

Pro-Survival Cocktail Improves Bone Marrow Stromal Cells (BMSC) Survival and Homing to Flank Tumors as demonstrated by Cellular MRI

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Introduction:

Over 98% of transplanted bone marrow stromal cells (BMSC) become apoptotic or die within 24-72 hours following direct implantation or IV injection. Incubating embryonic stem cells with pro-survival cocktails (PSC) resulted in improved engraftment and function in rat myocardial infarction (1). However, improvement in survival of BMSC has not been demonstrated and it is unknown if cellular MRI (CMRI) will detect the increased homing of ferumoxides-protamine (FePro) labeled cells to flank tumors.

Methods: Human BMSC were maintained in α -MEM medium supplemented with 20% FBS. Pro-survival cocktail consisted of Insulin-like growth factor I, caspase inhibitor I, anti-apoptotic protein BCL_{xL}, cyclosporin A, and pinacidil as previously described (1). Cells were treated with PSC in no serum (NS) or full serum (20%FBS, FS) for 1 to 3 days under normoxic (N, 21% O₂) or hypoxic (H, 1% O₂) conditions. Five different BMSC donors were used, with all experiments run in triplicate. In vitro evaluation of BMSC grown in PSC under N or H conditions was performed using MTS proliferation assay and AnnexinV/Propidium Iodide (PI) binding assay. Cell migration in response to no serum, SDF-1 α , PDGF-A/B, or 20% FBS was tested using a fluorescence based Boyden chamber assay. Nude mice (n=2 per group) were SQ implanted with human melanoma and when tumors reached 3mm in size, 7.5x10⁵ FEPro labeled BMSC were infused via tail vein. Animals were divided into 4 groups: BMSC N, BMSC H, BMSC H+PSC and BMSC N+PSC. T2w and T2* w MRI at 3T (Philips Intera) using a solenoid RF coil was performed 7 Days after BMSC infusions. MRI parameters were as follows: T2* TE15/TR558 FA=30; ME TE9.2 and 60/TR3626. Region of interest analysis was performed and histogram distribution of signal intensity was performed on T2*W images using MEDx software and tumors were evaluated by Prussian blue (PB) stain.

Results: Analysis at 72 hours showed improved survival of PSC-treated cells, which exhibited 5-10% less apoptosis and 7-10% less death as compared to corresponding NS controls. Cell growth over 72 hours showed significant (p<0.05) increases of cell numbers in PSC-treated BMSC in comparison to no-serum controls (Fig 1). Migration assay results demonstrated an increase in some PSC-treated BMSC compared to FS controls. Figure 2 contains mean signal intensity (SI) histogram distribution for the four cohorts of animals. There was a significant shift to lower T2*W SI in mice that received FePro labeled BMSC H+PSC, BMSC H and BMSC N+PSC compared to the control cells grown under N conditions. Histological exam demonstrated increase numbers of PB + cells in animals that were treated with H alone or PSC.

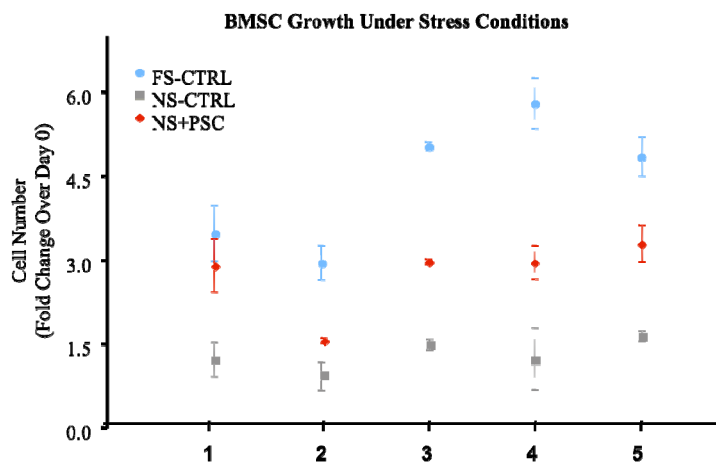


Figure 1. Cell growth over 72 hours in full serum (FS, blue), no serum (NS, grey), and with prosurvival cocktail (+PSC, red) in 5 different hBMSC donors.

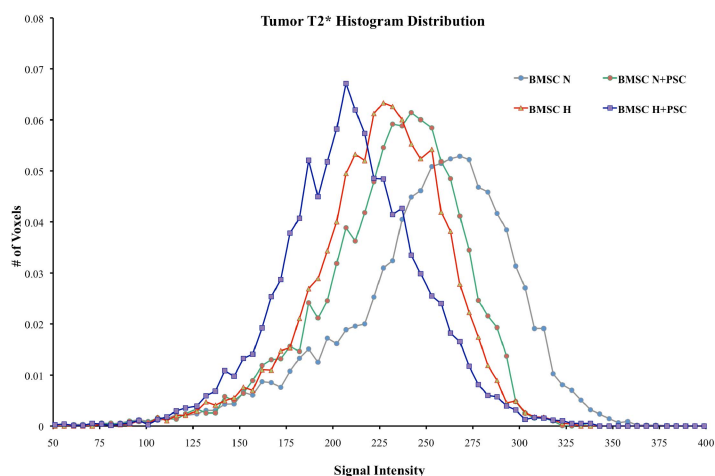


Figure 2. Histogram analysis of signal intensity from tumors show total numbers of voxels with lower signal intensity from T2*W MRI BMSC H+PSC<BMSC N+PSC, BMSC H< BMSC N.

Conclusion:

Incubation of BMSC with PSC under hypoxia improves growth and protects cells from serum starvation stress conditions. Treated cells show reduced apoptosis and an increase in number of cells in G2 and S phases (data not shown). CMRI demonstrated decreases in T2* w image SI, indicating improved survival and migration in mice that received labeled cells incubated in PSC and under hypoxic conditions. These results demonstrate that PSC+H improves the longevity and survival of BMSC in vivo and can be directly implemented in cellular gene therapy for treatment of diseases.

References:

1. LaFlamme et al, Nat Biotechnol. 2007 Sep;25(9):1015-24.