

## **CD49<sup>hi</sup>/CD90<sup>lo</sup> cell from primary human osteosarcomas is enriched for cancer stem/progenitor cells.**

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Osteosarcoma is one of the most prolific forms of cancer among adolescents, resulting in a severe decrease in life expectancy unless detected early. Although, many factors have been elucidated as contributing to osteosarcomagenesis the exact mechanism is unclear. Recently, a sub-population of stem-like cells within solid tumors are implicated in tumor formation and progression. In this study, we report the identification and characterization of a cancer stem/progenitor cell population from human osteosarcomas. Human osteosarcoma samples were collected during biopsies, expanded *in vitro*, phenotyped for the expression of multiple cell-surface markers and then injected subcutaneously into nu/nu mice. Osteosarcoma cells expressing the CD49<sup>hi</sup>/CD90<sup>lo</sup> cell surface marker consistently produced fast growing tumors. Amiloride, a potassium-sparing diuretic, decreased CD49f protein expression and *in vivo* treatments decreased tumor growth when injected into nu/nu mice, indicating that CD49f is an important marker in tumor progression. The transfection of CD49<sup>hi</sup>/CD90<sup>lo</sup> cells with an shCD49f lentiviral construct showed a 20% decrease in their ability to migrate in a transwell assay, and experienced a three-fold decrease in their capability to form sarcospheres. Xenograft assays in immunodeficient mice with or without shCD49f cells showed that shCD49f expressing cells decreased tumor growth by 30% compared to controls. In conclusion, our data suggests the CD49<sup>hi</sup>/CD90<sup>lo</sup> cell population is linked to a more aggressive osteosarcoma tumor growth and that this cell surface marker is a potential candidate for the characterization of TICs in osteosarcomas.

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