Human Liver Cancer Stem Cells as a potential target for novel drug therapy and drug discovery

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Hepatocellular carcinoma (HCC) is an aggressive tumor with a poor prognosis. Current therapeutic strategies against this disease target mostly rapidly growing differentiated tumor Cells. In the present study we have isolated Liver Cancer Stem Cells for screening novel drug candidates for the treatment of patients with Liver Cancer. The metastatic spread of Liver Cancer Cells from the primary tumors to major vital organs, such as Lung, Colon, Brain, and Bone, is responsible for the majority of cancer-related deaths.

Liver Cancer Stem Cells are likely to play essential roles in the metastatic spread of primary liver tumors because of their self-renewal capability and their potential to give rise to differentiated progenies that can adapt to different target organ microenvironments. In the present study we have developed a high throughput cell based assay system with Human Liver Cancer Stem Cells. This assay system has enabled us to identify 300 novel drug candidates for Liver Cancer patients. The gene expression and protein expression profiles enable one to constructively conclude the novel drugs' safety and efficacy. The current Cell based assay system enables one to perform novel drug candidates screening with Human Liver Parental and Cancer Stem Cells simultaneously.

Method: Primary tumor tissue was obtained from ten consented patients in Celprogen's Cancer Stem Cell Complete Growth Medium. The Liver Cancer Tumor sections were analyzed and confirmed for CEA, ESA & Alpha Fetal Protein 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 Liver Cancer Stem Cell Complete Growth Media and matrix. After 14 days in culture the cells were characterized for Liver Cancer Cell biomarkers and Liver 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
Male 56 yrs Caucasian	ESA & CEA, Alpha Fetal Protein, Telomerase	ESA, Telomerase , CD133, CD44, CD24, SSEA-1, TRA-1-61, TRA-1-81, Oct3/4, SSEA3/4, Alpha Fetal Protein
Male 60 yrs Caucasian		
Male 55 yrs Hispanic		
Female 55 yrs African American		
Male 45 yrs African American		
Female 59 yrs African American		
Male 53 yrs African American		
Male 52 yrs Caucasian		
Male 56 yrs Hispanic		
Male 59 yrs Hispanic		
Female 51 yrs Hispanic		

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 Liver Cancer Stem Cell utilized an ELISA based assay system with CD133 and Alpha Fetal protein markers.

Fig5. SCID nude mice injected with 1000 Liver Cancer Stem Cells per mouse (10). Subcutaneous Tumor 20 days.

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

Conclusions: This study has demonstrated that it is possible to isolate and characterize Human Liver Cancer Parental and Liver Cancer Stem Cells generated from patient biopsy samples when utilizing Celprogen Liver Cancer Stem Cell Culturing system. The Celprogen Liver cancer cell line generating system makes it possible to utilize these parental and cancer stem cell culture for screening novel drug treatments for Liver 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 Liver cancer patients. In this study we have identified 300 novel drug candidates that show promise as being potential agents that may have favorable clinical out come for patients with Liver Cancer.

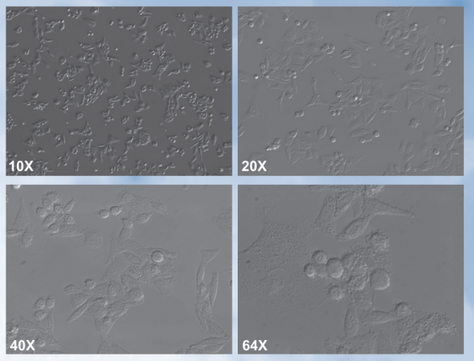


Fig1. Human Liver Cancer Stem Cells at the following magnifications grown in Celprogens Media and Matrix.

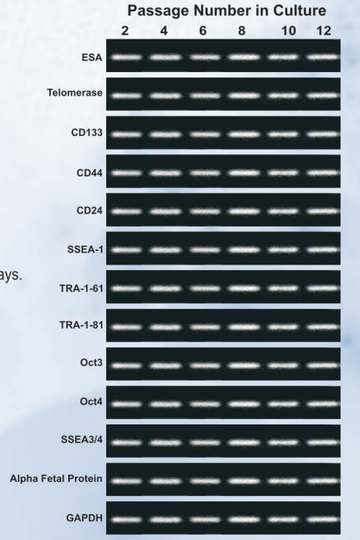
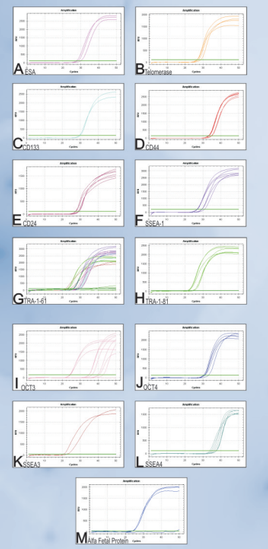
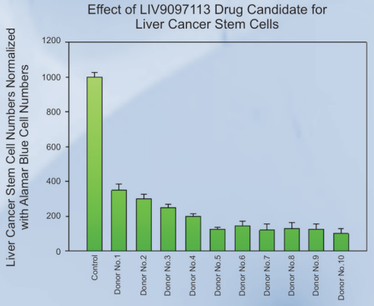


Figure3. Human Liver Cancer Stem Cell gene expression profile of up to twelve passages when maintained in Celprogen's Un-Differentiation Media and ECM.

•RT-PCR analysis of ESA, Telomerase, CD133, CD44, CD24, SSEA-1, TRA-1-61, TRA-1-81, Oct3, Oct4, SSEA3, SSEA4, Alpha Fetal Protein and GAPDH was used as an internal control. *n* = 10.



Graph1. Real Time PCR for the following genes A. ESA, B. Telomerase, C. CD133, D. CD44, E. CD24, F. SSEA-1, G. TRA-1-61, H. TRA-1-81, I. OCT3, J. OCT4, K. SSEA3, L. SSEA4, M. Alfa Fetal Protein.
(Real Time - PCR: CFX96™ Real-Time System C1000™ Thermal Cycler)



Graph2. LIV9097113 Drug candidate was used in a cell base assay system to determine its effectiveness in inducing cellular death of Liver 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.

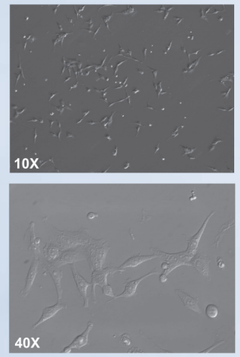


Fig2. Human Liver Parental Stem Cells at the following magnifications grown in Celprogens Media and Matrix.

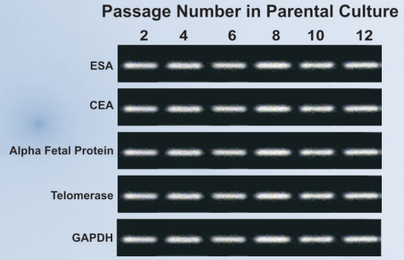


Figure4. Human Liver Cancer Stem Cell gene expression profile of up to four passages when maintained in Celprogen's Un-Differentiation Media and ECM.

•RT-PCR analysis of ESA, CEA, Alpha Fetal Protein, Telomerase and GAPDH was used as an internal control. *n* = 10.

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