A Quantitative Assay for Insulin-expressing Colony-forming Progenitors.

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Public Summary:
The field of pancreatic stem and progenitor cell biology has been hampered by a lack of in vitro functional and quantitative assays that allow for the analysis of the single cell. Analyses of single progenitors are of critical importance because they provide definitive ways to unequivocally demonstrate the lineage potential of individual progenitors. Although methods have been devised to generate "pancreatospheres" in suspension culture from single cells, several limitations exist. First, it is time-consuming to perform single cell deposition for a large number of cells, which in turn commands large volumes of culture media and space. Second, numeration of the resulting pancreatospheres is labor-intensive, especially when the frequency of the pancreatosphere-initiating progenitors is low. Third, the pancreatosphere assay is not an efficient method to allow both the proliferation and differentiation of pancreatic progenitors in the same culture well, restricting the usefulness of the assay. To overcome these limitations, a semi-solid media based colony assay for pancreatic progenitors has been developed and is presented in this report. This method takes advantage of an existing concept from the hematopoietic colony assay, in which methylcellulose is used to provide viscosity to the media, allowing the progenitor cells to stay in three-dimensional space as they undergo proliferation as well as differentiation. To enrich insulin-expressing colony-forming progenitors from a heterogeneous population, we utilized cells that express neurogenin (Ngn) 3, a pancreatic endocrine progenitor cell marker. Murine embryonic stem (ES) cell-derived Ngn3 expressing cells tagged with the enhanced green fluorescent protein reporter were sorted and as many as 25,000 cells per well were plated into low-attachment 24-well culture dishes. Each well contained 500 muL of semi-solid media with the following major components: methylcellulose, Matrigel, nicotinamide, exendin-4, activin betaB, and conditioned media collected from murine ES cell-derived pancreatic-like cells. After 8 to 12 days of culture, insulin-expressing colonies with distinctive morphology were formed and could be further analyzed for pancreatic gene expression using quantitative RT-PCR and immunoflourescent staining to determine the lineage composition of each colony. In summary, our colony assay allows easy detection and quantification of functional progenitors within a heterogeneous population of cells. In addition, the semi-solid media format allows uniform presentation of extracellular matrix components and growth factors to cells, enabling progenitors to proliferate and differentiate in vitro. This colony assay provides unique opportunities for mechanistic studies of pancreatic progenitor cells at the single cell level.

Scientific Abstract:
The field of pancreatic stem and progenitor cell biology has been hampered by a lack of in vitro functional and quantitative assays that allow for the analysis of the single cell. Analyses of single progenitors are of critical importance because they provide definitive ways to unequivocally demonstrate the lineage potential of individual progenitors. Although methods have been devised to generate "pancreatospheres" in suspension culture from single cells, several limitations exist. First, it is time-consuming to perform single cell deposition for a large number of cells, which in turn commands large volumes of culture media and space. Second, numeration of the resulting pancreatospheres is labor-intensive, especially when the frequency of the pancreatosphere-initiating progenitors is low. Third, the pancreatosphere assay is not an efficient method to allow both the proliferation and differentiation of pancreatic progenitors in the same culture well, restricting the usefulness of the assay. To overcome these limitations, a semi-solid media based colony assay for pancreatic progenitors has been developed and is presented in this report. This method takes advantage of an existing concept from the hematopoietic colony assay, in which methylcellulose is used to provide viscosity to the media, allowing the progenitor cells to stay in three-dimensional space as they undergo proliferation as well as differentiation. To enrich insulin-expressing colony-forming progenitors from a heterogeneous population, we utilized cells that express neurogenin (Ngn) 3, a pancreatic endocrine progenitor cell marker.

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