OMICS Group Conferences
Accelerating Scientific Discovery

International Conference on
Epidemiology and Evolutionary Genetics
August 21-23, 2013 Holiday Inn Orlando International Airport, Orlando, FL, USA

# Characterisation and in vitro propagation of mammary cancer stem cells in long term cultures

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**Background:** Tumor recurrence and treatment failure are well known in cancer therapy and recently it has been linked with cancer stem cells (CSCs) or tumor initiating cells (TICs). The TISCs may not respond to primary treatment as bulk cancer cells resulting in resistance and relapse. Breast cancer stem cells (BCSCs) are increasingly thought to play a major role in breast cancer growth, relapse and metastasis. Studying cancer stem cell behavior is important in understanding cancer pathogenesis. Recent studies identified that BCSCs can be identified and sorted out based on the presence of aldehyde dehydrogenase (ALDH) enzyme. It has recently been shown that human breast cancer stem cells can be enriched and propagated in suspension cultures as mammospheres. However, little is known about the behavior of these cells in long-term cultures. Since extensive self-renewal potential is the hallmark of stem cells, we undertook a detailed functional characterization of human mammospheres over long-term passages.

**Methodology:** Breast cancer cell line MCF7 and SUM159 were grown as adherent cultures. The cells were then stained with ALDH using Aldeflor kit as per the manufacturer's instructions and sorted out using FACS ARIA II. The sorted ALDH+ cells (termed as Mammosphere forming units- MFUs), were then cultured in non-adherent, non-serum conditions at 37°C in 5% CO2 to form mammospheres. The resulting primary mammospheres after 10 days (termed M1 mammospheres) were dissociated both enzymatically and mechanically to obtain single cells. These sphere forming single cells enriched for breast stem and early progenitor cells were subjected to serial passaging every 10th day leading to the generation of M2, M3, M4 mammospheres and so on) to test the functional definition of stem cells to self renew. Also, the dissociated cells from these mammospheres were again analysed for the presence of ALDH+/bright cells and were sorted using FACS at every passage.

**Principal Findings:** We show that primary mammospheres contain a distinct population that displays a ALDH -/low phenotype, but fails to generate mammospheres. Instead, the mammosphere-initiating potential rests within the ALDH+/bright cells, in keeping with the phenotype of breast cancer-initiating cells. With increasing passages, mammospheres showed a dynamic increase both in the number of MFUs and sphere forming efficiency until the second passage followed by a dramatic reduction. Also there is an increase in the number of smaller sized spheres relative to the larger ones over multiple generations of mammospheres.

**Conclusions:** The importance of the stem cell niche in regulating stem cell behavior is well recognized. The exhaustion of the self-renewal potential of human breast cancer stem cells within seven in vitro passages of mammospheres can be explained by the alteration in this "niche". The increase in the number of senescent /differentiated cells around the non-senescent stem/ progenitor pool creates an unfavorable 'niche' for these primitive cells, thereby altering their self renewal and differentiation potential over multiple passages. Also the difference in the size of the mammospheres could represent the cellular heterogeneity with smaller spheres originating from progenitor cells and the larger spheres could be the ones being drifting towards differentiation.

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