Epigenetic Regulation of Metastatic Osteosarcoma Cells with HDAC Inhibitor

Xiaodong Mu, Ph.D., Daniel Brynien, Johnny Huard, Ph.D., Kurt Weiss.

University of Pittsburgh, Pittsburgh, PA, USA, University of Pittsburgh, Pittsburgh, PA, USA.

Disclosures: X. Mu: None. D. Brynien: None. J. Huard: 3B; Cook Myocite. K. Weiss: None.

Introduction: Cancer has both genetic and epigenetic origins. The epigenetic modification of cancer cells has been found to regulate tumor development and metastasis. Epigenetic changes include DNA methylation and histone modification, both of which regulate gene expression but do not alter the genetic code. Our previous research has demonstrated differential DNA methylation status of tumor suppressor genes between highly metastatic osteosarcoma cells (K7M2) and less-metastatic osteosarcoma cells (K12). The de-methylation of tumor suppressor genes was able to repress the metastatic phenotype of highly metastatic osteosarcoma cells [1]. Histone deacetylase inhibitors (HDAC inhibitors, HDIs) have been evaluated for epigenetic regulation of various cancer cells [2-4], but the role of HDAC inhibitors in osteosarcoma is still unclear. In the current study, we have investigated the effect of the HDAC inhibitor, vorinostat (SAHA), on highly metastatic K7M2 murine osteosarcoma cells.

Methods: 1. Osteosarcoma cells: 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 metastastic. K7M2 cells were previously reported by our group to display greater resistance to stress and ALDH activity than K12 cells. K7M2 cells also possess more characteristics of cancer stem cells, such as higher Notch-1 expression. 2. Vorinostat treatment of cells: vorinostat (SAHA) (25, 50, or 100 µM) in proliferation medium (10% FBS in DMEM) was used to culture the K7M2 cells. Cells were incubated with vorinostat for 48 hours prior to testing survival and proliferation, mRNA analysis, and in vitro invasion assay.

Results: 1. Vorinostat treatment of K7M2 cells decreased cell survival and proliferation. K7M2 cells were cultured with or without vorinostat (25, 50, 100 µM) for 48 hours. A significantly lower number of cells was observed in vorinostat-treated K7M2 cells (Figure 1). Vorinostat treatment caused both apoptosis and decreased cell proliferation of K7M2 cells.

2. Vorinostat treatment of K7M2 cells modified cell morphology. K7M2 cells were cultured with or without vorinostat (25, 50, 100 µM) for 48 hours. Phalloidin staining of the actin cytoskeleton revealed significant structural differences in vorinostat-treated cells. Treated cells displayed fewer invadopodia, and their shape was more polygonal (Figure 2A).

3. Vorinostat treatment of K7M2 cells modified the expression of tumorigenic and metastasis-related genes. Semi-quantitative polymerase chain reaction (PCR) showed that the expressions of mammalian target of rapamycin (mTOR, a cancer stem cell marker), aldehyde dehydrogenase-1 (ALDH-1, a cancer stem cell marker), and peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1, a mediator of mitochondria biogenesis) were down-regulated in K7M2 cells treated with vorinostat (50 µM). Conversely, the expression of microtubule-associated protein 1A/1B-light chain (LC3, an autophagy maker) was up-regulated in vorinostat treated cells (Figure 2B).

4. Vorinostat treatment of K7M2 cells reduced their in vitro invasion capacity.
K7M2 cells were cultured with or without vorinostat (50 µM) for 2 days, and the in vitro invasion capacity of the cells through 2.5% matrigel was analyzed with the xCelligence system. Vorinostat treatment significantly reduced the K7M2 cells’ invasion capacity (Figure 3).

**Discussion:** These data demonstrate that highly metastatic osteosarcoma cells can be epigenetically modified with HDAC inhibition. Vorinostat (SAHA) treatment of K7M2 cells in vitro reduced both the proliferation and migration of osteosarcoma cells, indicating HDAC inhibition may have the potential to reduce tumor development and metastasis in vivo. The effect of vorinostat on osteosarcoma cells could be related to diminished expression of some genes regulating tumor development and metastasis such as mTOR, ALDH and PGC-1. In the future we will test this hypothesis with an in vivo model of metastatic osteosarcoma.

**Significance:** Understanding of the epigenetic origin of tumor development and metastasis is crucial for developing improved therapeutic treatments. Our current study revealed the effectiveness of HDAC inhibition in epigenetically regulates osteosarcoma cells, suggesting that HDAC inhibitors could be potentially tested to treat osteosarcoma.
ORS 2015 Annual Meeting
Poster No: 1942