro and in vivo. Cell viability was examined by MTT method. MTT assay showed that γ-secretase inhibitor suppresses the growth of the osteosarcoma cell lines in vitro (Fig.1). In order to determine the effects of Notch blockade in vivo, we examined the formation of tumor xenografts using osteosarcoma cell line and nude mice. Intraperitonealy γ-secretase inhibitor administration dramatically inhibited the osteosarcoma xenografts growth in vivo (Fig.2). Kaplan-Meier analysis showed that γ-secretase inhibitor administration confers statically significant survival benefit.

**Discussion:** The molecular basis for osteosarcoma tumor cell emergence, survival, and proliferation remains unclear despite active investigation. We demonstrated that osteosarcoma cells both in vitro and in vivo express elevated levels of Notch receptors. In this context, Notch is constitutively activated as demonstrated by NIC nuclear accumulation and expression of Notch target proteins. Moreover, treatment of osteosarcoma tumor cells with γ-secretase inhibitor that block Notch activation, results in tumor regression in in vitro and in vivo model systems. These results suggests that inactivation of Notch may be a therapeutic approach for treating patients suffering from osteosarcoma.