Extended Mammosphere Culture of Human Breast Cancer Cells

Application Note

The PromoCell Cancer Stem Cell Medium

The PromoCell Cancer Stem Cell Medium has been designed to meet your requirements for the extended serial 3D mammosphere culture. Most commonly used mammary cell lines are supported. In contrast to the current adherent 2D culture, this type of 3D culture selectively exploits inherent biologic features of stem cells, such as anoikis-resistance and self-renewal.

Indeed, in combination with certain breast cancer cell lines, conventional mammosphere culture media struggle with permitting a steady 3D culture pattern (see Fig 4, right).

The Cancer Stem Cell Medium however allows for robust 3D culture even with difficult breast cancer cell lines resulting in enhanced cellular selection for stem cell traits. The stem cell selective cues specific to 3D suspension culture are complemented with the serial 3D passageability offered by the Cancer Stem Cell Medium facilitating the enrichment and subsequent maintenance of cells sharing these aforementioned properties (see. Fig 3b). PromoCell's Cancer Stem Cell Medium is ready-to-use and defined, providing a standardized culture devoid of stimuli of uncharacterized origin. This is a significant benefit in terms of mammary stem cells, which are a population of highly responsive stem cells requiring reliable and reproducible control of the self-renewal/differentiation axis. The Cancer Stem Cell Medium is suitable for the cost-efficient and standardized routine culture of breast cells lines as mammospheres. In contrast to the limited passage number of classical formulations, the Cancer Stem Cell Medium supports the formation of long-term passageable mammospheres for a broad variety of cell lines.

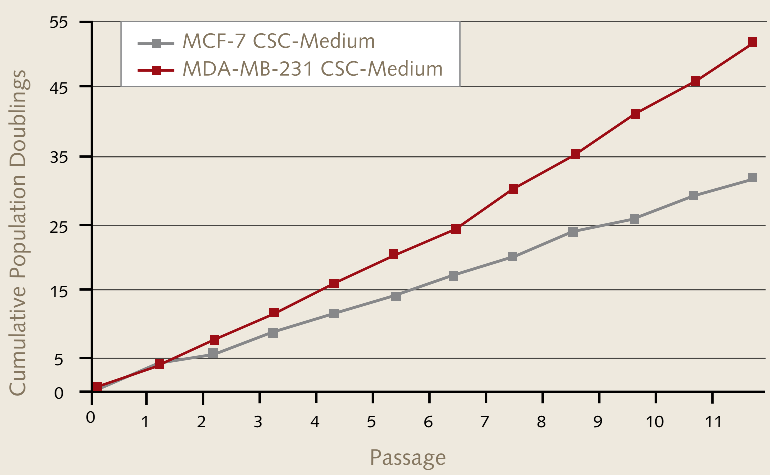

Fig. 1a: Plot of cumulative population doublings of MCF-7 and MDA-MB-231 breast cancer cell lines during serial passage of 3D mammosphere cultures in the PromoCell Cancer Stem Cell Medium. Forty thousand MCF-7 cells per well (10,000/ml) were plated in triplicate using 6-well suspension culture plates. Serial passage by enzymatic dissociation was performed according to the protocol. Mammosphere formation and proliferation were maintained during the culture, which was discontinued after passage 11 with no sign of growth rate inhibition.

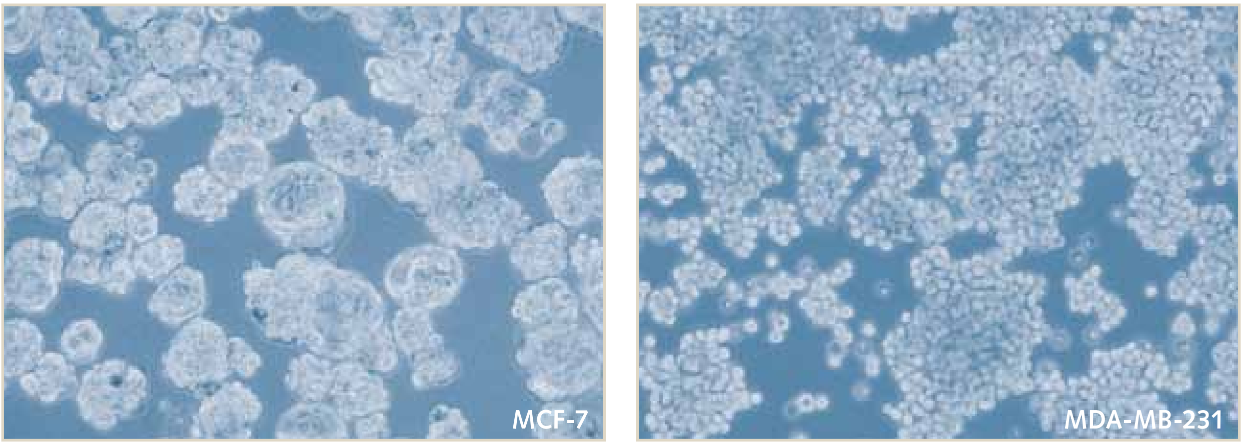

Fig. 1b: Mammosphere culture of MCF-7 (left) and MDA-MB-231 (right) mammary carcinoma cells in the PromoCell Cancer Stem Cell Medium (C-28070) after 10 serial passages. The mammosphere culture was subjected to serial passage by enzymatic dissociation according to the protocol. Robust suspension aggregate formation was maintained during serial culture. See Fig. 1a for proliferation data.

Background

As a highly regenerative and plastic organ, the mammary gland can undergo multiple cycles of changes in proliferation and function, a process controlled by stem cells. Signaling pathways involved in mammary stem cell regulation are also often found deregulated in breast cancer [1].
The progression and recurrence of breast cancer, the leading cause of cancer death in women, has been proven being linked to a small subpopulation of cancer stem cells (CSC) during the last 10 years (see Fig. 2). However, putative CSC-markers established so far still lack correlation with functional CSC features, such as tumorigenesis [2]. For now, the discovery of reliable specific marker profiles for identifying (breast cancer) CSC seems to be a distant prospect.
Reliable phenotypic testing of CSCs is still up to functional assays [3]. In 2003 Dontu *et al.* established a serum-free suspension culture system, allowing for growth of mammary stem cells as 3D spheres [4]. Serial passage of these mammospheres in serum-free media selected for cells with stem-like properties i.e. self-renewal and anoikis-resistance, prototypical stem cell features required for prolonged survival under these culture conditions. Since then, mammosphere culture became a standard tool for breast cancer research allowing for maintenance of mammary epithelial cells under culture conditions more selective for primitive cancer cells. In contrast, traditional 2D culture of established breast cell lines is essentially focused on the large majority of terminally differentiated non-stem cells within the culture as an economic and easily available replacement/model instead of primary human cells.
Mammospheres are now regarded as highly valuable *in vitro* models for studies on tumor formation and metastais as well as for drug-screenings under more phyisiologically revelant conditions as compared to 2D culture.

Schematic Overview

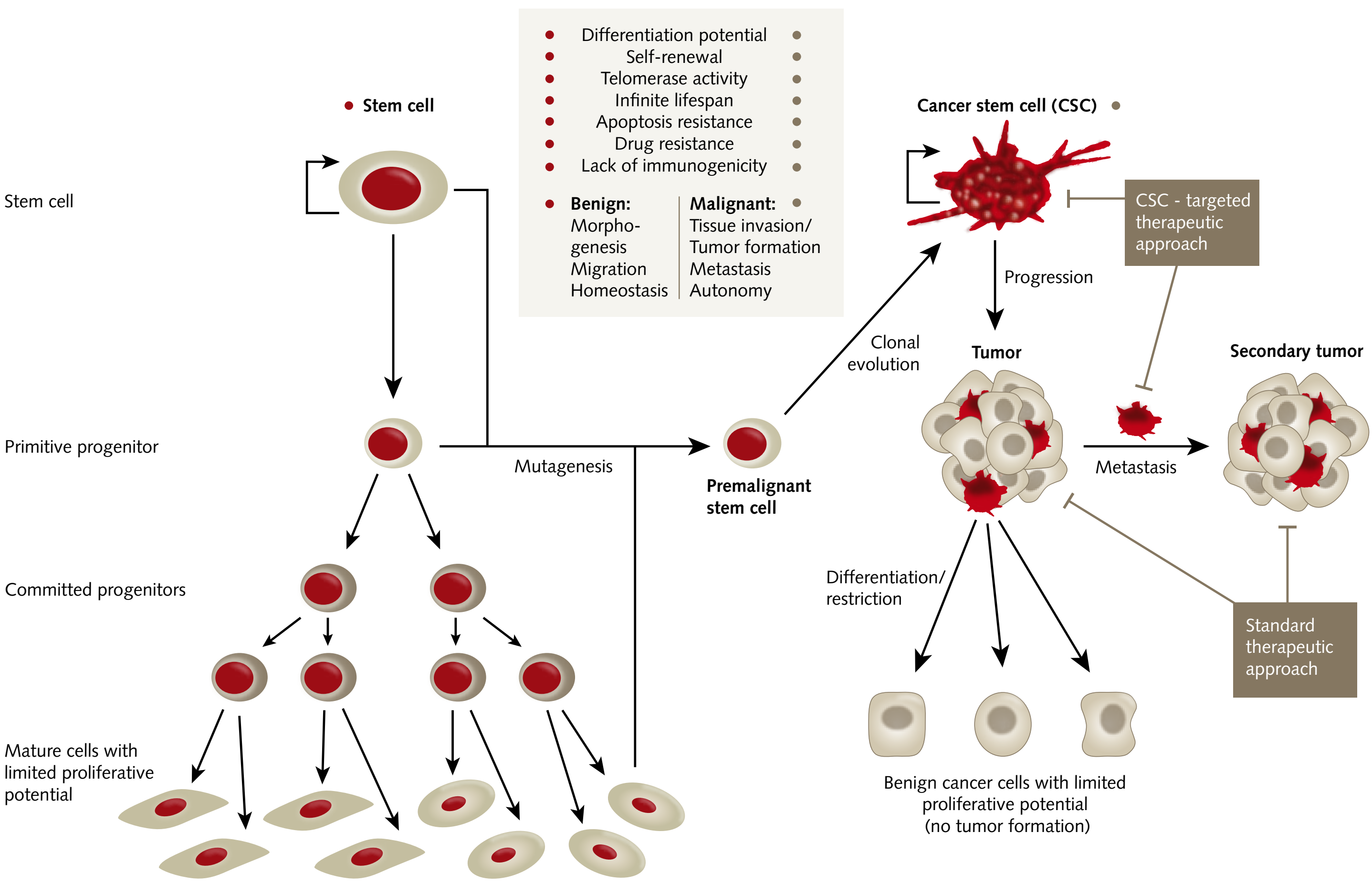

Fig. 2: Schematic overview on the origin, evolution and fate of cancer stem cells (CSC).

Use aseptic techniques and a laminar flow bench.

Mammosphere Culture Protocol

Mammosphere Culture Protocol

Materials

- Cancer Stem Cell Medium (C-28070)
- Phosphate Buffered Saline w/o Ca^{++}/Mg^{++} (PBS, C-40232)
- Detach-Kit (C-41210)
- 6-well Suspension Culture Plates (e.g. Greiner Bio One, No. 657 185)
- Adherently growing human breast cancer cell line (for initial mammosphere culture set-up)

A) Initiation of the mammosphere culture

1. **Harvest the adherent cells**
   Detach the cells of a human CSC-containing adherently growing breast cancer cell line using your standard procedures. The cells should be 80–90% confluent and in good condition. Centrifuge the cell suspension for 5 minutes at 300 x g and aspirate the supernatant. Resuspend the cells in a small volume, e.g. 3–5 ml, of the Cancer Stem Cell Medium.

2. **Count the cells**
   Count the cells using your routine method and adjust the volume with Cancer Stem Cell Medium to obtain a concentration of 1 million cells/ml.

3. **Set up the mammosphere culture**
   Seed the cells in appropriate suspension culture vessels at 10,000 cells/ml, e.g. 40,000 cells in 4 ml of Cancer Stem Cell Medium in each well of a 6-well suspension culture plate.

4. **Allow the mammospheres to grow**
   Incubate the culture for 4–10 days, depending on the cell type used. Add one half of the culture volume of fresh Cancer Stem Cell Medium every 3–4 days. Do not change the medium.

5. **Passage of the mammosphere culture**
   The mammospheres should be passaged (section B below) before they start to develop a dark center. Depending on the cell type used optimal passage should occur after 4–10 days.

B) Serial passage of mammosphere cultures

1. **Collect the mammospheres**
   Transfer the Cancer Stem Cell Medium containing the mammospheres into 15 ml conical tubes using a serological pipet.

2. **Gravity sedimentation of the mammospheres**
   Allow the spheres to settle by gravity sedimentation for 10 minutes at room temperature. Aspirate the supernatant, but leave approximately 200 µl in the conical tube. Do not aspirate the mammospheres.

3. **Wash the mammospheres**
   Repeat the sedimentation (step 2) with an equal volume of PBS. Gently aspirate the PBS leaving approximately 200 µl in the conical tube.

Use aseptic techniques and a laminar flow bench.

Mammosphere Culture Protocol

4. **Enzymatic digestion of the mammospheres**

   Add 1 ml of Trypsin-EDTA to the mammospheres and incubate for 2–4 minutes at room temperature. Keep the spheres resuspended in the trypsin solution by pipetting up and down once every 30 seconds. Avoid sedimentation of the spheres.

   Note: The optimal incubation time required to achieve complete dissociation in step B5 (below) must be determined empirically by the user for each cell type. While 2–3 minutes will be optimal in most cases, mammospheres of some cell types, e.g. MCF-7, may need longer incubation. If a completely defined dissociation process is preferred, a recombinant trypsin solution may be used as an alternative dissociation reagent according to the supplier's instructions.

5. **Break down remaining cell aggregates**

   Pipet the spheres up and down 10–20 times using a 1000 µl pipet tip to generate a single cell suspension. Aspirate the cell suspension as normal but tilt the pipet tip slightly at the bottom of the tube when expelling the cells. The shear forces generated facilitate the break up of any residual cell aggregates. Perform a visual check to confirm that no large cell aggregates remain. Immediately after trituration, add twice the volume of Trypsin Neutralization Solution (TNS).

   Note: Do not over-triturate as cell viability will be compromised. If in doubt, monitor the dissociation process microscopically. Non-dissociated cell aggregates may be removed by passing the cell suspension through a 40 µm cell strainer. When using recombinant trypsin use fresh Cancer Stem Cell Medium for inactivation instead of TNS.

6. **Determine the cell number and viability**

   Make up to 5 ml with fresh Cancer Stem Cell Medium and determine the cell number and viability. Centrifuge the cells for 5 minutes at 300 x g. Discard the supernatant and resuspend the cells in fresh Cancer Stem Cell Medium at 1 million cells/ml.

   Note: Alternatively, the cells may be resuspended in buffer, e.g. PBS w/o Ca^{++}/Mg^{++} plus 0.5% albumin plus 2 mM EDTA, and used for further experiments and/or analytical procedures.

7. **Plate the cells**

   Reseed the cells at 10,000 cells/ml in new suspension culture vessels. Typically, 6-well plates with 40,000 cells in 4 ml of medium per well are used.

Supplementary Data

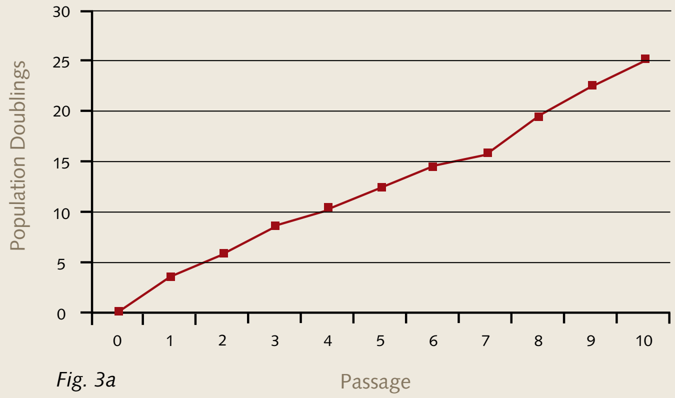

Fig. 3a

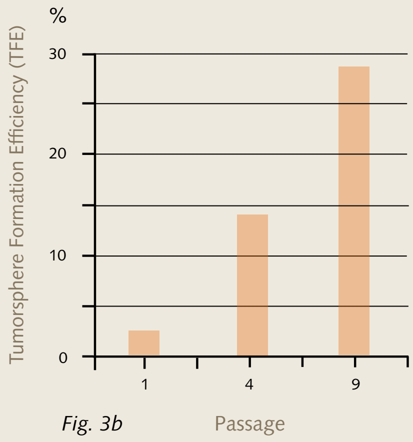

Fig. 3b

Fig. 3a: Plot of cumulative population doublings of MCF-7 cells during serial passage of 3D mammosphere culture. Forty thousand MCF-7 cells per well (10,000/ml) were plated in triplicate in the PromoCell Cancer Stem Cell Medium using 6-well suspension culture plates. Serial passage by enzymatic dissociation according to the protocol was performend every 9 days. Mammosphere formation and proliferation were maintained during the culture, which was discontinued after passage 10 with no sign of growth rate inhibition. The MCF-7 mammosphere culture achieved approximately 2.5 population doublings per passage. The proliferation rate is dependent on cell line and may vary accordingly with other types of tumor cells.

Fig. 3b: Serial passage of MCF-7 cells in results in significant increase of TFE from 2% in P1 to 28% in P9, respectively.

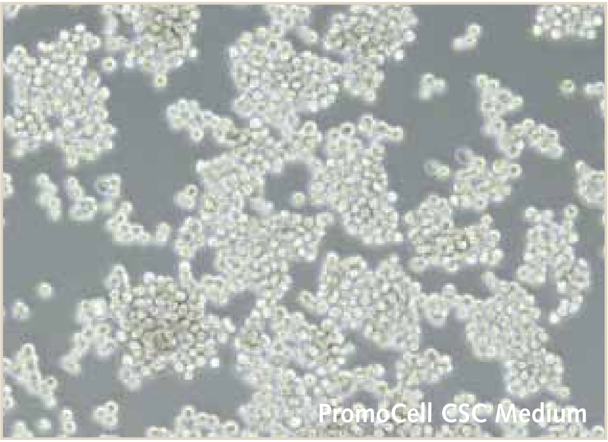

Fig. 4: 3D growth pattern of MDA-MB-231 cells in the PromoCell CSC Medium and a competitor medium containing heparin and hydrocortisone. While the cells grow as non-adherent suspension aggregates in the PromoCell CSC Medium (left), the competitor medium elicits an extensive but unwanted adherent growth pattern (right).

Products

Product	Size	Catalog Number
Cancer Stem Cell Medium	250 ml	C-28070
Cancer Stem Cell Medium, phenol red-free	250 ml	C-28075

Related Products

Product	Size	Catalog Number
Dulbecco's PBS, w/o Ca^{++}/Mg^{++}	500 ml	C-40232
DetachKit	3 x 125 ml	C-41210

References

[1] Ercan, C., P.J. van Diest, and M. Vooijs, Mammary development and breast cancer: the role of stem cells. Curr Mol Med, 2011. 11(4): p. 270-85.

[2] Liu, Y., et al., Lack of correlation of stem cell markers in breast cancer stem cells. Br J Cancer, 2014. 110(8): p. 2063-71.

[3] Owens, T.W. and M.J. Naylor, Breast cancer stem cells. Front Physiol, 2013. 4: p. 225.

[4] Dontu, G., et al., In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. Genes Dev, 2003. 17(10): p. 1253-70.

PromoCell GmbH

Sickingenstr. 63/65
69126 Heidelberg
Germany

Email: info@promocell.com
www.promocell.com

USA/Canada
Phone: 1 – 866 – 251 – 2860 (toll free)
Fax: 1 – 866 – 827 – 9219 (toll free)

Deutschland
Telefon: 0800 – 776 66 23 (gebührenfrei)
Fax: 0800 – 100 83 06 (gebührenfrei)

France
Téléphone: 0800 – 90 93 32 (ligne verte)
Téléfax: 0800 – 90 27 36 (ligne verte)

United Kingdom
Phone: 0800 – 96 03 33 (toll free)
Fax: 0800 – 169 85 54 (toll free)

Other Countries
Phone: +49 6221 – 649 34 0
Fax: +49 6221 – 649 34 40