

detected in two lines, 35 proteins were common. Mass spectrometry analysis of these protein spots led to identification of 92 proteins. Our results showed that proteins involved in signal transduction, Metabolism, cell motility and Transport are the main proteins that differentially expressed whereas Immune response and stress related proteins have a less abundance related to total differentially expressed proteins. Proliferation associated proteins such EBP1 (ErbB1 binding protein), RCL (putative c-myc responsive protein), Nucleophosmin (Multifunctional protein) and HSC70 tested by western blotting and immunocytochemistry. Concurrently transcriptomics analysis with microarray and real-time quantitative PCR approaches for candidate proteins are running.

**Conclusion:** Several novel ESC-associated genes and proteins have been presented in this study which warrants further investigation with respect to the etiology of stemness.

**Keywords:** Human Embryonic Stem Cells, Proteomics, Transcriptome

### **O-53: Endogenous Adult Neural Stem Cells Comprise the Minority of Label Retaining Cells in the Adult Mouse Brain**

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**Objective:** Given rapidly dividing and slow cycling populations of neural precursors have been identified in the adult brain, with several lines of evidence suggesting the latter population represents Neural Stem Cells (NSCs), we hypothesized that adult NSCs could be identified using Label Retaining Cell (LRC) approach.

**Materials and Methods:** Adult CBA mice were injected with the mitotic marker bromodeoxyuridine (BrdU), every two hours for 48 hours. We compared the number of LRCs cells detected in a 400µm region of the PVR at increasing chase periods, to the number of primary neurospheres and NSC-derived colonies (Large Colonies) that could be generated from the same region using Neurosphere Assay (NSA) and Neural Colony-Forming cell Assay (N<sub>2</sub>CFCA).

**Results:** While the prevalence of LRCs and neurospheres and overall colonies was equivalent at specific chase periods, the number of NSC-derived colonies remained reduced by at least two orders of magnitude for chase periods up to 7 months.

**Conclusion:** Our results suggest that < 5% of LRCs are bona fide Neural Stem Cells, and highlights the pitfalls of employing this methodology to discern stem from pro-

genitor cell populations.

**Keywords:** Neural Stem Cells-Mouse-Label Retaining Cell

### **O-54: Adenosine A2A Receptor Play an Active Role in Mouse Bone Marrow Stromal Cell Proliferation and Differentiation to Mesenchymal Stem Cells**

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**Objective:** Cellular therapy is gaining interest as a means of improving the prognosis of patients with failure of various organs. Numerous in vivo and in vitro studies have shown the potential of stem cells in the regeneration of organs such as heart, muscle, cartilage, and tendon; these cells are therefore used in tissue engineering. Adult bone marrow contains a minority population of Mesenchymal Stem Cells (MSCs) that contribute to tissue regeneration. Previous studies in our lab. have demonstrated that A2A receptor accelerate wound healing and tissue repair. MSCs express SH3 and SH4, two distinct epitopes of the ecto-5'-nucleotidase (CD73), the enzyme that produces adenosine. The aim of this study was to determine whether A2A receptor may also modulate MSCs proliferation and differentiation.

**Materials and Methods:** Bone Marrow MSCs were isolated and cultured from A2A deficient female C57Bl/6 mice. We also used cells from CD73 deficient female C57Bl/6 In mRNA studies. Adenosine receptor and CD73 expression was analyzed using PCR and Real Time PCR. We identified and quantified MSCs among the adherent cells cultured by colony forming Unit-Fibroblastic (CFU-F) assay. Procollagen α2 Type I expression was determined by western blotting and immunocytochemistry. In an effort to understand how A2A receptor control MSC properties, we focused on determining stem cell specific markers. To study Phenotypic characterization, cells from primary culture and third passage, labeled with the appropriate dilution of one of the following antibodies: anti mouse Procollagen α2 Type I, CD11b, CD34, CD44, CD45, CD73 (SH3-SH4), CD90 (Thy-1), CD105 (TGF-β Co-receptor also named SH2 or Endoglin), CD133. PCR and RT-PCR utilized to study adenosine receptor expression.

**Results:** Adherent cells cultured from Bone Marrow population showed diverse morphologies. More homogeneous cell population was obtained after Subcultures. We observed that CFU-F numbers, obtained at day 12 is reduced significantly in both BM MSCs cultures from A2A receptor knockout mice and A2A receptor antagonist (ZM) treated Cells as control. The selective adenosine A2A receptor agonist, CGS 21680, fails to induce a significant increase in number of colonies. Western blotting and Semi-quantification of Procollagen α2 Type I immu-