

Molecular Carcinogenesis and Stem Cell Biology Research

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Activities 2007. The cancer stem cell (CSC) hypothesis suggests that 'stem-like' cancer cells are responsible for the growth of tumours. Indeed, evidence is emerging that signaling pathways that control the homeostasis of normal stem cells are altered in cancer (Reya et al., 2001; Smalley and Ashworth, 2003), providing support for the concept that carcinogenesis is normal organogenesis gone awry (Pardal et al., 2003). We believe that a detailed understanding of the molecular mechanisms that govern normal stem cell function and morphogenetic programs in normal tissues will dramatically influence current approaches to the treatment of cancer. The focus of our studies is breast cancer. We have previously established techniques to cultivate primary normal human mammary epithelial cells as undifferentiated stem/progenitor cells that grow in suspension to form spherical colonies, referred to as mammospheres. These mammospheres contain within them a tiny fraction of 'true' stem cells (breast stem cells, BSCs), as well as a more differentiated bulk of non-stem progeny. We are able to efficiently purify the stem cell fraction of cells using a fluorescent lipophilic dye, PKH26, that remains associated with the quiescent stem cells, but that is progressively diluted out of dividing cells. We therefore use FACS-based cell separation, based on the intensity of cell staining, to physically separate stem cells from their differentiating progeny of committed progenitor cells. To prove that PKH26-stained (PKH26+) cells are indeed BSCs, we have characterized them by immunofluorescence, using a panel of established markers for luminal epithelial cells and basal/myoepithelial cells that are known to be expressed on BSCs and/or differentiated cells (Dontu et al., 2003). PKH26+ cells express markers common to both the epithelial (CD24+/EpCAM+) and the myoepithelial (CD49f+/CK5+/TP63+) phenotype, but they do not express detectable levels of markers of the terminally differentiated states (Muc1, E-cadherin, and ASMA). This suggests that our purified PKH26+ cells have not undergone epithelial or myoepithelial lineage specification, but



that they are, instead, bipotent BSCs. The efficiency of our stem cell purification approach can be seen from the results of our breast reconstitution assays, in which we have been able to reconstitute both luminal and myoepithelial cell types in vitro (in a 3D matrigel differentiation assay) from a single PKH26+ cell. PKH26+ cells generate two types of outgrowths which recapitulate several aspects of the mammary gland in vivo: hollow, branched lobulo-alveolar structures and cavitated acini. We can detect both epithelial (EpCAM-positive) and myoepithelial (TP63-positive) cells in the acinar-like structures. In contrast, we have found that PKH26- cells do not form three-dimensional structures,

but rather form monolayers of terminally differentiated cells, with patches of epithelial, or myoepithelial cells. We are currently in the process of characterizing the transcriptional profile of PKH26+ cells, compared to PKH26- cells. The stem cell profile that we obtain will be compared to independent human breast tumour datasets, in order to evaluate the 'stemness' of human breast cancers. Our aim is to identify any correlations between the molecular characteristics of 'stemness' and clinical features of breast cancer, such as tumour grade, prognosis and responsiveness to therapy. Our ultimate aim is to obtain new stem cell markers that can be used for diagnostic, prognostic and therapeutic purposes.