Early in vivo manganese-enhanced MRI (MEMRI) detection of embryonic stem cell induced teratoma formation

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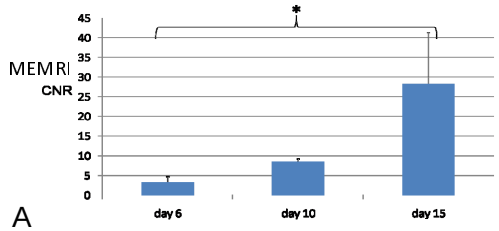
Background: Embryonic stem cell (ESC) therapy has shown to restore and regenerate damaged tissue. However, one significant complication of ESC therapy is teratoma formation. In order to address this issue, a sensitive *in vivo* imaging method to detect teratoma at an early stage is necessary. Manganese (Mn^{2+}) enters metabolically active cells through voltage-gated calcium channel and subsequently induces T_1 shortening effect. We hypothesized that *in vivo* manganese-enhanced MRI (MEMRI) can selectively enhance the ESC induced teratoma cells due to their higher metabolic activity and resultant higher intracellular concentration of Mn^{2+}relative to the normal surrounding cells .

Method: $1.5X10^6$ of firefly luciferase transduced mESC (mESC-RG) were transplanted into a SCID mouse right hindlimb. The same number of non-transduced mESC was injected into the left hindlimb (n=6). Longitudinal *in vivo* evaluation by MEMRI and bioluminescence imaging (BLI) was performed on post-transplant days 2, 4, 6, 8, 10, 15 and 21. For MEMRI, 1 mL of 10mM manganese chloride solution was injected intraperitoneally. Both hindlimbs were scanned using Signa 3.0 T Excite HD scanner (GE Healthcare system, Milwaukee, WI) with customized surface coil using T1 weighted spin echo inversion recovery (SE IR) sequence: TR 600 ms, TE 5 ms, TI 80-120ms, NEX 2, FOV 3, 128X128, slice thickness 0.5 mm. For BLI, 150mg/kg of d-luciferin was injected intraperitoneally and luciferase activity was measured for 30 minutes at 5 minute acquisition interval using IVIS spectrum (Xenogen, Alameda, CA). At post-transplant days 6, 15, and 21, 2 mice were sacrificed for histological evaluation.

Results: MEMRI detected teratoma cells as early as day 6 while positive luciferase activity was seen on day 5 (Fig.1A-B: PLEASE REPLACE THE LABEL FOR Y-AXIS FROM CNR TO MEMRI CNR). All SCID mice formed teratoma in both hindlimb (Fig.2A). Teratoma cells from mESC-RG and mESC were detected by MEMRI (Fig. 2B). This abnormal proliferation of mESC-RG was validated by BLI (Fig. 2C). Gross section of teratoma correlated with *in vivo* MRI and histology confirmed pluripotent cells of mESC induced teratoma (Fig. 2D-E).

Conclusion: MEMRI enabled early and robust *in vivo* detection of abnormal pluripotent proliferation of teratoma cells.



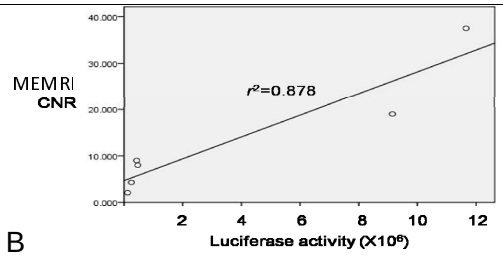


Fig.1 A. Significant enhancement of teratoma cells was detected as early as day 6 and increased rapidly (*p<0.05). **B.** Significant linear regression was observed between MEMRI and luciferase activity from days 6 to 15.

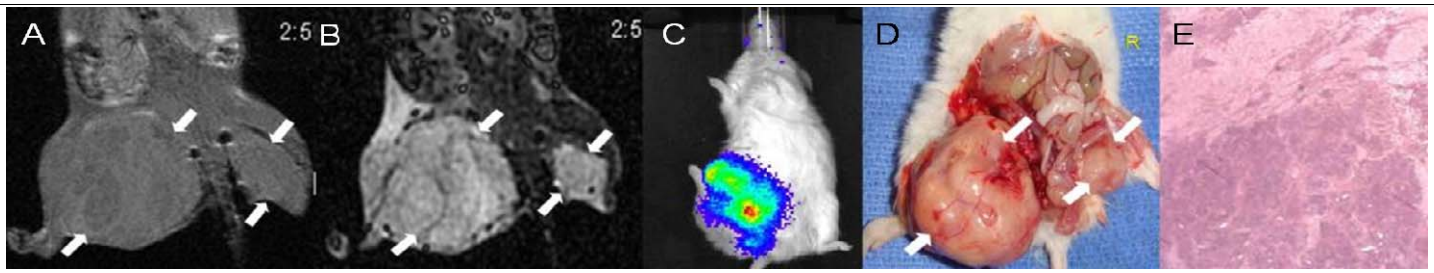


Fig.2.A. Pre-Mn^{2+} injection *in vivo* MRI of teratoma (white arrows), **B.** Post-Mn^{2+} injection *in vivo* MRI demonstrating significant contrast enhancement in the teratoma cells (white arrows), **C.** *In vivo* BLI validates MRI findings, **D.** Gross section shows corresponding teratomas (white arrows), and **E.** Histology confirms pluripotency of teratoma.