Human Pancreatic Cancer Stem Cell Utilized in Cell Based Assay system for Screening Novel and Potential Drug Candidates for Pancreatic Cancer Patients

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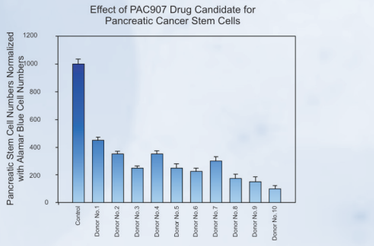
Malignant tumors are composed of a small subset of distinct cancer cells, termed "cancer stem cells" (typically less than 5-15% of total Cancer Cells based on cell surface marker expression), which have great proliferative potential, when compared to the more differentiated parental cancer cells. The Cancer Stem Cells (CSC) have the potential of differentiating into their parental cancer phenotypes, which have very limited proliferative potential. Data have been provided to support the existence of Cancer Stem Cells in several different types of Cancer, including Human Blood, Brain, Prostate, Ovarian, Melanoma, Colon, Liver and Breast Cancers. In this study we have recently reported the identification of a subpopulation of Pancreatic Cancer Stem Cells that express the Cell surface markers Cd133+, Cd44+, Cd24+, ESA+, SSEA-1+, TRA-1-61+ & TRA1-81+ (0.5-2.0% of all Human Pancreatic Parental Cancer Cells) that function as Pancreatic Cancer Stem Cells. The CD133+CD44+CD24+ESA+ SSEA-1+Oct3/4+TRA-1-61+& TRA1-81+ Pancreatic Cancer Stem Cells are highly tumorigenic and possess the Stem cell-like properties of self-renewal and the ability to produce differentiated progeny. Pancreatic Cancer Stem Cells also demonstrate upregulation of SSEA3+,SSEA4+ upon differentiation into parental Cancer phenotype. As for clinical importance, Cancer Stem Cells have shown resistance to standard therapies and may play a role in treatment failure or disease recurrence. The cell based assay system for Pancreatic Cancer Stem Cell enables one to further their drug discovery signaling pathway mechanisms that regulate their growth and survival. This Pancreatic Stem Cell based assay system may provide novel therapeutic approaches into treatment of Pancreatic Cancer patients, which are resistant to standard chemotherapy and radiation. The Pancreatic Cancer Stem Cell based assay system will provide a high through put screening of novel and potential drug candidates for the Pancreatic Cancer Patients.

Method: Primary tumor tissue was obtained from ten consented patients in Celprogen's Cancer Stem Cell Complete Growth Medium. The Pancreatic Cancer Tumor sections were analyzed and confirmed for CEA, ESA & CA199 positive antigen. The tumor was sectioned into two halves one section was processed as parental cancer cell line and the other section was processed and cultured in Pancreatic Cancer Stem Cell Complete Growth Media and matrix. After 14 days in culture the cells were characterized for Pancreatic Cell biomarkers and Pancreatic Cancer stem cell biomarkers as indicated in table 1 below:

Table1. Donor Demographics and Cell Culture Characteristics for Parental and Cancer Stem Cells

Individual	Parental Cell Culture Markers	Cancer Stem Cell Culture Markers
Female 56 yrs Caucasian	CA199, ESA & CEA, Telomerase, CD133,CD44, CD24,SSEA-1, TRA-1-61, TRA-1-81,GAD	ESA, Telomerase , CD133,CD44, CD24,SSEA-1, TRA-1-61, TRA-1-81, Oct3/4, SSEA3/4, GAD, Nestin
Female 60 yrs Caucasian		
Female 55 yrs Hispanic		
Female 45 yrs African American		
Female 45 yrs African American		
Female 59 yrs African American		
Female 53 yrs African American		
Female 52 yrs Caucasian		
Female 56 yrs Hispanic		
Female 59 yrs Hispanic		
Female 51 yrs Hispanic		



Graph1. PAC907 Drug candidate was used in a cell base assay system to determine its effectiveness in inducing cellular death of Pancreatic Cancer Stem Cells in 96Well format. The Cancer Stem Cells viability was determined by Alamar Blue dye. The Cell Numbers in this assay were normalized with Alamar Blue Cell viability.

The cell based assay system enabled one to perform a high throughput screening of novel compounds in a 96 well format. The cell based assay system for pancreatic cancer stem cell utilized an ELISA based assay system with CD133, GAD and Nestin markers.

Results: The results are indicated in the following Figures 1-4, and Graphs 1-2 .

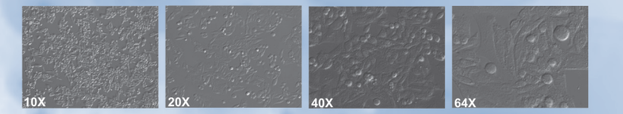


Fig1. Human Pancreatic Cancer Stem Cells at the following magnifications grown in Celprogens Media and Matrix.

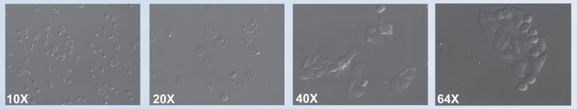


Fig2. Human Pancreatic Parental Stem Cells at the following magnifications grown in Celprogens Media and Matrix.

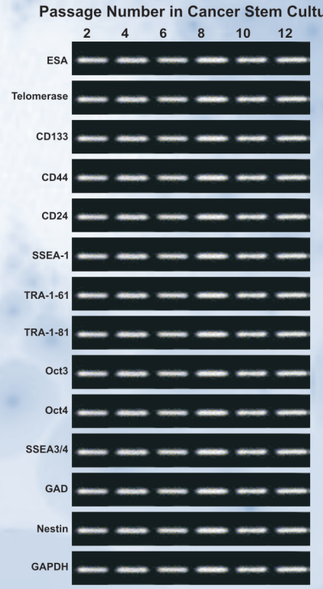
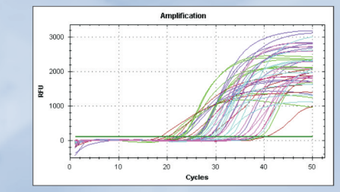


Fig3. Human Pancreatic Cancer Stem Cell gene expression profile of up to twelve passages when maintained in Celprogen's Media and ECM.

•RT-PCR analysis of ESA, Telomerase, CD133, Cd44, CD24, SSEA-1, TRA-1-61, TRA-1-81, OCT3, OCT4, SSEA3, SSEA4, GAD, Nestin and GAPDH was used as an internal control. *n* = 10.



Graph2. Human Pacreatic Cancer Stem Cells Real Time - PCR for the following genes: ESA, Telomerase, CD133, CD44, CD24, SSEA-1, TRA-1-61, TRA-1-81, OCT3, OCT4, SSEA3, SSEA4, GAD and Nestin (Real Time - PCR: CFX96™ Real-Time System C1000™ Thermal Cycler)

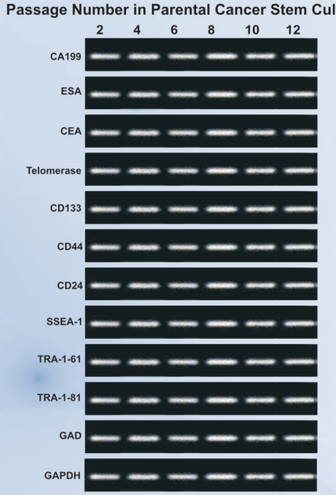


Fig4. Human Pancreatic Cancer Stem Cell gene expression profile of up to twelve passages when maintained in Celprogen's Media and ECM.

•RT-PCR analysis of CA199, ESA, CEA, Telomerase, CD133, CD44, CD24, SSEA-1, TRA-1-61, TRA-1-81, GAD AND GAPDH was used as an internal control. *n* = 10.

Conclusions: This study has demonstrated that it is possible to isolate and characterize Human Pancreatic Cancer parental and Pancreatic Cancer Stem Cells generated from patient biopsy samples when utilizing Celprogen's Pancreatic Cancer Stem Cell Culturing system. The Celprogen Pancreatic Cancer Cell Line generating system makes it possible to utilize these parental and cancer stem cell culture for screening novel drug treatments for pancreatic cancer patients. These cell lines (parental and cancer stem cells) from single donors may also be utilized in drug discovery programs utilizing gene expression and protein profiles molecular signatures for finding effective clinical therapy / treatments for pancreatic cancer patients. In this study we have identified 3 novel drug candidates that show promise as being potentially agents that may have favorable clinical out come.

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