Optimal Drug Concentration Screening and Evaluation in Cancer Stem Cells & 3D Tumor Stem Cell Cultures Drug Response Assays in Association with Clinical Efficacy for Pancreatic Cancer Stem Cell

John P. Cleary, Michael Sharma, Esteban J. Gomez, Cristian Sharma, Shruthi Satish, Aabha Khemani, Rubio Punzalan, Jitesh Jani, Mariam Navel, Natalee Amezcua, Jay Sharma

Requests for off-prints should be addressed to Jay P. Sharma; Email: cancerstemcells@celprogen.com
Cancer Stem Cell Biology, Celprogen Inc.
1871 N Gaffey Street, Suites A&B, San Pedro, CA 90731
www.celprogen.com

Introduction

Three-dimensional (3D) culture of cancer cell lines has long been advocated as a better model of the malignant phenotype that is most closely related to tumorigenicity in vivo. Moreover, new drug development requires simple in vitro models that more closely resemble the in vivo situation in order to select active drugs against solid tumors and to decrease the use of experimental animals. The induction of chemotherapy or concomitant chemo-radiotherapy has been used increasingly to improve survival, and organ preservation. This approach encounters significant morbidity and mortality. Therefore, reliable chemosensitivity assays are needed to accurately predict the response to chemotherapy and to guide the selection and treatment of cancer patients. The purpose of this study is to examine and evaluate optimum drug candidates in vitro chemosensitivity on patient tumor tissues directly in culture and on their cancer stem cells (CSCs) cultures.

Methods:

The tumor samples, obtained after surgery or biopsy, were placed immediately in Celprogen Tumor Transportation Media and shipped at 4-8°C for processing. Tissues were washed with 1XPBS solution, aseptically cut into 0.05mm sections, and cultured in 6 well tissue culture plates with an insert pre-coated with ECM. All cancer cell types remain viable and maintain their native architecture for at least 14 days and incorporated DNA measured by adding EdU (5-ethynyl-2'-deoxyuridine) to the culture. The efficacy of various therapeutic agents targeting major pathways (wnt, Notch, PI3K, MAPK, STAT) and chemotherapy agents were tested utilizing DNA uptake. The TUNNEL assays for these anti-cancer agents were calculated according to the inhibition index. The same compounds were tested for utilizing the patient's Pancreatic Cancer Stem Cell Cultures established with Celprogen's Media and ECM. Expression of PDX-1, SHH, CD24, CD44, CD133, EpCAM, CBX7, OC4, SNAIL, SLUG, TWIST, Ki67, E-cadherin, β-Catenin and vimentin were quantified by qPCR or immunohistochemistry per cell culture.

In-vitro study: Pancreatic CSCs and Pancreatic tumor cells (parental) were isolated from 10 terminal donor patients who had undergone chemotherapy and radiation treatments. The ages ranged from 55 years old to 65 years old, including both genders. The tissues were consented and obtained under IRB and HIPPA regulations and guidelines. The tissues were transported from the surgical suites to Celprogen in Human Pancreatic CSC complete growth media (Cat# M36115-42S) within 24 hours after it had been surgically removed from the patients. Upon receipt of the tissue, the tissue was sectioned into two halves and processed into monolayer cell cultures. One section was maintained as the heterogeneous tumor population and cultured as parental cell culture. The other section was processed further and isolated with CSC biomarkers, in Celprogen Media (M36115-42S) and Extracellular Matrix (ECM) (E36115-42-T25) combination. Once the monolayer cell cultures were established within 7-14 days the cells were characterized by Flow, IHC, Western Blot and Real Time PCR. Both the parental and the Human Pancreatic CSCs were checked for tumorigenicity by injecting 1000 cells subcutaneously in SCID mice. Once the cells were characterized they were seeded at 10,000 cells per well in a 96 well format, pre-coated with Celprogen ECM and cultured in complete growth media. The drugs were tested by incubating at various concentrations for 72 hours at 5% carbon dioxide, humidified 37°C incubator. At the end of 72 hours, cell viability was obtained utilizing Alamar blue assay and also BioRad Cell counter utilizing Trypan Blue assay. The potential drug candidates CEP1101, CEP1301, CEP1302, CEP1303, CEP1401, CEP1501 were developed at Celprogen Inc. and are highly potent inhibitors for human pancreatic CSC. CEP1101 is currently in phase I clinical development for human pancreatic cancer patients. The IC50 curves were generated for the test compounds CEP1301.

In-vivo study: One thousand viable human pancreatic CSCs and parental cells were subcutaneously injected at the hind limb of SCID mice. After 10 days post injection, animals bearing established tumors (200-300 mm3) were divided and injected with test compounds CEP1101, CEP1301, CEP1302, CEP1303, CEP1401, CEP1501 and carrier vehicle (control). The treatment was the experimental group that received IP injections three times per week for a period of two weeks. Each week the tumor growth measurements were performed and tabulated at regular intervals. At the end of the two weeks the mice were sacrificed and the tumor tissues were sectioned into three compartment; 1. One section was fixed and H&E stained, 2. One section was cultured into monolayer and IHC studies and flow studies with various Stem cell markers were performed, 3. One section was stored liquid nitrogen for genomic DNA and total RNA analysis for Real-time PCR.

Results:

The results are indicated in the figures and graphs below:

Figure 1: Model for Human Pancreatic CSC. Human Pancreatic Cancer stem cells were inoculated subcutaneously (1000 cells/mouse)10 days post injection blood samples were obtained from animals 200-300 mm3 sized tumors for PK/PD and ex-vivo Biochemical/IHC analysis.

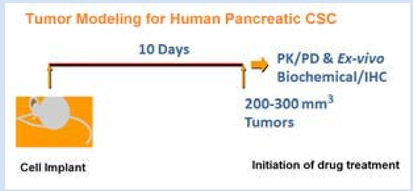


Figure 2. A. SCID mice injected with 50 CD44+CD24+ESA+ cells and 50 CD44-CD24-ESA- cells. Tumor formation was observed with positive markers within 20 days after subcutaneous injections. No visible tumor was observed with negative cells within the 20 day time frame. **B.** Human Pancreatic CSC stained positive for Ephrin type-B receptor 4 (Eph B4) marker from the Pancreatic CSC culture.

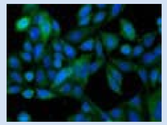


Table 1. Cancer Stem Cell general characterization Markers

Cancer Stem Cell Markers	Commonly Expressed Markers in Cell Culture
CD133	☑
Ability to form tumors <1000 cells in mice	☑
Telomerase	☑
SSEA 3 / 4	☑
OCT-4	☑
SOX2	☑
CD44	☑

Table 2. Positive Cells Markers for Human Pancreatic Parental Cancer cells and Cancer Stem Cells.

Parental Cancer	Cancer Stem Cells
CA19-9	CD133, CD44, CD24
CEA	SSEA 3 /4 , OCT4, CBX7
Alpha-10-antitrypsin	Alkaline Phosphatase
Mucin	Aldehyde Dehydrogenase
Keratin	Telomerase
CK7	Nestin
Ki67	SNAIL, SLUG, TWIST, Ki67
E-cadherin	E-cadherin, β-catenin, vimentin
EpCAM	PDX-1, SHH, EpCAM

Figure 3: Comparison of cell death of treated and untreated Pancreatic Cancer by TUNNEL assay

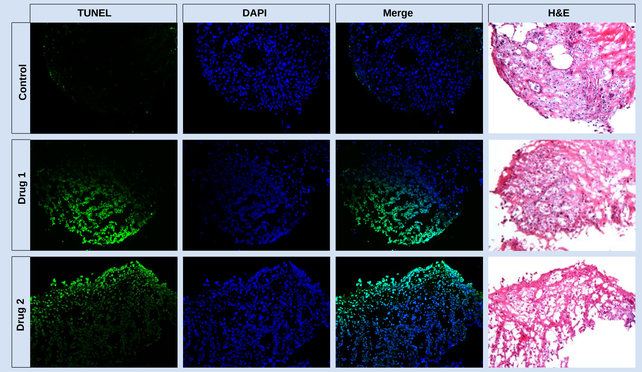


Figure 4: 3D culture system Human Pancreatic CSC for Evaluation of Drugs (CEP1101, CEP1301, CEP1302, CEP1303, CEP1401, CEP1501) inhibition of tumor growth with 100nM

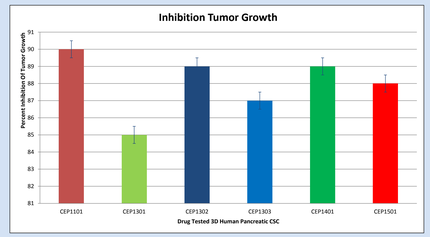
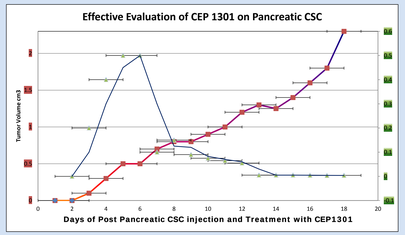
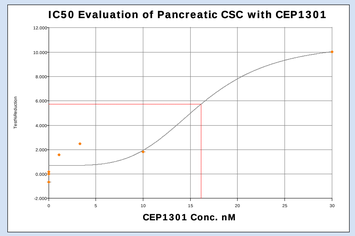


Figure 5: IC50 evaluation of Pancreatic Cancer Stem Cells with CEP1301 Drugs





Conclusions:

The epithelial-mesenchymal transition (EMT) is linked to induction of a stem-cell like phenotype. The cells were cultured in low oxygen since Tumor hypoxia includes EMT, which induces invasion and metastasis, and is linked to cancer stem cells (CSCs). Among the 600 compounds tested Gemcitabine, Taxol, Fluorouracil, Leucovorin, Irinotecan, and Oxaliptin were not effective against Pancreatic Cancer Stem Cells (CSC) but were effective on tumor cells (differentiated CSCs). The results showed 6 (CEP1101, CEP 1301, CEP1302, CEP1303, CEP1401, CEP1501) compounds that were effective against Pancreatic CSC targeting selected pathways.

AACR Annual Meeting 2013
Visit Celprogen at Booth 744.