Human Triple Negative Breast Cancer Stem Cells Utilized for Drug Discovery Therapeutics for Triple Negative Breast Cancer Patients

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Breast cancer is the most common malignancy among women in developed countries, affecting more than a million women per year worldwide. Of these, triple negative breast carcinoma represents 10-17 %. Triple negative breast carcinomas, characterized by estrogen, progesterone and HER2 receptor negativity are very aggressive tumors with poor prognosis. Breast Cancer Stem Cell derived from triple negative parental Breast Cancer tumors, are a subpopulation of cells within the parental breast cancer population within the individual which are positive for the following markers: CD133+, CD44+, CD24+, ESA+, SSEA-1+, TRA-1-61+, TRA1-81+, Oct3/4, Telomerase and GAPDH these Breast Cancer Stem Cells are highly tumorigenic and possess the stem cell-like properties of self-renewal and the ability to produce differentiated progeny. Breast Cancer Stem Cells also demonstrate up regulation of SSEA3+,SSEA4+ upon differentiation into parental cancer phenotype. Individualized treatment (tailored therapy) based on molecular biology markers of tumor and patient is the trend in clinical practice these days. However, molecular targets and predictors for the treatment of triple negative breast carcinoma do not currently exist. With the identification and characterization of Breast Cancer Stem Cells from parental triple negative tumors, enables one to screen novel drug candidates for potential development of therapeutics for triple negative Breast Cancer Patients. In this study we have utilized Breast Cancer Stem Cells from triple negative Tumors to screen potential drug candidates. The Breast Cancer Stem Cell based assay system may provide novel therapeutic approaches into treatment of triple negative breast cancer patients, which are resistant to standard chemotherapy and radiation.

Method: Primary tumor tissue was obtained from ten consented patients in Celprogen's Cancer Stem Cell Complete Growth Medium. The Breast Cancer Tumor sections were analyzed and confirmed for Estrogen, Progesterone and HER2 receptor negative. 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 Breast Cancer Stem Cell Complete Growth Media and matrix. After 14 days in culture the cells were characterized for Breast Cancer Cell biomarkers and Breast 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 Cancer Stem Cell Culture Markers	Cancer Stem Cell Culture Markers
Female 45 yrs Caucasian	CA15-3, HER2-, Estrogen-, Progestrone-, Telomerase, GAPDH, Ephrine B4, CA 27.29	HER2-, Estrogen-, Progestrone-, CD133+, CD44+, CD24+, ESA+, SSEA-1+, TRA-1-61+, TRA-1-81, OCT3 & 4, Telomerase, GAPDH
Female 55 yrs Caucasian		
Female 60 yrs Hispanic		
Female 65 yrs African American		
Female 70 yrs African American		
Female 55 yrs African American		
Female 56 yrs African American		
Female 55 yrs Caucasian		
Female 65 yrs Hispanic		
Female 55 yrs Hispanic		
Female 55 yrs Hispanic		

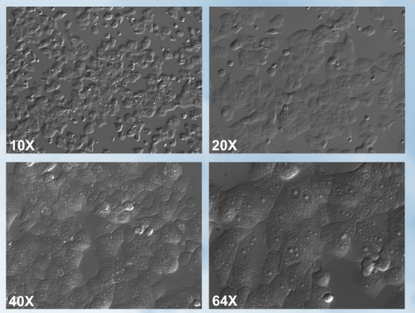


Fig1. Breast Cancer Stem Cells (3-) at the following magnifications grown in Celprogens Media and Matrix.

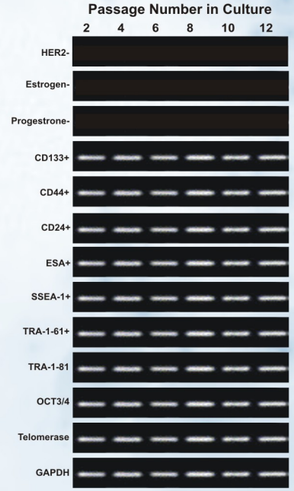
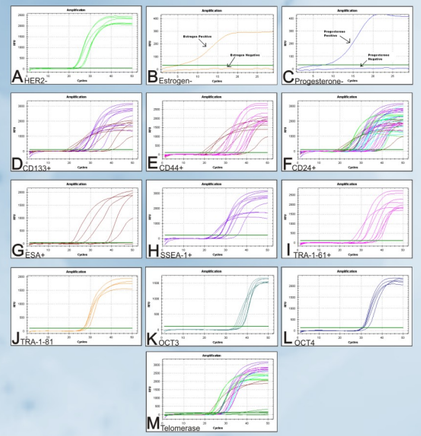


Figure3. Human Breast Cancer Stem Cell (3-) gene expression profile of up to twelve passages when maintained in Celprogen's Media and ECM.

•RT-PCR analysis of HER2-, Estrogen-, Progestrone-, CD133+, Cd44+, CD24+, ESA+, SSEA-1+, TRA-1-61+, TRA-1-61+, OCT3/4, Telomerase and GAPDH was used as an internal control. *n* = 10.



Graph1. Real Time PCR for the following genes A. HER2-, B. Estrogen-, C. Progestrone-, D. CD133+, E. CD44+, F. CD24+, G. ESA+, H. SSEA-1+, I. TRA-1-61+, J. TRA-1-81, K. OCT3, L. OCT4 and M. Telomerase.
(Bio-Rad Real Time - PCR: CFX96™ Real-Time System C1000™ Thermal Cycler)

Human Breast Cancer Stem Cells (3-)

Parental Cancer	Cancer Stem Cells
CA15-3	CD133, CD44,
HER 2 -ve	SSEA3/4, OCT4
Progesterone-ve	Tumorgenicity (<1000 cells)
Estrogen -ve	Alkaline Phosphatase
Ephrine B4 +ve	Aldehyde dehydrogenase
CA 27.29	Telomerase

Table2. Markers for Human Parental Breast Cancer Stem Cells (3-) and Human Breast Cancer Stem Cells (3-), in Culture.

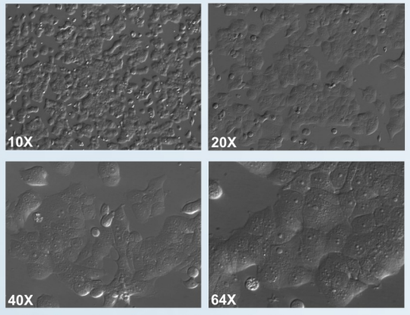


Fig2. Breast Cancer Parental Cells (3-) at the following magnifications grown in Celprogens Media and Matrix.

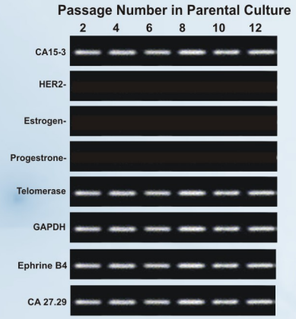
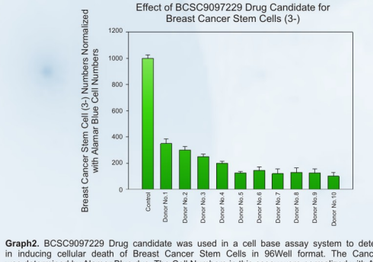


Figure4. Human Breast Cancer Parental Stem Cell (3-) gene expressionprofile of up to twelve passages when maintained in Celprogen's Media and ECM.

•RT-PCR analysis of CA15-3, HER2-, Estrogen-, Progestrone-, Telomerase, GAPDH, Ephrine B4, CA 27.29 was used as an internal control. *n* = 10.



Graph2. BCSC9097229 Drug candidate was used in a cell base assay system to determine its effectiveness in inducing cellular death of Breast 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.

Results: The results are indicated in the following Figures 1-7, and Graph 1&2.

Fig7. SCID nude mice injected with 1000 Breast Cancer Stem Cells (3-) per mouse (10). Subcutaneous Tumor formation within 20 days.

Fig6. Cell Based Assay System for testing potential drug compounds.

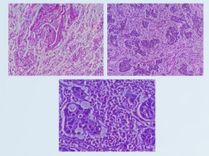


Fig5. Human Breast Cancer Stem Cells H&E

Conclusions: This study has demonstrated that it is possible to isolate and characterize Human Breast Cancer Parental and Breast Cancer Stem Cells generated from patient biopsy samples when utilizing Celprogen Breast Cancer Stem Cell Culturing system. The Celprogen Breast Cancer Cell Line generating system makes it possible to utilize these parental and cancer stem cell culture for screening novel drug treatments for Breast Cancer triple negative 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 triple negative breast cancer patients.

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