CORNING

Culture and Assay Systems Utilized for

Cancer Stem Cell Research

Review Article

Marguerite Kosovsky, Ph.D.

Introduction

Advances in stem cell biology over the last two decades have provided new insights into cancer biology.[1-3] Tumors show marked heterogeneity in morphology, proliferation rates, genetic lesions and therapeutic response. Thus, not all tumor cells are equal. This heterogeneity is even seen in an individual tumor that is clonal. The cellular and molecular basis for tumor heterogeneity represents a fundamental problem for cancer researchers.[4,5] What controls the tumor cells? Are they responding to external and internal influences or are they a caricature of normal adult tissue that retains a hierarchical organization with stem cells at the top? Recent work with cancer stem cells suggests that there may not be one unifying theory to explain tumor heterogeneity.[6-8] Both clonal evolution and the cancer stem cell model may be complicated by the plasticity of the cancer stem cell.[9,10] Despite these theoretical arguments, the cancer stem cell model is vital to cancer research, especially in explaining tumor heterogeneity. The following overview will focus on cancer stem cells and the culture systems for growing and studying these cells. Particular focus will be on the variety of extracellular matrix substrates used to analyze the growth properties and behavior of cancer stem cells, including both *in vitro* and *in vivo* models.

Definition

It is now believed that most tumors contain a small subpopulation of cells with stem cell properties, namely cells with the ability to perpetuate through self-renewal and the ability to generate diverse mature cell types by differentiation.[11] These cells, termed cancer stem cells (CSC), have the ability to produce all of the distinct cell types found in their original tumor. They are defined experimentally by their ability to regrow tumors and are also referred to as tumorigenic cells. It is currently unclear whether CSCs arise from the transformation of stem cells or from the dedifferentiation of mature neoplastic cells.[8,11,12] Although CSCs usually represent a small fraction of the cells within a malignant tumor, they have the ability to initiate tumors upon transplantation and may be the driving force behind malignancies. CSC rich tumors are associated with higher rates of metastasis and poor patient prognosis.[13] Furthermore, CSCs have been found to have increased resistance to chemotherapeutic agents.[14,15,16] Understanding the biology and cellular chemistry of CSCs is necessary for developing more effective therapies to treat cancer.

Experimental Evidence for Cancer Stem Cells

Hematopoietic stem cells have been identified and isolated using specific surface marker profiles in conjunction with fluorescence activated cell sorting.[1] More recently, investigators have identified and used surface markers from embryonic or adult stem cells.[17] These surface markers have been used to isolate cells from tumors that exhibit stem cell-like properties. CSCs were first discovered in the hematopoietic system.[18,19] In these studies, a CD34+CD38- subpopulation was isolated from acute myeloid leukemia that could form tumors when transplanted into immunodeficient mice (the xenograph model). Using this experimental approach, a large number of CSCs have been identified in solid tumors including breast, brain and colon.[20,21,22] More recently, CSCs have also been identified in prostate, pancreas, head and neck, lung, skin, liver, kidney, ovary, and bone.[5,23] CSCs have also been isolated from established cell lines and from genetically modified cells.[17] In each case, a small number of cells were isolated from a tumor using specific cell surface markers. These cells were then tested *in vitro* to demonstrate self renewal and shown to transfer disease into immunodeficient mice by forming tumors *in vivo*.

Although CSCs all share the fundamental properties of self-renewal and the ability to differentiate into a diversity of mature cell types that can recapitulate the original tumor, CSCs derived from different tumor types can exhibit significant variability. The properties that are used to identify and characterize CSCs from one type of tumor may be different from other tumor types. This may be due in part to the fact that CSCs identified with different methods display variable phenotypes. Furthermore, some cells not originally identified as CSCs have been shown to have tumorigenic potential and stem cell features, suggesting that there is a bidirectional conversion between stem and non-stem compartments.[8] These recent studies have suggested that the characteristics required for a cancer cell to be tumorigenic may not be a set of stable properties (expression of a specific cell surface marker) but may involve dynamic pathways that are governed by the tumor microenvironment.[24]

Role of the Microenvironment

There is a mutual exchange of information that guides the functional organization of normal tissues through collaboration with stromal cells, epithelial and tissue specific cells.[25] Cells communicate with each other via cell junctions, through interactions with the extracellular matrix (ECM) via receptors, and via dynamic interactions with hormones and soluble factors.[26] The ECM is composed primarily of glycoproteins, collagens, proteoglycans, and elastin. This material serves to stabilize tissues, promote cell attachment, and modulate cell functionality by specifically interacting with cell surface receptors and activating the associated signaling pathways.[27] It has been shown that the ECM plays structural, biochemical and mechanical roles in normal growth and differentiation of stem cells[28] and in cancer progression.[29]

Normal stem cells reside in a “stem cell niche” that maintains them in a stem cell state.[2] This niche has a complex architecture containing stromal cells such as immune cells and mesenchymal cells, a vascular network, and ECM.[30] Like normal stem cells, CSCs are influenced by interactions between the nonmalignant cells that comprise their microenvironment. In fact, recent data suggest that CSCs rely on a similar niche, the “CSC niche,” which controls their self-renewal and differentiation.[31,32] *In vitro* approaches have been used to investigate the specific, well defined interactions between CSCs and the surrounding stromal cells (detailed below). Syngeneic mouse models have helped clarify the role of the microenvironment in CSCs. Furthermore, signal transduction pathways (eg, Notch, Hedgehog, and Wnt pathways) are regulated by extrinsic signals originating in the stem cell microenvironment or niche.[25]

This niche may even protect the CSCs from genotoxic insults by promoting a higher rate of DNA repair[1.4,15,33] This suggests that many cancer therapies will fail if they kill the bulk of the tumor cells but do not eliminate the CSCs.[7,34] As a result, the CSC microenvironment, including ECM binding sites and associated signaling pathways, is considered a potential target for anti-cancer therapies.[11]

Strategies for Studying Cancer Stem Cells

CSCs are most often defined by the enrichment of a subpopulation of tumor cells from tumor tissue using specific cell surface markers and isolation with FACS.[3] In addition to tumor tissue, CSCs have been isolated from existing tumor-derived cell lines.[35-37] CSCs have also been isolated from genetic modifications of normal cells.[8,38,39] Once isolated, the CSC enriched populations are then tested for their ability to self renew and form tumors.

Two *in vitro* assay systems are typically used to demonstrate self-renewal of CSCs: colony formation assays and sphere formation assays. The colony formation assay measures the functional capacity of stem cells and has recently been used to study breast and colon CSCs.[37,40] Sphere formation (or tumorsphere) assays involve three dimensional (3D) culture systems. There are two main approaches for growing 3D tumorspheres, as a suspension in serum-free media or on a 3D substrate comprised of reconstituted basement membrane (Corning® Matrigel® matrix). Matrigel matrix has been used for tumorsphere formation of CSCs isolated from many solid tumor types as well as tumor derived cell lines and genetically modified cells (Table 1). The major component of Matrigel matrix is laminin, followed by collagen IV, heparin sulfate proteoglycans, entactin, nidogen and growth factors. Based on its physiological composition and functionality, Matrigel matrix effectively models the physical interplay that occurs between CSCs and the ECMs that exist in the tumor microenvironment *in vivo*. This is especially important in light of the recent studies demonstrating the role of the microenvironment in maintaining the CSC niche.[25,30,77]

Xenotransplantation into immuno-compromised mice is the primary *in vivo* assay used to demonstrate tumorgenecity of CSCs. Many of the CSC transplantation experiments have utilized Corning® Matrigel® matrix as a carrier (Table 1). In a recent study, Quintana, et al., used FACS to fractionate melanoma cells into CSC-enriched CD271+ and CD271- subpopulations.[61]

These subpopulations were each mixed with Matrigel matrix High Concentration (HC) and then injected into NOD/SCID IL2R g null mice. The results demonstrated that both subfractions of melanoma cells were able to generate tumors *in vivo*, whether enriched for CSCs or not. These findings question the hierarchical model that CSCs are a minority cell type in all solid tumors, but rather may exist as heterogeneous CSC sub-types within one tumor type. Furthermore, these findings suggest that CSCs may be characterized by a unique plasticity that allows for reversible changes of their phenotype. Interestingly, the plasticity of CSCs has been demonstrated recently in breast cancer cells.[8,51]

The functionality of CSCs may be influenced by the characteristics of the immunodeficient recipient, the site of implantation, the cell carrier (e.g., collagen, Matrigel matrix), the number of input cells, as well as the time *in vivo*.[9] Since the growth of human tumors in mice is under the control of murine stroma and vasculature, it may be difficult to analyze the effects of the microenvironment on the growth and functionality of human cancer stem cells in this system. Although *in vivo* assays using immonodeficient mice are the gold standard for identifying stem cells, serial transplantation assays with animal models do not lend themselves to highthroughput screening.[17] Alternatively, defined *in vitro* systems allow for the study of CSCs plasticity, the regulation of CSCs by the microenvironment, and may ultimately be used for compound screening and the development of anti-cancer drugs.[25,49]

Table 1. Cancer Stem Cells/Representative Culture and Assay Conditions

Cell Type	Extracellular Matrix or other condition	Cell Function or Behavior
BRAIN	Corning Matrigel Matrix	Tumorsphere Formation (3D) (31) Cell Invasion (41) Cell Migration (42) Differentiation (42)
	Poly-D-lysine-Laminin	Differentiation (31,43)
	Laminin	Attachment (44)
	Serum-free Suspension	Tumorsphere Formation (3D) (21,43)
BREAST	Matrigel Matrix	Colony Formation (40) Tumorsphere Formation (3D) (39,8) Cell Invasion (36,45-47) Differentiation (3D) (39,48) Xenotransplantation (20,38,49)
	Corning Primaria™	Proliferation (38)
	Fibronectin	Adherence (50) Differentiation (48)
	Collagen I, Laminin	Differentiation (48)
	Collagen IV	Differentiation (51)
	Serum-free Suspension	Tumorsphere Formation (3D) (48,51)
COLON	Matrigel Matrix	Colony Formation (37,52) Tumorsphere Formation (3D) (33,53) Differentiation (3D) (32,53) Xenotransplantation (22,37,54,55-57)
	Collagen	Differentiation (55,58) Proliferation (53)
	Fibronectin	Adherence (50)
	Serum-free Suspension	Tumorsphere Formation (3D) (22,32,33,54,55)
MELANOMA	Matrigel Matrix	Migration (59) Differentiation (3D) (60) Xenotransplantation (61)
	Serum-free Suspension	Tumorsphere Formation (3D) (62)
PANCREAS	Matrigel Matrix	Migration (63) Xenotransplantation (64)
KIDNEY	Matrigel Matrix	CSC Microvesicle-induced Angiogenesis (65) Xenotransplantation (66)
LUNG	Matrigel Matrix	Xenotransplantation (67)
	Fibronectin	Proliferation (50) Migration (67)
	Serum-free Suspension	Tumorsphere Formation (3D) (68)
PROSTATE	Matrigel Matrix	Tumorsphere Formation (3D) (69,70) Cell Invasion (70,71) Differentiation (70) Xenotransplantation (70,72)
	Laminin	Proliferation (50)
BONE	Matrigel Matrix	Xenotransplantation (73)
OVARY	Matrigel Matrix	Xenotransplantation (74,75)
	Serum-free Suspension	Tumorsphere Formation (3D) (75,76)

Specific *In Vitro* Culture Conditions And Assays

3D Culture of Cancer Stem Cell Tumorspheres

Many laboratories have utilized reconstituted basement membrane (Corning® Matrigel® Matrix) to grow tumorspheres *in vitro*.[8,31,33,39,48,53,69,70] Other studies have grown tumorspheres in suspension using serum-free media.[21,22,32,33,43,48,51,54,55,62,68,75,76] Regardless of the conditions in which the tumorspheres are initially grown, they can be further cultured *in vitro* in the presence of Matrigel matrix, which allows for their propagation and differentiation. Dontu and colleagues developed a 3D culture system in Matrigel matrix that allowed single cells isolated from mammospheres to generate complex acinar structures (Fig. 1).[48] Two more recent studies have demonstrated the plasticity of breast epithelial CSCs in a 3D culture environment comprised of Matrigel matrix. Mani and colleagues have shown that breast epithelial cells transformed with the SNAI1 and TWIST genes, which are known to induce the Epithelial-Mesenchymal Transition (EMT), acquired characteristics of CSCs.[39] The EMT is important in development, and it is often activated in cancer invasion and metastasis. Specifically, the transformed, immortalized cell lines exhibited an increased ability to form mammospheres in a matrix composed of Matrigel matrix. These cells looked like CSCs isolated from human tumors and showed an increase in EMT markers. These researchers also demonstrated that single mammospheres from the transformed cell lines could differentiate in Matrigel matrix to form complex 3D structures similar to mammary ducts (Fig. 2).[39] These findings illustrate a direct link between the EMT and the acquisition of epithelial stem cell properties. In another study, breast CSCs cultured in 3D using Matrigel matrix were found to exhibit an unexpected degree of plasticity between stem-like and breast cancer cells that do not exhibit stem-like properties.[8] Using 2D and 3D culture, a subpopulation of mammary epithelial cells was shown to spontaneously differentiate into stem-like cells. Furthermore, the results demonstrated that genetic transformation enhances the spontaneous conversion of non-stem cancer cells to CSCs *in vitro* and *in vivo*. Both of these studies dispute the hierarchical model for CSCs and have the potential to drastically change the strategies used for anti-cancer drug discovery.

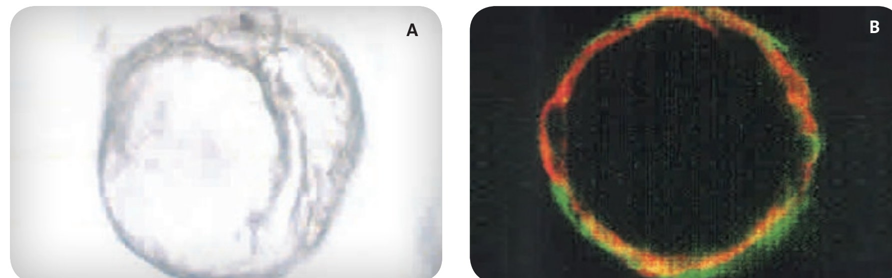


Figure 1. Mammospheres Contain Multipotent Cells Capable of Differentiating into Ductal-Alveolar Structures. (A) Acinar structure generated by a single human mammary epithelial cell isolated from the mammosphere and grown on Corning Matrigel matrix for 3 weeks (3D culture). (B) Acinar structure visualized by immunostaining for myoepithelial linage (CD10, FITC, green) and ductal epithelial linage (ESA, TEXAS RED®). Data courtesy of Dr. Gabriela Dontu (originally published in reference 48), University of Michigan.

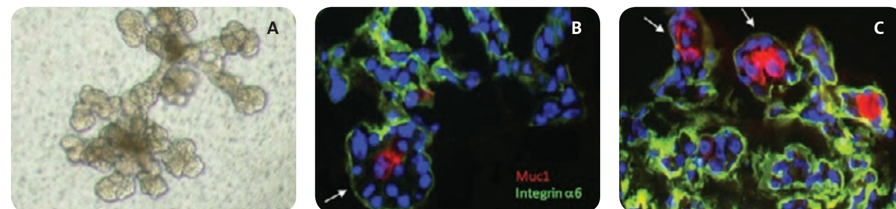


Figure 2. *In Vitro* Differentiation of Mammospheres in Matrigel Matrix Promotes the Formation of Secondary Structures. (A) Phase contrast images of the differentiated mammospheres following culture in Matrigel matrix. (B and C) The differentiated structures were immunostained for Muc 1 (red) and CD49f/integrin 6 (green). Data courtesy of Dr. Robert Weinberg (originally published in reference 39), Massachusetts Institute of Technology.

Co-Culture of Cancer Stem Cell Tumorspheres

Co-culture systems have been used to better understand how CSCs are regulated by their microenvironment. In a recent study, two approaches were used to examine brain CSCs in the presence of primary human endothelial cells (PHEC).[31] When these cells were cultured together in Corning® Matrigel® matrix, the PHECs formed vascular tubes (Fig. 3).[31] This result was only observed using the CD133+ subfraction (enriched with CSCs), but not the CD133- subfraction. Since normal neural stem cells are maintained by soluble factors secreted by endothelial cells, the authors then tested if endothelial-secreted factors were able to maintain brain CSCs using a cell culture insert model. In this system, the PHECs were cultured in the apical compartment (Falcon® inserts, 0.4 µm), and brain CSCs in the basolateral compartment. This insert system allows for the exchange of diffusible factors, but not cells, between chambers. The experiment demonstrated that PHECs allow for the maintenance of self-renewal and the undifferentiated phenotype (tumorspheres) of brain tumor CSCs. In addition, tumorsphere differentiation was demonstrated on a substrate composed of poly-D-lysine/laminin (Corning BioCoat™ coverslips). Taken together, these experiments demonstrate how the niche microenvironment participates in the regulation of CSC behavior.

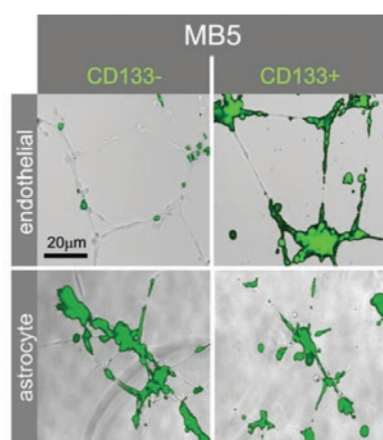


Figure 3. CD133+ Brain Tumor Cells Associate with Endothelial Cells in 3D Matrigel Cultures. Overlay of phase contrast and autofluorescence photomicrographs of unlabeled endothelial cells (top panels) or astrocytes (bottom panels) that were cocultured with CD133- or CD133+green fluorescence-labeled primary medulloblastoma cells (MB5). Data courtesy of Dr. Richard Gilbertson (originally published in reference 31), St. Jude Children's Research Hospital, Memphis, TN.

Cancer Stem Cell-Mediated Cell Invasion Assays

In vitro cell invasion assays have been used to examine the interaction of cancer stem cells with stromal cells in order to better understand the stem cell niche in tumors. Previous studies have shown that mesenchymal cells may be recruited to the sites of developing tumors and stimulate tumor growth via the production of IL-6. Recently, Lui et al. demonstrated that bone marrow-derived mesenchymal stem cells (MSCs) exhibit invasive behavior in the presence of breast CSCs when cultured in invasion chambers precoated with growth factor-reduced Matrigel matrix.[46] Specifically, they showed that breast CSCs cultured in the basolateral chamber increased mesenchymal cell invasion from the apical chamber. This effect could be blocked with anti-IL-6 antibody. These results indicate that MSC invasion towards breast CSCs is mediated via the IL-6 signaling pathway, which supports the conclusion that cytokine networks regulate CSC interactions with stromal cells.

Previously, Charafe-Jauffret and colleagues used BioCoat Matrigel Invasion Chambers to demonstrate that IL-8 increases the invasiveness of CSCs that were isolated from breast cancer cells lines.[36] Specifically, they demonstrated that the Aldefluor+ subpopulation (enriched for CSCs) had a 6 to 20 fold increase in invasion through Matrigel matrix as compared to the Aldefluor- subpopulation (not enriched in CSCs). The addition of IL-8 to the culture system significantly increased the invasion of the CSCs, but had no effect on the Aldefluor- subpopulation. They concluded that the IL-8 pathway, reported by others to play a role in metastasis, is involved in CSC invasion. More recently, McGowan, et al. have used invasion assays to demonstrate the involvement of the Notch signaling pathway in maintaining the stem cell-like phenotype of breast CSCs.[47] This study demonstrated that silencing of Notch1 with shRNA significantly reduces the ability of breast cancer cells to invade through a barrier of Matrigel matrix. Taken together, these examples highlight key experimental systems currently used for studying the regulation of CSCs by signal transduction pathways *in vitro*.

Furthermore, invasion assays have been utilized to study how CSCs modify their tumor environment by triggering angiogenesis through the release of microvesicles (MVs). MVs have been implicated in cancer progression, and tumors are known to release large amounts of MVs.[78] Grange and colleagues isolated a renal CSC+ subpopulation (CD105+) and then derived MVs from the CSCs.[65] The MVs from the CD105+ subpopulation, but not MVs from the CD105- subpopulation, were shown to increase the angiogenic phenotype of human endothelial cells. Moreover, the CSC-derived MVs increased the invasion of endothelial cells through cell culture inserts coated with Corning® Matrigel® matrix. This paper demonstrated that MVs from renal CSCs trigger an angiogenic switch and formation of a pre-metastatic niche which may be involved in tumor progression and metastasis.

3D Differentiation Assays of Cancer Stem Cells

Colon CSCs that were isolated using the CD133 marker[22,56] have been shown to exhibit differentiation *in vitro*.[33] This work investigated the differentiated properties of colon CSCs grown as spheroid cultures (tumorspheres) in serum-free media. The spheroid cells could be forced to differentiate *in vitro* into large polygonal colon cells when grown as adherent cultures on collagen-coated flasks with 10% serum. When the tumorspheres were grown in 3D in the presence of serum in Matrigel matrix, the resulting colonies were organized in a complex structure reminiscent of a colonic crypt (Fig. 4).[33] Others have recently adapted this *in vitro* 3D culture system using Matrigel matrix to propagate normal intestinal crypts[79,80] and CSCs derived from colorectal cancer-derived cell lines.[37]

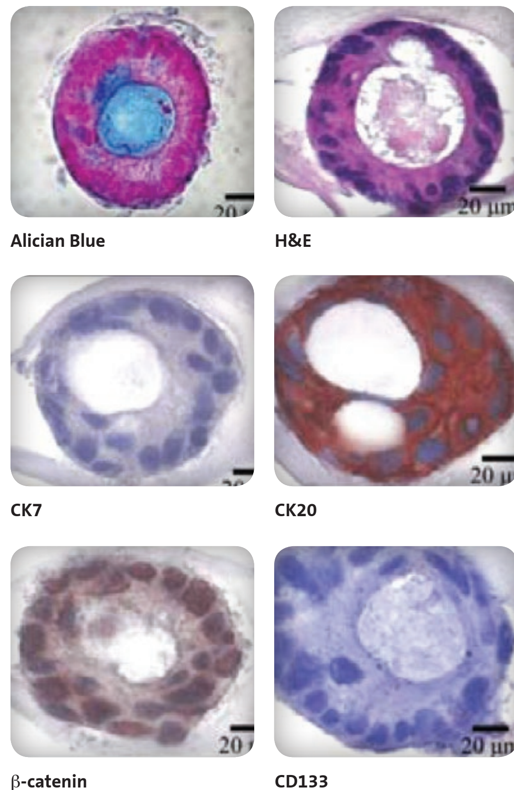


Figure 4. Colon Cancer Spheroids Cultured in the Presence of FBS in Corning Matrigel Matrix Organize in a Complex Structure Reminiscent of a Colonic Crypt. Acadian blue, H&E staining, and immunohistochemical analysis of CK7, CK20, b-catenin, and CD133 performed on paraffin-embedded sections of spheroids cultured in Matrigel matrix for 20 days. Data courtesy of Dr. Giorgio Stassi and Dr. Jan Paul Medema (originally published in reference 33), University of Palermo and Academic Medical Center, Amsterdam, the Netherlands.

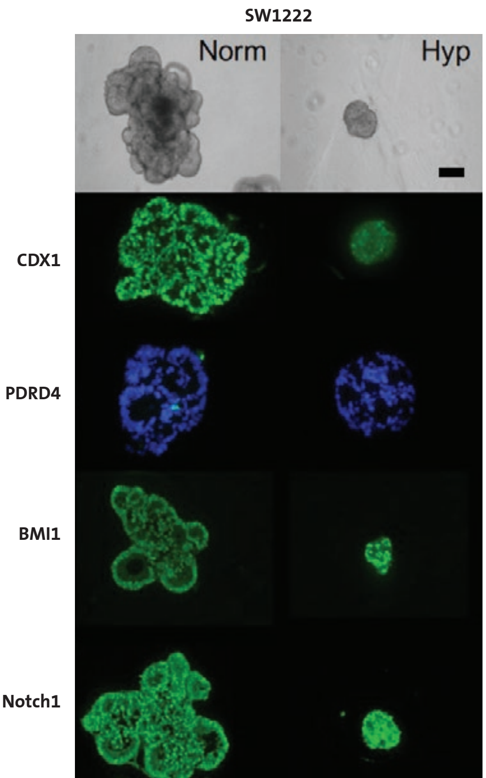


Figure 5. Hypoxia Prevents Differentiation of Colorectal Cancer Cells and Maintains a Stem-like Phenotype. Light microscopy and immunofluorescence of SW1222 grown for 4 weeks in Matrigel matrix under normoxia and hypoxia (1% oxygen). (Magnification: 20x objective; scale bar: 200 µm.) Data courtesy of Dr. Walter Bodmer (originally published in reference 52), University of Oxford, UK.

In a subsequent study using colon CSCs, Vermeulen and colleagues demonstrated that CSC differentiation is controlled by an extracellular signal input utilizing *in vitro* differentiation in the presence of growth inhibitors.[32] Single cell clones of CSCs were isolated and shown to form tumors *in vivo* when xenotransplanted into mice and were also shown to exhibit multilineage differentiation *in vitro* when cultured in growth factor-reduced Corning® Matrigel® matrix. Importantly, they showed that the *in vitro* differentiation of colon CSCs could be directed by inhibiting phosphoinositide 3-kinase (PI3K) signaling. These findings have provided clues to the regulatory pathways that govern CSCs *in vivo*.

Yeung et al. have demonstrated that colorectal CSCs derived from cell lines were able to differentiate under normal oxygen conditions *in vitro* using Matrigel matrix.[52,37] Previous studies have demonstrated that hypoxia can induce the CSCs phenotype in Glioma stem cells[81] as seen by the smaller, rounder, less differentiated colonies grown under hypoxic conditions. In this report, hypoxia inhibited the differentiation of these CSCs and increased their clonogenicity as seen by the smaller, rounder, less differentiated colonies grown in 1% oxygen (Fig. 5).[52] Furthermore, this study implicated the Notch1 and CDX1 ligands in controlling the differentiation of these cells. Taken together, these findings help to explain why hypoxia is associated with a more aggressive tumor and poor clinical outcome by showing that hypoxia leads to an increase in the proportion of CSCs in a tumor.

Future Directions

The distinct roles of CSCs in cancer progression can be studied by a variety of complementary *in vitro* approaches. Investigation of CSCs offers the possibility of generating novel targets for cancer that may overcome drug resistance and effectively combat the process of tumor cell metastasis.[12] Because CSCs appear similar to normal stem cells, great care must be taken to protect normal cells when patients are exposed to anti-cancer treatments. New and improved experimental approaches, and especially *in vitro* assay systems, will allow scientists to micro-dissect the exact relationship between the various cell types within a tumor and their microenvironment.

References

1. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem sells, cancer, and cancer stem cells. Nature 414:105-111 (2001).
2. Dick JE. Stem cell concepts renew cancer research. Blood 112:4793-4807 (2008).
3. Clevers H. The cancer stem cell: premises, promises and challenges. Nature Medicine 17:313-319 (2011).
4. Lander AD. The ‘stem cell’ concept: is it holding us back? J. Biology 8:70-76 (2009).
5. Visvader JE. Cells of origin in cancer. Nature 469:314-322 (2011).
6. Rosen JM, Jordan CT. The increasing complexity of the Cancer Stem Cell Paradigm. Science 117:1670-1673 (2009).
7. Gupt PB, Filmore CM, Jiang G, Shapira SD, Tao K, Kiperwasseer C, Lander ES. Stochasticstate transitions give rise to phenotypic equilibrium in populations of cancer cells. Cell 146:633-644 (2011).
8. Chaffer CL, Brueckamnn I, Scheel C, Kaestli A, Wiggins PA, Rodrigues LO, Brooks M, Reinhardt F, Su Y, Polyal K, Arendt LM, Kuperwasser C, Bierie B, Weinberg RA. Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. PNAS 108:7950-7955 (2011).
9. Sarry JE, Murphy K, Perry R, Sanchez PV, Secreto A, Keefer C, Swider CR, Strzelecki AC, Cavelier C, Recher C, Mansat-De Mas V, Delabesse E, Danet-Desnoyers G, Carroll M. Human acute myelogenous leukemia stem cells are rare and heterogeneous when assayed in NOD SCID/IL2R c-defieient mice. J. Clinical Investigation 121:384-395 (2011).
10. Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrisosn SJ. Efficient tumor formation by single human melanoma cells. Nature 456:593-598 (2008).
11. Alison MR, Islam S, Wright NA. Stem cells in cancer: instigators and propagators? J. Cell Science 123:2357-2368 (2010).
12. Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CHM, Jones D L, Visvader J, Weissman IL, Wahl GM. Cancer stem cells-Perspectives on current status and future directions: AACR Workshop on Cancer Stem Cells. Cancer Research 66:9339-9344 (2006).
13. Ailles LE, Weissman IL. Cancer stem cells in solid tumors. Current Opinion in Biotechnology 18:460-466 (2007).
14. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. Nature Reviews 5:275-284 (2005).
15. Bao S, Wu Q, McLendon RE, Hao Y, Quig S, Hjelmeland AB, Dewhirst MW, Bignet DD, Rich JN. Glioma stem cells promote radioresistance by preferential activiation of the DNA damage response. Nature 444:756-760 (2006).

16. Hermann PC, Huber SL, Herrier T, Aicher A, Ellwart JW, Guba M, Bruns CJ, Heeschen C. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell 1:313-323 (2007).
17. Mather JP. Cancer stem cells: in vitro models. Stem Cells 30:95-99 (2012).
18. Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J, Minden M, Paterson B, Caligiuri MA, Dick JE. A cell initiating human acute myeloid leukemia after transplantation into SCID mice. Nature 367:645-648 (1994).
19. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nature Medicine 3:730-737 (1997).
20. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. PNAS 100:3983-3988 (2003).
21. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. Nature 432:396-401 (2004).
22. Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle De Maria R. Identification and expansion of human colon-cancer-initiating cells. Nature 445:111-115 (2007).
23. Campbell Marotta LL, Polyak K. Cancer Stem Cells: a model in the making. Current Opinions in Genetics and Development 19:44-50 (2009).
24. Prestegarden L, Enger PO. Cancer Stem Cells in the Central Nervous System – A Critical Review. Cancer Res. 70:8255-8258 (2010).
25. Bissell MJ, Hines WC. Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. Nature Medicine 17:320-329 (2011).
26. Hynes RO. The extracellular matrix: not just pretty fibrils. Science 326:1216-1219 (2009).
27. Discher DE, Mooney DJ, Zandstra PW. Growth Factors, Matrices, and Forces Combine and Control Stem Cells. Science 324:1673-1677 (2009).
28. Reilly GC, Engler AJ. Intrinsic extracellular matrix properties regulate stem cell differentiation. J. Biomechanics 43:55-62 (2010).
29. Levental KR, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 139:891-906 (2009).
30. Borovski T, Mello PDSE, Vermeulen L, Medema JP. Cancer stem cell niche: The place to be. Cancer Res. 71:634-639 (2011).
31. Calabrese C, Poppleton H, Kocak M, Hogg T, Fuller C, Hamer B, Oh EY, Gaber MW, Finklestein D, Allen M, Frank A, Bayazitov IT, Zakharenka SS, Gajjar A, Davidoff A, Gilbertson RJ. A perivascular niche for brain tumor cells. Cancer Cell 11:69-82 (2007).
32. Vermeulen L, Todaro M, Mello PDS, Sprick MR, Kemper K, Perez Alea M, Richel DJ, Stassi G. Single-cell cloning of colon cancer stem cells reveals a multi-lineage differentiation capacity. PNAS 105:13427-13432 (2008).
33. Todaro M, Alea MP, Di Stephano AB, Cammareri P, Vermeulen L, Iovino F, Tripodo C, Russo A, Gulotta G, Medena JP, Stassi G. Colon cancer stem cells dictate tumor growth and resist cell death by production of interleukin-4. Cell Stem Cell 1:389-402 (2007).
34. Fang DD, Kim YJ, Lee CN, Aggarwal S, McKinnon K, Mesmer D, Norton J, Birse CE, He T, Ruben SM, Moore PA. Expansion of CD133+ colon cancer cultures retaining stem cell properties to enable cancer stem cell target discovery. Br. J. Cancer 102:1265-1275 (2010).
35. Li H, Chen X, Calhoun-Davis T, Claypool K, Tang DG. PC3 human prostate carcinoma cell holoclones contain self-renewing tumor-initiating cells. Cancer Res. 68:1820-1825 (2008).
36. Charate-Jouffret E, Ginestier C, Iovino F, Wicinski J, Cervera N, Finetti P, Hur M-H, Diebel ME, Monville F, Dutcher J, Brown M, Viens P, Xerri L, Bertucci F, Stassi G, Dontu G, Birnbaum Wicha MS. Breast cancer cell lines contain functional cancer stem cells with metastatic capacity and a distinct molecular signature. Cancer Res. 69:1302-1313 (2009).
37. Yeung TM, Gandhi SC, Wilding JL, Muschel R, Bodmer WF. Cancer stem cells from colorectal cancer-derived cell lines. PNAS 107:3722-3727 (2010).
38. Ince TA, Richardson AL, Bell GW, Saitoh M, Godar S, Karnoub AE, Iglehart JD, Weinberg RA. Transformation of different human breast epithelial cell types leads to distinct tumor phenotypes. Cancer Cell 12:160-170 (2007).
39. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhow AY, Brooks M, Reinhard F, Zhang CC, Shipitsin M, Campbell LL, Polyak K, Brisken C, Yang J, Weinberg RA. The epithelial- mesenchymal transition generates cells with properties of stem cells. Cell 133:704-715 (2008).
40. Asselin-Labat ML, Sutherland KD, Vaillant F, Gyorki DE, Wu D, Holroyd S, Breslin K, Ward T, Shi W, Bath ML, Deb S, Fox SB, Smyth GK, Lindeman GJ, Visvader JE. Gata-3 negatively regulates the tumor initiating capacity of mammary luminal progenitor cells and targets the putative tumor suppressor caspase-14. Mol. Cell. Biol 31:4609-4622 (2011).

41. Jin X, Jeon H-Y, Joo KM, Kim J-K, Jin J, Kim SH, Kang BG, Beck S, Lee SJ, Kim JK, Park A-K, Park W-Y, Choi Y-J, Nam D-H, Kim H. Frizzled 4 regulates stemness and invasiveness of migrating glioma cells established by serial intra-cranial transplantation. Cancer Res. 71:3066-3075 (2011).
42. Bao S, Wu Q, Sathornsumetee S, Hao Y, Li Z, Hjelmeland AB, Shi Q, McLendon RE, Bigner DD, Rich JN. Stem cell-like glioma cells promote tumor angiogenesis through vascular endothelial growth factor. Cancer Res. 66:7843-7848 (2006).
43. Lee J, Kotliarova S, Kotlirov Y, Li A, Su Q, Donin NM, Pastorino S, Purow B, Christopher N, Zhang W, Park JK, Fine HA. Tumor stem cells derived form glioblastomas cultured in bFGF and EGF more closely mirror the phenotype and genotype of primary tumors then do serum-cultured cell lines. Cancer Cell 9:391-403 (2006).
44. Pollard SM, Yoshikawa K, Clarke ID, Canovi D, Stricker S, Russell R, Bayani J, Head R, Lee M, Bernstein M, Squire JA, Smith A, Dirks P. Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. Cell Stem Cell 4:568-580 (2009).
45. Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinburg RA. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature 449:557-563 (2007).
46. Lui S, Ginestier C, Ou SJ, Clouthier SG, Patel SH, Monville F, Korkaya H, Heath A, Dutcher J, Kleer CG, Jung Y, Dontu G, Taichman R, Wicha MS. Breast cancer stem cells are regulated by mesenchymal stem cells through cytokine networks. Cancer Res 71:614-624 (2011).
47. McGowan PM, Simedrea C, Ribot EJ, Foster PJ, Palmieri D, Steeg PS, Allan AL, Chambers AF. Notch1 inhibition alters the CD44hi/DC24lo population and reduces the formation of brain metastases from breast cancer. Mol Cancer Res. 9:834-844 (2011).
48. Dontu G, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ, Wicha MS. In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. Genes and Development 17:1253-1270 (2003).
49. Gupta PB, Onder TT, Jiang G, Tao K, Kuperwasser C, Weinberg RA. Identification of selective inhibitors of cancer stem cells by high-throughput screening. Cell 138:645-659 (2009).
50. Roberts PE. Isolation and establishment of human tumor stem cells. Methods in Cell Biology 86:325-342 (2008).
51. Iliopoulos D, Hirsch HA, Wang G, Struhl K. Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. PNAS 108:1397-1402 (2011).
52. Yeung, et al. Hypoxia and lineage specifications of cell line-derived colorectal cancer stem cells. PNAS 108:4382-438 (2011).
53. Cammareri P, Lombardo Y, Francipane MG, Bonventre S, Todaro M, Stassi G. Isolation and culture of colon cancer stem cells. Methods in Cell Biology 86:311-324 (2008).
54. Lin L, Lui A, Peng Z, Lin H-J, Li P-K, Lin J. STAT3 is necessary for proliferation and survival in colon cancer initiating cells. Cancer Res. 71:7226-7237 (2011).
55. Fang FF, Kim YJ, Lee CJ, Aggarwal S, McKinnon K, Mesmer D, Norton J, Birse CE, He T, Ruben SM, Moore PA. Expansion of CD133+ colon cancer cultures retaining stem cell properties to enable cancer stem cell target discovery. Br. J. Cancer 102:1265-1275 (2010).
56. O'Brien CA, Pollet A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumor growth in immunodeficient mice. Nature 445:106-110 (2007).
57. Vermeulen L, Melo FDSE, Heijden MVD, Cameron K, De Jong J, Borovski T, Tuynman JB, Todaro M, Merz C, Rodermond H, Sprick MR, Kemper K, Richel DJ, Stassi G, Medena JP. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. Nature Cell Biology 12:468-476 (2010).
58. Kirkland SC. Type I collagen inhibits differentiation and promotes a stem cell-like phenotype in human colorectal carcinoma cells. Br. J. Cancer 101:320-326 (2009).
59. Wang X, Katayama, Wang Y, Yu l, Favoino E, Sakakura K, Favole A, Tsuchikawa T, Silver S, Watkins SC, Kageshita T, Ferrone S. Functional characterization of an scFv-Fc antibody that immunotherapeutically targets the common cancer cell surface proteoglycan CSPG4. Cancer Research 71:7410-7422 (2011).
60. Frank NY, Schatton T, Kim S, Zhan Q, Wilson BJ, Ma J, Saab KR, Osherov V, Widlund HR, Gasser M, Waaga-Gasser A-M, Kupper TS, Murphy GF, Frank MH. VEGFR-1 expressed by malignant melanoma-initiating cells is required for tumor growth. Cancer Research 71:1474-1485 (2011).
61. Quintana E, Shackleton M, Foster HR, Fullen DR, Sabel MS, Johnson TM, Morrison SJ. Phenotypic heterogeneity among tumorigenic melanoma cells from patients that is reversible and not hierarchically organized. Cancer Cell 18:510-523 (2010).
62. Fang D, Nguyen TK, Leishear K, Finko R, Kulp AN, Hotz S, Van Belle PA, Xu X, Elder DE, Herlyn M. A tumorigenic subpopulation with stem cell properties in melanomas. Cancer Research 65:9328-9337 (2005).
63. Hermann PC, Huber SL, Herrier T, Aicher A, Elwart JW, Guba M, Bruns CJ, Heeschen C. Distinct populations of cancer stem cells determine tumor growth and metastatic activity in human pancreatic cancer. Cell Stem Cell 1:313-323 (2007).

64. Li C, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM. Identification of pancreatic cancer stem cells. Cancer Research 67:1030-1037 (2007).

65. Grange C, Tapparo M, Collino F, Vitillo L, Damasco C, Deregibus MC, Tetta C, Bussolati B, Camussi G. Micro-vesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche. Cancer Research 71:5346-5356 (2011).

66. Bussolati B, Bruno S, Grange C, Ferrando U, Camussi G. Identification of tumor-initiating stem cell population in human renal carcinomas. FASEB J. 22:3696-3705 (2008).

67. Damelin M, Geles KG, Follettie MT, Yuan P, Baxter M, Golas J, DiJoseph JF, Karnoub M, Huang S, Diesl V, Behrens C, Choe SE, Rios C, Gruzas J, Sridharan L, Dougher M, Kunz A, Hamann PR, Evans D, Armellino D, Khandke K, Marquette K, Tchistiakova L, Boghaert ER, Abraham RT, Wistuba II, Zhou B-BS. Delineation of a cellular hierarchy in lung cancer reveals on oncofetal antigen expressed on tumor-initiating cells. Cancer Research 71:4236-4246 (2011).

68. Eramo A, Sette F, Pilozzi E, Biffoni M, De Virgilio A, Conticello C, Pesche C, De Maria R. Identification and expansion of the tumorgenic lung cancer stem cell population. Cell Death and Differentiation 15:504-514 (2008).

69. Hu W-Y, Shi G-B, Lam H-M, Hu D-P, Ho S-M, Madueke IC, Kajdacsy-Balla A, Prins GS. Estrogen-Initiated Transformation of Prostate Epithelium Derived from Normal Human Prostate Stem-Progenitor. Cells Endocrinology 152:2150-2163 (2011).

70. Yu C, Yoa Z, Dai J, Zhang H, Escara-Wilke J, Zhang Z, Keller ET. ALDH activity indicates increased tumorigenic cells, but not cancer stem cells, in prostate cancer cell lines. In Vivo 25:69-76 (2011).

71. Li H, Chem X, Calhoun-David T, Claypool K, Tang DG. PC3 human prostate carcinoma cell holoclones contain self-renewing tumor-initiating cells. Cancer Research 68:1820-1825 (2008).

72. Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer Research 65:10946-10951 (2005).

73. Tirino V, Desiderio V, Paino F, De Rosa A, Papaccio F, Fazioli F, Pirozzi G, Papaccio G. Human primary bone sarcomas contain CD133+ cancer stem cells displaying high tumorigenicity. in vivo FASEB J. 25:2022-2030 (2011).

74. Silva IA, Bai S, McLean K, Yang K, Griffith K, Thomas D, Ginestier C, Johnson C, Kueck A, Reynolds RK, Wicha MS, Buckanovich RJ. Aldehyde dehydrogenase in combination with CD133 defines angiogenic ovarian cancer stem cells that portend poor patient survival. Cancer Research 71:3991-4001 (2011).

75. Zhang S, Balch C, Chen MW, Lai H-C, Matei D, Schilder JM, Yan PS, Huang TH-M, Nephew KP. Identification and characterization of ovarian cancer-initiating cells from primary human tumors. Cancer Research 68:4311-4320 (2008).

76. Bapat SA, Mali AM, Koppikar CB, Kurrey NK. Stem and progenitor-like cells contribute to the aggressive behavior of human epithelial ovarian cancer. Cancer Research 65:3025-3029 (2005).

77. Medema JP, Vermeulen L. Microenvironmental regulation of stem cells in intestinal homeostasis and cancer. Nature 474:318-326 (2011).

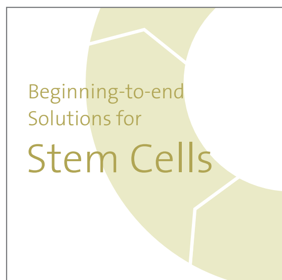
78. Muralidharan-Chari V, Clancy JW, Sedgwick A, D’Souza-Schorey C. Microvesicles: mediators of extracellular communication during cancer progression. J. Cell Sci. 123:1603-1611 (2010).

79. Ootani A, Li X, Sangiorgi E, Ho QT, Ueno H, Toda S, Sugihara H, Fujimoto K, Weissman IL, Capecchi MR, Kuo CJ. Sustained in vitro intestinal epithelial culture within a Wnt-dependent stem cell niche. Nature Medicine 15:701 (2009).

80. Sato T, Vries RG, Snippeert HJ, Wetering M, Barker N, Stange DE, Van Es JH, Abo A, Kujala P, Peters PJ, Clevers H. Single Lgr5 stem cells built crypt-villus structures in vitro without a mesenchymal niche. Nature 459:262-266 (2009).

81. Li Z, Bao S, Wu Q, Wang H, Eyler C, Sathornsumetee S, Shi Q, Cao Y, Lathia J, McLendon RE, Hjelmeland AB, Rich JN. Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. Cancer Cell 15(6):501-13 (2009).

Warranty/Disclaimer: Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications.



www.corning.com/lifesciences/solutions

The science of stem cell culture has advanced rapidly since its beginnings in the 1980s, as has the technology behind this research. From feeder-free substrates, to defined media, to scalable cell expansion systems, continual advances in stem cell culture have inspired Corning to develop innovative new tools to support this groundbreaking work.

Corning was a leader in disposable cell cultureware during the exciting early days of stem cell culture. Today, we continue to work with researchers, providing high quality cell culture consumables, as well as the latest technologies, including defined cell culture surfaces, xeno-free culture media, and scalable cell expansion vessels for stem cells, primary cells, and other cell types.

For additional product or technical information, visit **www.corning.com/lifesciences** or call 800.492.1110. Outside the United States, call +1.978.442.2200 or contact your local Corning sales office.

Corning Incorporated
Life Sciences
836 North St.
Building 300, Suite 3401
Tewksbury, MA 01876
t 800.492.1110
t 978.442.2200
f 978.442.2476
www.corning.com/lifesciences

Worldwide Support Offices

ASIA/PACIFIC

Australia/New Zealand
t 61 427286832

China
t 86 21 3338 4338
f 86 21 3338 4300

India
t 91 124 4604000
f 91 124 4604099

Japan
t 81 3-3586 1996
f 81 3-3586 1291

Korea
t 82 2-796-9500
f 82 2-796-9300

Singapore
t 65 6572-9740
f 65 6861-2913

Taiwan
t 886 2-2716-0338
f 886 2-2516-7500

EUROPE

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 31 20 655 79 28
f 31 20 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

All Other European Countries
t 31 (0) 20 659 60 51
f 31 (0) 20 659 76 73

LATIN AMERICA
grupoLA@corning.com

Brasil
t (55-11) 3089-7400
f (55-11) 3167-0700

Mexico
t (52-81) 8158-8400
f (52-81) 8313-8589

For a listing of trademarks, visit us at www.corning.com/clstrademarks.
All other trademarks in this document are the property of their respective owners.

© 2013-2015 Corning Incorporated. All rights reserved. Printed in U.S.A. 8/15 CLS-DL-AN-228 REV1