Ernst Schering Foundation Symposium Proceedings, Vol. 5, pp. 141–153
DOI 10.1007/2789_2007_049

Published Online: 25 July 2007

Breast Stem Cells and Cancer

G. Farnie, R. B. Clarke(✉)

Breast Biology Group, School of Cancer and Imaging Sciences,
Faculty of Medicine and Human Sciences, University of Manchester,
Paterson Institute for Cancer Research, Wilmslow Road, M20 4BX Manchester, UK
email: *robert.clarke@manchester.ac.uk*

Abstract. Recent results have increased our understanding of normal stem cells and the signalling pathways which regulate them during the development of the mammary gland. Tumours in many tissues are now thought to develop from dysregulated stem cells and depend on activated stem cell self-renewal pathways such as Notch for their tumourigenic capacity. These cancer stem cells are recognised by specific cell surface proteins that they express and their capacity to grow tumours *in vivo* or spheres *in vitro*. We have described human breast DCIS mammospheres grown from cancer stem cells and demonstrated their dependence on the EGF and Notch receptor pathways. Stem cell self-renewal pathways such as these may represent novel therapeutic targets to prevent recurrence of pre-invasive and invasive breast cancer.

1 Introduction

Adult stem cells are defined by their capacity for self-renewal and differentiation into cell lineages present in a specific tissue (Morrison et al. 1997; Weissman 2000). The adult mammary gland has a lobulo-alveolar structure, composed of three cell lineages: myoepithelial cells which form the basal layer of ducts and alveoli; ductal epithelial cells which line the lumen of ducts and alveolar epithelial cells which synthesise

milk proteins (Richert et al. 2000; Rudland et al. 1998; Daniel and Smith 1999). The existence of a mammary stem cell is suggested by the cyclic development, involution and subsequent redevelopment of the mammary gland after pregnancy and lactation. Evidence to suggest that a pluripotent stem cell gives rise to both the luminal and myoepithelial cells was first demonstrated over 40 years ago when small fragments of the rodent duct or terminal end buds (TEBs) transplanted in cleared mammary fat pads of a syngeneic host could develop an entire and functional mammary tree (Deome et al. 1959; Hoshino and Gardner 1967; Daniel et al. 1968; Ormerod and Rudland 1986). Further studies observing the pattern of X chromosome inactivation throughout the ductal and lobular epithelium show that contiguous patches of epithelium with inactivation of the same X chromosome were present throughout the human breast, suggesting that the cells within each patch had been derived from the same stem cell (Novelli et al. 2003; Tsai et al. 1996).

Techniques to enrich for breast stem cells have subsequently been developed, including isolating a sub-population of mouse mammary epithelial cells defined by its ability to efflux the dye Hoecht 33342. The cells were termed the 'side population' (SP) and were found to include cells capable of regenerating a functional mammary gland system in a cleared fat pad (Welm et al. 2002). A similar side population has also been identified from human breast tissue (Clarke et al. 2005; Clayton et al. 2004; Alvi et al. 2003; Dontu et al. 2003a); this provided a functional parallel to the SP-containing haematopoietic stem cells in bone marrow (Goodell et al. 1997). More recently purification of mouse mammary epithelial stem cells with cell surface markers Lin^{-}, CD29hi and $CD24^{+}$ demonstrated these cells are highly enriched for mammary stem cells by transplantation and showed that a single cell, marked with a LacZ transgene, can reconstitute a complete mammary gland *in vivo* (Shackleton et al. 2006; Stingl et al. 2006).

There are many similarities between stem cells and cancer cells: Both self renew, although cancer cells are poorly controlled, unlike somatic stem cells, which are highly regulated. Differentiation also occurs where somatic stem cells generate normal, mature cells of the specific tissue; however, this is usually abnormal in cancer cells (reviewed by Pardal et al. 2003). Therefore, stem cells are an attractive candidate as the origin of cancer, as they would already have active self-renewal pathways

and over their long life span mutations and epigenic changes in the dysregulated pathways mentioned can occur, allowing for increasing evolution towards malignancy. Reviews have proposed a model describing cancer stem cells derived from mutated adult stem cells (Dontu et al. 2003b; Reya et al. 2001); the fact that leukaemic stem cells have a surface marker phenotype that is similar to normal haematopoietic stem cells supports the idea that they arise from haematopoietic stem cells. The model also suggests that in addition to stem cells, early or late progenitors could also serve as targets for these transforming events; however, if this was the case these cells would need to acquire mutations not only to promote malignancy but also to enable them to undergo self-renewal.

Signalling pathways involved in normal mammary stem cell regulation, including WNT (Liu et al. 2004; Li et al. 2003), Hedgehog (Dontu and Wicha 2005), Notch (Dontu et al. 2004; Stylianou et al. 2006), LIF (Kritikou et al. 2003), TGF-β (Ewan et al. 2005; Boulanger et al. 2005) and EGF families (Dontu et al. 2003a), prolactin (Dontu et al. 2003a), estrogen and progesterone (Clarke et al. 2005) are known to be dysregulated in many cancers (Fig. 1). In particular our group have reported that Notch receptor signalling is aberrantly activated in breast cancers compared to normal breast (Stylianou et al. 2006). Levels of Jagged 1 and Notch 1 have previously been correlated with poor prognosis (Reedijk et al. 2005). In a very recent study discussed below, we have demonstrated the importance of the Notch receptor signalling pathway in breast ductal carcinoma in situ stem cells (DCIS).

2 Cancer Stem Cells

There is now a large body of evidence showing that leukaemia originates from a cancer stem cell (Reya et al. 2001). The first evidence for cancer stem cells described a small but variable proportion of human acute myeloid leukaemia (AML) cells, which could be identified and purified with cell surface markers $CD34^{+}CD38^{-}$, and were found to be the only cells capable of transferring AML from human patients to NOD/SCID mice (Bonnet and Dick 1997), providing evidence that not all AML cells have clonogenic capacity and only a small subset of

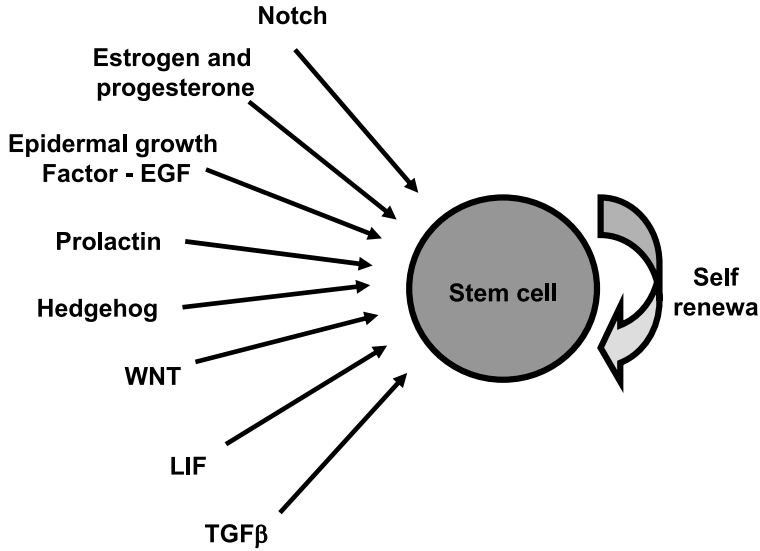


Fig. 1. Self-renewal pathways involved in regulating the normal breast stem cell

cells (the cancer stem cells, CSCs) are capable of regenerating the cancer. Solid cancers are known to be phenotypically heterogeneous and clonogenic in culture; therefore, many groups have extrapolated the cancer stem cell hypothesis from the haematopoietic system to solid cancers. Cells with stem cell characteristics from brain tumours were first isolated with clonogenic neurosphere culture technique from human glioblastoma (Ignatova et al. 2002), and now other groups have independently confirmed that brain tumours contain neurosphere-forming cells (Singh et al. 2003, 2004; Hemmati et al. 2003). These cells are highly enriched for cell surface marker CD133 and nestin, have a marked capacity for proliferation and self-renewal and are capable of differentiating *in vitro* into phenotypes identical to the tumour in situ. Cancer stem cell populations have also been found in prostate (Collins et al. 2005; Lawson et al. 2007), pancreas (Li et al. 2007)[39], colon (O'Brien et al. 2007; Ricci-Vitiani et al. 2007) and breast cancer (Al-Hajj et al. 2003).

For example O'Brien et al. (2007) reported a xenograft model using subrenal implantation of human colon cancer cell suspensions into pre-irradiated NOD/SCID mice where 17 out of 17 primary or metastatic colon cancer samples formed tumours which resembled the original tumour from which they were derived; these tumours could be passaged and re-form tumours in secondary and tertiary recipients. Fractionation of colon cancer cells based on expression of CD133, a potential cancer stem cell marker, revealed that the proportion of $CD133^+$ cells ranged from 1.8% to 24.5% within the colon cancer samples and that after implantation into NOD/SCID mice only 1 out of 47 mice injected with a $CD133^-$ population formed a tumour compared to 45 out of 49 when $CD133^+$ cells were implanted. Limiting dilution experiments determined that 1 in every 262 $CD133^+$ colon cancer cells was capable of re-initiating a tumour (O'Brien et al. 2007). Ricci-Vitiani and colleagues took a similar approach in sorting for $CD133^+$ primary colon cancer cells, where again they found that the $CD133^+$ population was enriched for cells which give rise to subcutaneous tumours in SCID mice, which could re-form tumours after re-implantation into secondary and tertiary mice. An *in vitro* culture system was also used to grow colon cancer cells as colon spheres similar to neurospheres, which allows the cells to grow in an undifferentiated state. Spheres were enriched for $CD133^+$ cells and were capable of growing tumours in mice, in contrast differentiated colon cancer cells were not tumourigenic. The study also demonstrated that primary colon cancer cells grown as spheres for over 1 year were still capable of initiating tumours with morphology similar to tumours formed before long-term culture (Ricci-Vitiani et al. 2007). Both studies are in line with the cancer stem cell hypothesis which suggests that tumours are generated and maintained by a small subset of undifferentiated cells able to self-renew and differentiate into the bulk tumour population (Wang and Dick 2005).

In the breast, the study by Al Hajj et al. (2003) was the first to identify a subpopulation of human breast cancer cells which initiated tumours in NOD/SCID mice, using a set of cell surface markers to sort cells with an increased tumourogenic capacity. Cells which were $CD44^+$, $CD24^{low}$, ESA^+ and $lineage^-$ (cells lacking markers CD2, DC3, CD10, CD16, CD18, CD31, CD64 and CD140b) isolated from one primary breast cancer and nine metastasis were able to form heterogeneous tumours

eight out of nine times. The tumours contained not only the $CD44^+$, $CD24^{low}$, ESA^+ and $lineage^-$ tumour initiating cells but also the phenotypically diverse non-tumourigenic cells which comprise the bulk of tumours. As few as 200 $CD44^+/CD24^{low}/ESA^+/lineage^-$ cells implanted into NOD/SCID mice could form tumours four out of four times, while no tumours formed when 200 cells from the $CD44^-/CD24^+/ESA^-$ cell population were used (Al-Hajj et al. 2003). A subsequent study carried out on 16 breast lesions with the sphere culture technique which has been used to enrich for normal breast stem cells (Dontu et al. 2003a) resulted in the production of three long-term primary cultures which had self-renewing capacity and could differentiate into the different breast lineages. The sphere forming cells were found to be 96%–98% $CD44^+/CD24^-$; however, cells with self-renewal only accounted for 10%–20% of the total cell number, showing that only a sub-group within the $CD44^+/CD24^-$ sorted cells had self-renewal capacity (Ponti et al. 2005), which is consistent with only 1 in 200 cells being capable of initiating a tumour in the previous study (Al-Hajj et al. 2003). This indicates that sorting for a $CD44^+$/CD24 population enriches for tumour-initiating cells; however, it highlights the need for additional markers to isolate the true CSC. Tumour-initiating capacity was measured with a long-term sphere culture of the breast cancer cell line MCF7, termed MCF-S. $CD44^+/CD24^-$ cells from the MCF-S or MCF7s (used as a control) were implanted into the mammary fat pad of SCID mice. The MCF7 cells gave rise to tumours when at least 1 million cells were implanted; however, the MCF-S cells gave rise to tumours with smaller numbers of cells (10^5, 10^4 and 10^3) with at least a 60% success rate, whereas the MCF7s showed no growth when a comparable cell number was implanted (Ponti et al. 2005), thus indicating that both the mammosphere culture system and the cell surface marker selection enriched for tumour-initiating cells.

Further studies in breast cancer cell lines and most recently in WNT-induced mouse mammary tumours have added to the growing evidence for CSC in the breast. Hoechst dye exclusion was used to isolate a side population (SP) in MCF7 cells (0.2%); this population had a greater tumourigenic capacity than the non-SP fraction when determined by tumour production subcutaneously in NOD/SCID mice. The MCF7 SP

also expressed higher levels of Notch1 and β-catenin mRNA compared to the non-SP population, suggesting that the SP cancer cells have some intrinsic properties of stem cells (Patrawala et al. 2005). The SP population within hyperplasic tissue from mouse mammary tumour virus (MMTV)-driven Wnt-1 transgenic mice was >2-fold increased compared to matched background controls (Woodward et al. 2007); radiation was shown to selectively enriched progenitors in the activated WNT cells compared to background-matched control mice. A recent paper suggests that breast cancer-initiating cells are radioresistant, firstly showing that MCF7 and MDA-MB-231 breast cancer cells grown as mammospheres have elevated numbers of $CD24^{-/low}/CD44^{+}$ cells; they were also found to be more radioresistant than the corresponding cells grown in monolayer when compared by clonogenic assay (Phillips et al. 2006). A comparable but more extensive study in glioblastomas showed not only that $CD133^{+}$ tumour cells were more radioresistant than the $CD133^{-}$ but that ionising radiation also increased the proportion of $CD133^{+}$ cells from glioblastoma specimens (Bao et al. 2006). The $CD133^{+}$ population preferentially activate the DNA damage checkpoint response to DNA damage and repair radiation-induced DNA damage more effectively than $CD133^{-}$ cells, suggesting that $CD133^{+}$ cells could be the source of tumour recurrence in patients after radiation. A specific Chk1 and Chk2 inhibitor used in this study was found to reverse this radioresistance *in vitro* and *in vivo*, indicating that targeting DNA damage check points may disrupt this resistance mechanism and improve tumour control with radiation treatment.

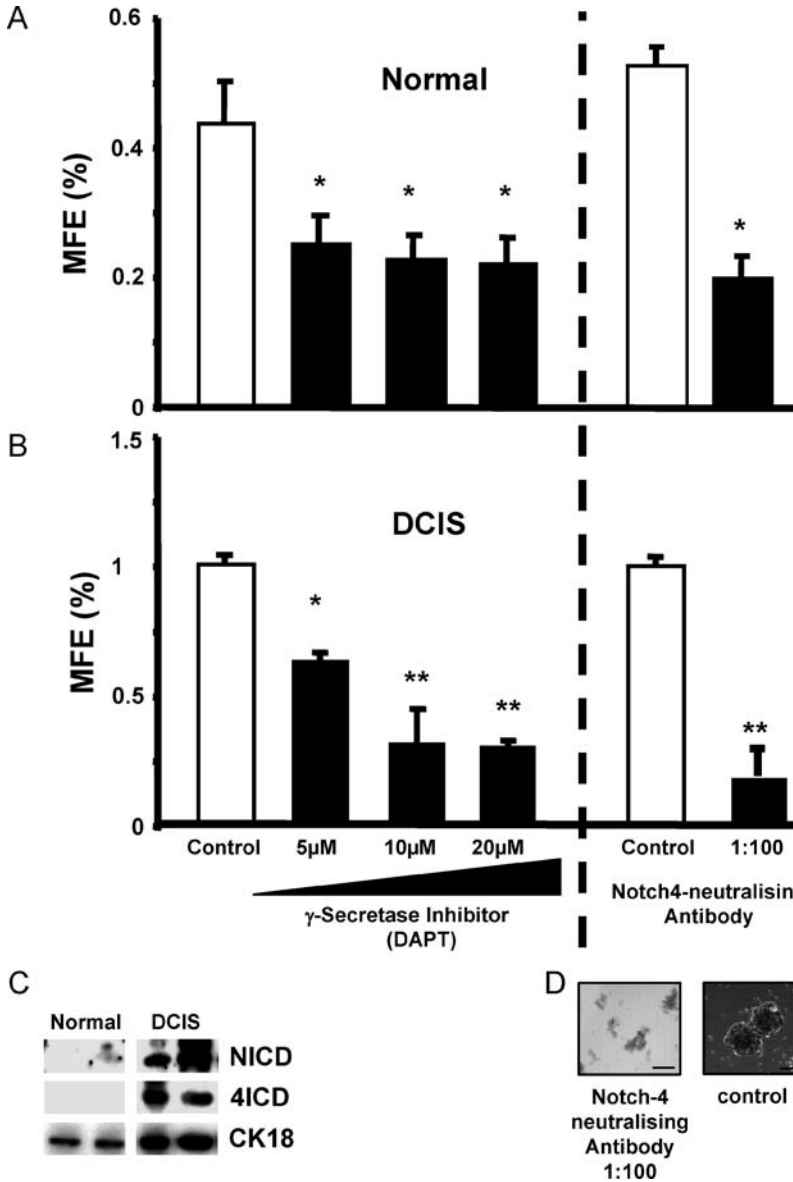
3 DCIS Mammospheres–Importance of EGF and Notch Signalling

We have adapted the mammosphere culture system which has been used to enrich for normal stem cells and populations of cells from cancers with a greater tumourigenic capacity to grow primary DCIS mammospheres (Dontu et al. 2003a; Singh et al. 2004; Ricci-Vitiani et al. 2007; Ponti et al. 2005; Farnie et al. 2007). Compared to normal breast cells grown under the same non-adherent culture conditions, primary DCIS tissue contained a greater number of cells with the ability to form mam-

Fig. 2. Mammosphere-forming efficiency (*MFE*) in normal breast (**A**) and DCIS (**B**) after treatment with DAPT and a Notch 4 neutralising antibody compared to equivalent DMSO or IgG control, respectively. Mean %MEF±SE, two-tailed test: $*P<0.05$, $**P<0.01$. (**C**) Western blotting showing expression levels of Notch 1 and 4 intracellular domain (NICD, 4ICD) in normal breast and DCIS tissue. Cytokeratin (CK) 18 is shown as a control for epithelial content of tissue. (**D**) Brightfield image of DCIS mammospheres after treatment with a Notch 4 neutralising antibody or an IgG control

mospheres (Fig. 2 A and B), DCIS mammosphere-forming cells also had a greater self-renewal capacity than normal breast mammospheres as they could produce a greater number of new mammosphere generations after passage. High-grade DCIS also had an increased MFE compared to low-grade DCIS; this is consistent with findings from both brain and colon cancers, where the most aggressive clinical samples have tumour stem cells with the highest sphere formation and self-renewal capacity (Singh et al. 2003; Ricci-Vitiani et al. 2007).

The non-adherent DCIS culture system has allowed the investigation of pathways which are involved in the survival and self-renewal of DCIS. Our previous xenograft work has shown that EGFR signalling is required for the growth of DCIS tumours (Chan et al. 2002). We found that high-grade DCIS mammospheres have a greater sensitivity to an EGFR inhibitor, gefitinib, compared to low-grade DCIS in the absence of exogenous EGF, suggesting secretion of an EGF-like ligand from the high-grade DCIS which is regulating mammosphere initiation and/or growth via the EGFR signalling pathways. Notch signalling has been shown not only to play a role in normal stem cell regulation but also to be frequently dysregulated in a number of cancers, including invasive breast cancer (Dontu et al. 2004; Stylianou et al. 2006). Western blotting confirmed that Notch signalling was highly activated in DCIS compared to normal breast tissue, showing that both Notch 1 and 4 intracellular domain (NICD and 4ICD, respectively) were elevated (Fig. 2C). We then used two Notch inhibitors in DCIS and normal breast mammosphere culture, mammosphere formation was reduced with both a γ-secretase inhibitor (DAPT) and a Notch 4 neutralising antibody. How-


A
Normal
MFE (%)
0.6
0.4
0.2
0
*
*
*
*
B
DCIS
MFE (%)
1.5
1
0.5
0
*
**
**
**
Control
5μM
10μM
20μM
Control
1:100
γ-Secretase Inhibitor
(DAPT)
Notch4-neutralising
Antibody
C
Normal
DCIS
NICD
4ICD
CK18
D
Notch-4
neutralising
Antibody
1:100
control

ever ,the DCIS mammospheres were more sensitive to both inhibitors; in particular the Notch 4 blocking peptide where in two out of six cases caused 100% inhibition of mammosphere growth (Fig. 2D). Our results suggest that targeting both of these pathways may have therapeutic value as adjuvant therapy for DCIS.

References

Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF (2003) Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 100:3983–3988

Alvi AJ, Clayton H, Joshi C, Enver T, Ashworth A, Vivanco MM, Dale TC, Smalley MJ (2003) Functional and molecular characterisation of mammary side population cells. Breast Cancer Res 5:R1–R8

Bao S, Wu Q, McLendon RE, Hao Y, Shi Q, Hjelmeland AB, Dewhirst MW, Bigner DD, Rich JN (2006) Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. Nature 444:756–760

Bonnet D, Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 3:730–737

Boulanger CA, Wagner KU, Smith GH (2005) Parity-induced mouse mammary epithelial cells are pluripotent, self-renewing and sensitive to TGF-β1 expression. Oncogene 24:552–560

Chan KC, Knox WF, Gee JM, Morris J, Nicholson RI, Potten CS, Bundred NJ (2002) Effect of epidermal growth factor receptor tyrosine kinase inhibition on epithelial proliferation in normal and premalignant breast. Cancer Res 62:122–128

Clarke RB, Spence K, Anderson E, Howell A, Okano H, Potten CS (2005) A putative human breast stem cell population is enriched for steroid receptor-positive cells. Dev Biol 277:443–456

Clayton H, Titley I, Vivanco M (2004) Growth and differentiation of progenitor/stem cells derived from the human mammary gland. Exp Cell Res 297:444–460

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

Daniel CW, Smith GH (1999) The mammary gland: a model for development. J Mammary Gland Biol Neoplasia 4:3–8

Daniel CW, De Ome KB, Young JT, Blair PB, Faulkin LJ Jr (1968) The *in vivo* life span of normal and preneoplastic mouse mammary glands: a serial transplantation study. Proc Natl Acad Sci USA 61:53–60

Deome KB, Faulkin LJ Jr, Bern HA, Blair PB (1959) Development of mammary tumors from hyperplastic alveolar nodules transplanted into gland-free mammary fat pads of female C3H mice. Cancer Res 19:515–520

Dontu G, Wicha MS (2005) Survival of mammary stem cells in suspension culture: implications for stem cell biology and neoplasia. J Mammary Gland Biol Neoplasia 10:75–86

Dontu G, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ, Wicha MS (2003a) In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. Genes Dev 17:1253–1270

Dontu G, Al-Hajj M, Abdallah WM, Clarke MF, Wicha MS (2003b) Stem cells in normal breast development and breast cancer. Cell Prolif 36(Suppl:1):59–72

Dontu G, Jackson KW, McNicholas E, Kawamura MJ, Abdallah WM, Wicha MS (2004) Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. Breast Cancer Res 6:R605–R615

Ewan KB, Oketch-Rabah HA, Ravani SA, Shyamala G, Moses HL, Barcellos-Hoff MH (2005) Proliferation of estrogen receptor-α-positive mammary epithelial cells is restrained by transforming growth factor-β1 in adult mice. Am J Pathol 167:409–417

Farnie G, et al. (2007) Novel cell culture technique for primary ductal carcinoma in situ: Role of Notch and EGF receptor signaling pathways. J Natl Cancer Inst (in press)

Goodell MA, Rosenzweig M, Kim H, Marks DF, DeMaria M, Paradis G, Grupp SA, Sieff CA, Mulligan RC, Johnson RP (1997) Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. Nat Med 3:1337–1345

Hemmati HD, Nakano I, Lazareff JA, Masterman-Smith M, Geschwind DH, Bronner-Fraser M, Kornblum HI (2003) Cancerous stem cells can arise from pediatric brain tumors. Proc Natl Acad Sci USA 100:15178–15183

Hoshino K, Gardner WU (1967) Transplantability and life span of mammary gland during serial transplantation in mice. Nature 213:193–194

Ignatova TN, Kukekov VG, Laywell ED, Suslov ON, Vrionis FD, Steindler DA (2002) Human cortical glial tumors contain neural stem-like cells expressing astroglial and neuronal markers *in vitro*. Glia 39:193–206

Kritikou EA, Sharkey A, Abell K, Came PJ, Anderson E, Clarkson RW, Watson CJ (2003) A dual, non-redundant, role for LIF as a regulator of development and STAT3-mediated cell death in mammary gland. Development 130:3459–3468

Lawson DA, Xin L, Lukacs RU, Cheng D, Witte ON (2007) Isolation and functional characterization of murine prostate stem cells. Proc Natl Acad Sci USA 104:181–186

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

Li Y, Welm B, Podsypanina K, Huang S, Chamorro M, Zhang X, Rowlands T, Egeblad M, Cowin P, Werb Z, Tan LK, Rosen JM, Varmus HE (2003) Evidence that transgenes encoding components of the Wnt signaling pathway preferentially induce mammary cancers from progenitor cells. Proc Natl Acad Sci USA 100:15853–15858

Liu BY, McDermott SP, Khwaja SS, Alexander CM (2004) The transforming activity of Wnt effectors correlates with their ability to induce the accumulation of mammary progenitor cells. Proc Natl Acad Sci USA 101:4158–4163

Morrison SJ, Shah NM, Anderson DJ (1997) Regulatory mechanisms in stem cell biology. Cell 88:287–298

Novelli M, Cossu A, Oukrif D, Quaglia A, Lakhani S, Poulsom R, Sasieni P, Carta P, Contini M, Pasca A, Palmieri G, Bodmer W, Tanda F, Wright N (2003) X-inactivation patch size in human female tissue confounds the assessment of tumor clonality. Proc Natl Acad Sci USA 100:3311–3314

O'Brien CA, Pollett A, Gallinger S, Dick JE (2007) A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. Nature 445:106–110

Ormerod EJ, Rudland PS (1986) Regeneration of mammary glands *in vivo* from isolated mammary ducts. J Embryol Exp Morphol 96:229–243

Pardal R, Clarke MF, Morrison SJ (2003) Applying the principles of stem-cell biology to cancer. Nat Rev Cancer 3:895–902

Patrawala L, Calhoun T, Schneider-Broussard R, Zhou J, Claypool K, Tang DG (2005) Side population is enriched in tumorigenic, stem-like cancer cells, whereas ABCG2+ and ABCG2– cancer cells are similarly tumorigenic. Cancer Res 65:6207–6219

Phillips TM, McBride WH, Pajonk F (2006) The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. J Natl Cancer Inst 98:1777–1785

Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, Pilotti S, Pierotti MA, Daidone MG (2005) Isolation and *in vitro* propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. Cancer Res 65:5506–5511

Reedijk M, Odorcic S, Chang L, Zhang H, Miller N, McCready DR, Lockwood G, Egan SE (2005) High-level coexpression of JAG1 and NOTCH1 is observed in human breast cancer and is associated with poor overall survival. Cancer Res 65:8530–8537

Reya T, Morrison SJ, Clarke MF, Weissman IL (2001) Stem cells, cancer, and cancer stem cells. Nature 414:105–111

Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R (2007) Identification and expansion of human colon-cancer-initiating cells. Nature 445:111–115

Richert MM, Schwertfeger KL, Ryder JW, Anderson SM (2000) An atlas of mouse mammary gland development. J Mammary Gland Biol Neoplasia 5:227–241

Rudland PS, Barraclough R, Fernig DG, Smith JA (1998) Growth and differentiation of the normal mammary gland and its tumours. Biochem Soc Symp 63:1–20

Shackleton M, Vaillant F, Simpson KJ, Stingl J, Smyth GK, Asselin-Labat ML, Wu L, Lindeman GJ, Visvader JE (2006) Generation of a functional mammary gland from a single stem cell. Nature 439:84–88

Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB (2003) Identification of a cancer stem cell in human brain tumors. Cancer Res 63:5821–5828

Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB (2004) Identification of human brain tumour initiating cells. Nature 432:396–401

Stingl J, Eirew P, Ricketson I, Shackleton M, Vaillant F, Choi D, Li HI, Eaves CJ (2006) Purification and unique properties of mammary epithelial stem cells. Nature 439:993–997

Stylianou S, Clarke RB, Brennan K (2006) Aberrant activation of notch signaling in human breast cancer. Cancer Res 66:1517–1525

Tsai YC, Lu Y, Nichols PW, Zlotnikov G, Jones PA, Smith HS (1996) Contiguous patches of normal human mammary epithelium derived from a single stem cell: implications for breast carcinogenesis. Cancer Res 56:402–404

Wang JC, Dick JE (2005) Cancer stem cells: lessons from leukemia. Trends Cell Biol 15:494–501

Weissman IL (2000) Stem cells: units of development, units of regeneration, and units in evolution. Cell 100:157–168

Welm BE, Tepera SB, Venezia T, Graubert TA, Rosen JM, Goodell MA (2002) Sca-1(pos) cells in the mouse mammary gland represent an enriched progenitor cell population. Dev Biol 245:42–56

Woodward WA, Chen MS, Behbod F, Alfaro MP, Buchholz TA, Rosen JM (2007) WNT/beta-catenin mediates radiation resistance of mouse mammary progenitor cells. Proc Natl Acad Sci USA 104:618–623