mTOR Inactivation Reduces Motility and Proliferation In Murine Osteosarcoma Cells

Xiaodong Mu, Christian Isaac, Riddhima Agarwal, Johnny Huard, and Kurt Weiss
Stem Cell Research Center, Department of Orthopaedic Surgery, University of Pittsburgh, Pittsburgh, PA 15219
Correspondence: wenkr@upmc.edu

INTRODUCTION:
Osteosarcoma (OS) is the most common primary malignancy of bone and mainly affects children and teens. Nearly all deaths are caused by pulmonary metastatic disease. We have demonstrated that bone morphogenetic protein-2 (BMP-2) and vascular endothelial growth factor (VEGF) are important to OS metastatic biology (1). Our previous data suggest that BMP-2 is associated with OS cell motility and VEGF is an OS cell survival factor. Rapamycin, an inhibitor of mTOR (mammalian target of rapamycin) signaling, has been studied extensively in oncology. Its relevance in osteosarcoma is not yet clear, but studies demonstrate that mTOR signaling interacts with BMP-2 and VEGF activity (2). We hypothesized that mTOR inhibition with rapamycin would alter the metastatic potential of OS cells, as well as their resistance to oxidative stress. K7M2 and K12 are related murine OS cell populations with differing metastatic potentials; K7M2 is highly metastatic to the lung but K12 is much less metastatic. In the current study, the differential expressions of metastasis-associated factors in K7M2 and K12 cells were compared. K7M2 cells were treated with rapamycin. Their motility, proliferation, expression of metastasis-associated factors, and resistance to oxidative stress were analyzed.

METHODS:
Single cell migration tracking: K7M2 cells cultured in 12-well plates were treated with rapamycin (100 nM) and immediately observed using a time-lapsed microscopic live-cell imaging (LCI) system (Automated Cell, Inc.) for four days. Cell migration velocity and proliferation were compared with or without rapamycin treatment.

Oxidative stress resistance assay: To examine the role of rapamycin treatment on the cell resistance to oxidative stress, K7M2 was performed using rapamycin (100 nM) pretreatment for 72hrs prior to exposure to oxidative stress (300 μM of H2O2 in Proliferation Medium) conditions. Propidium iodide (PI) was added to the medium (1μg/ML), and apoptotic cells were identified with positive PI staining.

RESULTS:
1 - Rapamycin treatment reduces both migration and proliferation potentials of the K7M2 cells.

Figure 1: Rapamycin treatment (100nM) of K7M2 cells caused statistically significant inhibition of cell velocity (A) and proliferation rate (B).

2 - Differential expression of metastasis-associated factors in K7M2 and K12 cells, and the effects of rapamycin treatment on K7M2 cells (Figure 2).

DISCUSSION:
We have previously reported that BMP-2 inhibition decreases the motility of OS cells, and VEGF inhibition diminishes the viability of OS cells (1). Our current results demonstrate that inhibition of mTOR signaling with rapamycin diminishes the expression of both BMP-2 and VEGF in OS cells, which could help to explain the molecular mechanism of rapamycin’s effects. Also, the gene expression of TNF-alpha and ALDH are down-regulated by rapamycin treatment, indicating a potential change in OS cell resistance to oxidative stress. The oxidative stress resistance of OS cells was greatly reduced by rapamycin. Therefore, targeting mTOR signaling could be beneficial for the treatment of OS by reducing metastatic potential through OS cell motility, proliferation, attenuation of BMP-2 and VEGF activity, and promoting cell apoptosis in an environment of oxidative stress.

SIGNIFICANCE:
The greatest challenge in OS treatment is pulmonary metastatic spread. The prognosis of OS has not improved in over twenty years, clearly illustrating the requirement for better understanding of OS metastatic biology. The demonstration of the interactions of factors including mTOR, BMP and VEGF in our results may help to uncover novel therapeutic targets for OS treatment.


ACKNOWLEDGEMENTS: The authors gratefully acknowledge the generous support of the Pittsburgh Foundation and the Huoy family in loving memory of Jon Huoy.