# CANCER STEM CELLS AS A THERAPEUTIC TARGET IN 3D TUMOR MODELS OF HUMAN CHONDROSARCOMA: AN ENCOURAGING FUTURE FOR PROLINE RICH POLYPEPTIDE-1

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Chondrosarcomas are malignant bone neoplasms relatively insensitive to chemotherapy and radiation, a property attributed by the self-renewing and stroma-perpetuating cancer stem cells (CSC). In the absence of effective adjuvant therapies, surgical resection remains the standard of care and investigations into novel targets are critical to the development of effective systemic therapies. Proline rich polypeptide (PRP-1), a 15-amino acid mammalian target of rapamycin complex-1 (mTORC1) inhibitor, has previously demonstrated cytostatic properties and antineoplastic regulation of the Wnt pathway in JJ012 human chondrosarcoma cells. This study utilizes spheroids, a dependable in vitro model of 3D solid tumors, to determine PRP-1's ability to eliminate properties of anchorage independent growth and metastatic potential. A better understanding of the mechanism by which this occurs in the CSC population of human chondrosarcoma could identify novel targets for future therapeutics.

Cultured JJ012 cells, a subset treated with PRP-1, underwent ALDEFLUOR® assay with N,N-diethylaminobenzaldehyde (DEAB) as negative control, to measure aldehyde dehydrogenase (ALDH) activity (a recognized marker of CSC's) and sorted into bulk JJ012, $ALDH^{high}$ and PRP-1 treated $ALDH^{low}$ via flow cytometry. All PRP-1 treatments were administered in a dose response manner. All cell fractions underwent clonogenic colony formation comparing PRP-1 treated colonies vs control. Cell cycle and apoptosis analysis using propidium iodide was completed using spheroids grown and treated with PRP-1 and analyzed via flow cytometry. Additionally, PRP-1 treated and control spheroids were grown for assessment of early apoptosis and cell death using a modified annexin V/Pi apoptosis assay followed by flow cytometry.

Clonogenic dose-response assay demonstrated a dose of 5 μg/mL PRP-1 to be most effective in eliminating colonies formed by JJ012 bulk

(92%, $p < 0.0002$) and $ALDH^{high}$ CSC population (80.5%, $p < 0.0005$). $ALDH^{low}$ non-CSC population was affected to a lesser extent at all doses (maximum reduction 53.5%, $p < 0.0013$). Qualitative analysis of spheroid growth displayed unequivocal reduction with increasing dosage of PRP-1 (Figure 1). Cell cycle analysis of spheroids displayed a 6% increase in apoptosis after treatment with PRP-1 and most notably a shift in cycling to G1/S phase arrest. Annexin V analysis displayed an overall decrease in spheroid viability by 59.2% with 7.6% cells shifting from viable cells into early apoptosis and 51.6% shifting from viable to dead by another mechanism after treatment with PRP-1.

The results display the effectiveness of PRP-1 in eliminating anchorage independent colony formation and thus malignant potential of chondrosarcoma CSCs. Spheroid formation, a reliable 3D tumor model and hallmark of metastatic potential conferred by CSCs, was markedly reduced by PRP-1. Additional reduction occurred in the non-CSC bulk tumor population, indicating a concomitant decline in tumor stromal cells following exposure to PRP-1. Spheroid cell cycle analysis demonstrates PRP-1's cytostatic function in CSCs by G1/S phase arrest, and insinuates death induction properties in CSCs by increased apoptosis. Annexin V analysis further displays these properties, accomplished both by late apoptosis and cell death by another mechanism. These findings ratify that PRP-1 effectively reduces CSC viability in a reliable spheroid chondrosarcoma tumor model. Further studies are necessitated in chondrosarcoma animal models to improve our understanding of the effects of PRP-1 on both neoplastic and non-neoplastic tissue.