

## 11477 - Epigenetic Reprogramming of Osteosarcoma Tumor Initiating Cells Alters Histologic and Metastatic Phenotype

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**Background:** Osteosarcoma (OS), a bone cancer primarily affecting children and adolescents, has a survival rate of 60% that has remained unchanged in the last 30 years. Furthermore, survivors suffer long term morbidities associated with the current standard of care. Our lab is investigating the role of intratumoral heterogeneity in OS biology and therefore has developed a transcriptional reporter where the Oct4 promoter drives expression of a green fluorescent protein (GFP). Activation of the construct, and therefore expression of GFP, allows for the isolation of OS tumor initiating cells (TICs): cells that are 100 times more tumorigenic than their GFP- counterparts. Pure populations of GFP+ TICs injected into mice consistently generate heterogeneous tumors, consisting of benign GFP- cells and malignant GFP+ cells. The two populations have unique gene expression signatures which are highly reproducible. This loss of tumorigenic phenotype is a rapid process as the time from injection of a clonal population of GFP+ cells to harvest of a heterogeneous tumor is less than 6 weeks. The fast kinetics of this process coupled with the reproducibility of the gene expression changes occurring suggests that OS tumorigenicity is governed at the epigenetic level.

### **Purpose:**

To investigate if agents that modify the cell epigenome can manipulate the tumorigenic potential of OS TICs.

### **Methods:**

In order to investigate our first hypothesis, cells were treated with TSA *in vitro* at a dose that induced hyperacetylation within the cells without inducing cell death. The effects of the treatment were assessed using cell cycle analysis, cell proliferation assays, and western blotting to evaluate cell cycle proteins. TSA treated and untreated cells were also injected into immunocompromised mice for xenograft studies (n=5). Time until tumor onset and endpoint was recorded. Upon harvest, histological analyses were carried out on the tumor specimens and the mice lungs were analyzed for the presence of metastatic nodules. Cells were sorted based on GFP expression and RNA was then collected for gene expression studies. Finally, global gene expression studies and differential analyses were carried out between tumors arising from TSA treated cells (TSA tumors) and tumors arising from control treated cells (control tumors) using Affymetrix microarrays and The Database for Annotation, Visualization, and Integrated Discovery. Expression changes between populations from the two tumors, as well as between the populations within the same tumor using GFP expression, were carried out. Finally, expression changes were confirmed at the protein level using immunofluorescence and western blotting.

**Results:** The studies investigating our first hypothesis demonstrated that although TSA treatment showed promising tumor inhibitory effects *in vitro*, treated cells generated tumors that displayed enhanced metastatic dissemination, coupled with histologic changes associated with metastatic behavior in xenograft studies. Differential expression analyses between the TICs isolated from TSA tumors and TICs isolated from control tumors showed that the new tumor phenotype was associated with a decrease in extracellular matrix production and an increase in cellular proliferation. TSA treatment also resulted in a reduction in the GFP- population within the tumor: 40% of the cells in control tumors are GFP-, whereas less than 5% of the TSA tumors are GFP-. TSA treated cells also gave rise to tumors with less intratumoral heterogeneity in characteristics important for tumor growth (stress and proliferation) and were significantly more sensitive to Doxorubicin compared to their respective control population (p<0.01).

**Conclusions:** Treatment of OS cells with agent that alters both the epigenome of the cells change the tumor phenotype and change important tumorigenic characteristics such as metastatic ability, suggesting that OS tumorigenicity is indeed at least partially governed at the epigenetic level. This study emphasizes the phenotypic plasticity of cancer cells and that even slower dividing, seemingly benign cancer cells need to be targeted by therapies as they can revert back to their malignant phenotype. However, this plasticity can be exploited in cases of tumors that are refractory to treatment or have acquired resistance as their phenotype could potentially be changed by agents that change their epigenome. Furthermore, it provides a proof of principle that it is possible to reduce the intratumoral heterogeneity, a key culprit in drug resistance and disease relapse, and to re-sensitize tumors to the.