**Title:** Multiscale Modeling of Multiple Myeloma and Osteoclast Interactions in the Hypoxic Bone Marrow Microenvironment

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**Background:** In multiple myeloma (MM), which is a cancer of the plasma cells, the most visible aspect is destruction of the bones throughout the body. Two significant events implicated in bone destruction are accumulation of malignant plasma cells (or myeloma cells) inhibiting normal bone-forming cells and rapid growth of osteoclasts (OCs), cells that resorb or break down bones. These events occur in the bone marrow, a microenvironment that is hypoxic by nature. Our aim is to develop a multiscale mathematical model based on experimental data of the mechanisms underlying the ability of MM cells to sustain the hypoxic niche and whether their interactions with OCs play role in supporting the growth and survival of MM cells.

**Methods:** We tested myeloma RPMI-8226 cell proliferation in different conditions, such as normal (21% O2), hypoxia (5% O2), and in both conditions co-cultured with osteoclast (OC) cells. After 72h, we assayed dsDNA content of RPMI-8226 cells. The results displayed slower growth of RPMI-8226 cells in hypoxia condition. Interestingly, OC cells were observed to be supporting RPMI-8226 cell growth, in either normal or hypoxia condition. The samples were analyzed using reverse phase protein array (RPPA) technique. Using the experimental data, we constructed intracellular signaling pathways of proteins governing MM-OC interactions under hypoxic condition. We also performed parameter estimation for our mathematical model.

**Results:** From the RPPA data we generated a generic pathway for MM cell apoptosis, growth, and migration. The data showed that hypoxia inhibits MM cell growth by (i) activation of apoptosis through the activation proteins responsible for apoptosis such as casp7, and (ii) arresting cell cycle. For MM cells co-cultured with OC cells under hypoxic stress, the data revealed activation of Wnt signaling pathway which is responsible for cell cycle, leading to cell growth. OC cells may support MM growth through mTOR pathway and STAT5 activation. The cell migration was deduced from activation of proteins involved in cell adhesion and migration, such as fibronectin. From these pathways and the performed parameter estimation, a system of ordinary differential equations was developed for modeling at the intracellular level. Going upstream, the intracellular processes are then used for modeling at the cellular level describing phenotypic behaviors of MM and OC cells. Conclusions: Our experimental results give data for apoptosis that are consistent with studies that have been done. The new contribution from our study is the insight into the relation between MM and OC cells in hypoxic condition that leads to the malignancy and metastasis of MM cancer cells. This work can provide a basic framework for the study of MM and OC interactions, and later for future work we will combine it with the study of the interactions between myeloma initialing cells, multiple myeloma cells, and osteoclast cells.