Roles of SPRR1A in tumorigenesis of osteosarcoma: a study using newly generated artificial cancer stem cells

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INTRODUCTION:
Osteosarcoma (OS) is the most common primary malignant bone tumor in children and adolescents. With the standardization of treatment approaches combining chemotherapy and limb-sparing surgery, the 5-year survival rate for OS patients has improved. However, the 5-year survival rate for OS patients with non-metastatic lesions has plateaued at around 70%, and less than 20% for those with metastatic and/or recurrent lesions. Previous studies have suggested that cancer stem cells (CSCs) play crucial roles in cancer progression, chemoresistance, and/or metastatic capabilities in various cancer types. Recently, we successfully generated CSC-like cells from OS cell lines by transduction of defined factors. These OS CSC-like cells showed significantly enhanced CSC properties, including slow cell proliferation, higher chemoresistance, enhanced sphere formation, and increased migration ability. More interestingly, microarray analyses revealed significantly increased expression in OS CSC-like cells of small proline-rich protein 1A (SPRR1A), a cross-linked envelope protein of keratinocytes that is commonly expressed in skin, esophagus, and vagina, but not in bone (1). We therefore hypothesized that SPRR1A should be associated with tumor initiation, formation, and poor prognosis in OS. In this study, we analyzed the functions of SPRR1A to elucidate its roles in OS.

METHODS:
Following our previously published methods, we generated the OS CSC-like cells "MG-OKS" by transducing *OCT3/4*, *KLF4*, and *SOX2* from the OS cell line MG-63 (1). MG-OKS cells were then transfected with SPRR1A- selective siRNA to selectively suppress SPRR1A expression (siMG-OKS group). siRNA-free MG-OKS cells (MG-OKS group) and scrambled siRNA-transfected MG-OKS cells (scMG-OKS group) were used as controls. qRT-PCR analysis was performed to evaluate the mRNA expression levels of SPRR1A after siRNA transfection. Morphological changes following siRNA transfection were observed under phase contrast microscopy. Cell proliferative activities were assessed by the WST-8 assay to examine the effect of SPRR1A inhibition on cell proliferation. Cell migratory ability was also evaluated using the scratch wound healing assay. To evaluate in vivo tumorigenic function, cells from these three groups (2 million cells each) were transplanted subcutaneously into the back of nude mice (n=6 per group) , and tumor volume was measured 4 weeks after transplantation for comparison among the groups.

RESULTS:
The mRNA expression level of SPRR1A was significantly decreased in the siMG-OKS group compared to the MG-OKS (no transfection) and scMG-OKS groups. In the cell morphology examination, MG-OKS cells showed elongated cell bodies with invasive processes compared to MG-63 cells in our previous study (1). However, the invasive processes were reduced in the siMG-OKS group compared to the MG-OKS and scMG-OKS groups (**Fig. 1a**). The WST-8 assay showed SPRR1A inhibition significantly decreased cell proliferation compared to controls ($P<0.05$) (**Fig. 1b**). Wound healing assays revealed that the cell migratory ability of the siMG-OKS group was significantly decreased compared to that of the MG-OKS and scMG-OKS groups ($P<0.05$) (**Fig. 2**). In vivo comparison among the three groups resulted in a significant reduction regarding tumorigenic function in the siMG-OKS group ($P<0.05$) (**Fig. 3**).

DISCUSSION:
SPRR1A is a cross-linked envelope protein of the SPRR family known as a keratinocyte terminal differential marker, with various reported functions, including a protective effect against myocardial ischemia and a prognostic biomarker for colorectal cancer (2, 3). However, there is no report on the role of SPRR1A in OS. In this study, we investigated the functions of SPRR1A in OS cells; inhibition of SPRR1A gene expression resulted in impaired proliferative and migratory capacity as well as altered cell morphology. Our findings suggest that SPRR1A should be associated with tumor progression in OS. Although further investigations are needed, SPRR1A may serve as a biomarker and/or therapeutic target for OS.

SIGNIFICANCE/CLINICAL RELEVANCE:
In this study, we investigated the roles of SPRR1A in osteosarcoma using cancer stem cell-like cells, which we succeeded in generating for the first time from an established osteosarcoma cell line. Our findings suggest that SPRR1A should be associated with tumor progression and that SPRR1A may serve as a biomarker and/or therapeutic target for osteosarcoma.

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IMAGES AND TABLES: