Cell Cycle

ISSN: 1538-4101 (Print) 1551-4005 (Online) Journal homepage: http://www.tandfonline.com/loi/kccy20

Xenografts in zebrafish embryos as a rapid functional assay for breast cancer stem-like cell identification

Arrate Eguiara, Olaia Holgado, Izaskun Beloqui, Leire Abalde, Yolanda Sanchez, Carles Callol & Angel G. Martin

To cite this article: Arrate Eguiara, Olaia Holgado, Izaskun Beloqui, Leire Abalde, Yolanda Sanchez, Carles Callol & Angel G. Martin (2011) Xenografts in zebrafish embryos as a rapid functional assay for breast cancer stem-like cell identification, Cell Cycle, 10:21, 3751-3757, DOI: 10.4161/cc.10.21.17921

To link to this article: http://dx.doi.org/10.4161/cc.10.21.17921

Published online: 01 Nov 2011.

Submit your article to this journal

Article views: 601

View related articles

Citing articles: 13 View citing articles

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=kccy20

Download by: [88.12.19.17] **Date:** 19 September 2016, At: 03:13

Cell Cycle 10:21, 3751-3757; November 1, 2011; © 2011 Landes Bioscience

Xenografts in zebrafish embryos as a rapid functional assay for breast cancer stem-like cell identification

Arrate Eguiara,[1] Olaia Holgado,[2] Izaskun Beloqui,[1] Leire Abalde,[1] Yolanda Sanchez,[1] Carles Callol[2] and Angel G. Martin[1,*]

[1]Regulation of Cell Growth Laboratory; Fundación Inbiomed; [2]Biobide SL; San Sebastian, Gipuzkoa, Spain

Key words: xenograft, zebrafish, breast cancer, cancer stem cells, mammosphere, curcumin

Abbreviations: DPF, days post fertilization; CSC, cancer stem cell; TIC, tumor initiating cell; ALDH, aldehyde dehydrogenase; EMT, epithelial mesenchymal transition

The cancer stem cell is defined by its capacity to self-renew, the potential to differentiate into all cells of the tumor and the ability to proliferate and drive the expansion of the tumor. Thus, targeting these cells may provide novel anticancer treatment strategies. Breast cancer stem cells have been isolated according to surface marker expression, ability to efflux fluorescent dyes, increased activity of aldehyde dehydrogenase or the capacity to form spheres in non-adherent culture conditions. In order to test novel drugs directed toward modulating self-renewal of cancer stem cells, rapid, easy and inexpensive assays must be developed. Using 2 days post-fertilization (dpf) zebrafish embryos as transplant recipients, we show that cells grown in mammospheres from breast carcinoma cell lines migrate to the tail of the embryo and form masses with a significantly higher frequency than parental monolayer populations. When stem-like self-renewal was targeted in the parental population by the use of the dietary supplement curcumin, cell migration and mass formation were reduced, indicating that these effects were associated with stem-like cell content. This is a proof of principle report that proposes a rapid and inexpensive assay to target in vivo cancer stem-like cells, which may be used to unravel basic cancer stem cell biology and for drug screening.

Do not distribute.

Introduction

The hypothesis that cancer is driven by a minor population of cells with the ability to initiate, maintain and give rise to the cell heterogeneity present in the tumor [the so-called cancer stem sells (CSCs) or tumor initiating cells (TICs)], has recently attracted a great deal of attention, owing to the promise of novel cellular targets for the treatment of malignancies.[1,2] Clonal expansion of cancer stem cell populations is believed to be the first step in carcinogenesis;[3] therefore drug screening efforts directed toward reducing this population may provide novel anticancer treatment strategies. In the case of breast cancer, the isolation of CSCs has been accomplished according to several parameters, such as surface marker expression ($CD44^+CD24^{-/low}$), the ability to efflux vital dyes (side population), the ability to growth in non-adherent conditions (mammospheres) and increased activity of Aldehyde Dehydrogenase (ALDH).[4-6] Cytotoxic agents that target the bulk of rapid proliferating cells constitute the majority of current chemotherapeutic drugs in use. However, there are a number of reports using in vitro culture of tumor cells and animal models showing that CSCs are more resistant to conventional cancer therapies, therefore placing these cells at the root of tumor recurrence and metastases. Several preliminary reports have indeed shown that this is the case with human cancer patients. In breast cancer, Li et al. found that conventional chemotherapy increased the fraction of $CD44^+CD24^-$ cells in a neoadjuvant setting of advanced breast cancer patients and increased mammosphere formation in vitro. Tanei et al. have shown that paclitaxel and epirubicin-based chemotherapy enriches for aldehyde dehydrogenase 1 (ALDH1) positive cells in breast tumors. Novel approaches for targeting cancer stem cell self-renewal pathways, and assays to test those agents are needed.[9]

The non-adherent mammosphere assay is commonly used as a surrogate in vitro culture assay for tumor formation to investigate cancer stem cells. This assay is used as a stem cell-like functional assay that allows the propagation of mammary epithelial and breast tumor cells in an undifferentiated state based on their ability to proliferate in suspension.[5,10,11] In these culture conditions, cancer stem cells are able to maintain stem-like properties such as tumor initiation, self-renewal and limited differentiation potential.[5,11] Indeed, the mammosphere assay has been demonstrated to enrich and propagate MCF7 breast carcinoma cells with enhanced tumor initiating ability.[12]

*Correspondence to: Angel G. Martin; Email: agmartin@inbiomed.org
Submitted: 08/30/11; Accepted: 08/30/11
http://dx.doi.org/10.4161/cc.10.21.17921

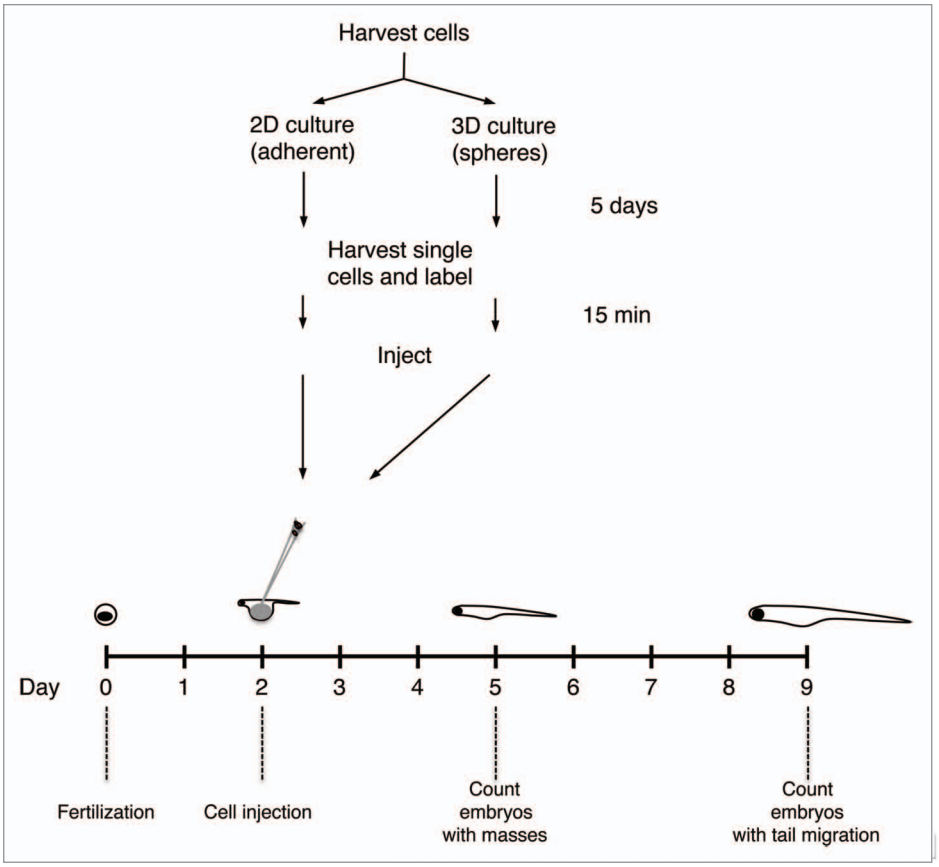

Figure 1. Schematic representation of experimental design. Breast carcinoma cells were grown as monolayer cultures or as non-adherent mammospheres for 5 days, on the same culture medium. After 5 days cells were harvested and sphere disaggregated using enzymatic and mechanical procedures, as described under Methods. Single cell suspensions were stained with the red-fluorescent tracking dye CM-DiI (4.8 μg/ml) for 15 min, washed and loaded into a pulled glass micropipette. Two dpf zebrafish embryos, prepared as described under Methods, were injected with stained cells in the yolk sac and the presence of cells observed under the microscope. Embryos with no cells or cells located elsewhere than the yolk sac were excluded from the experiments. One hundred animals were used for each experiment. Three days after the injection the number of embryos with evident cell masses were counted. Seven days after the injection the number of embryos with red cells in the tail section were counted.

Currently, the gold standard functional assay to demonstrate tumor initiating ability is the cell xenograft in immunodeficient mice, although cancer stem cell frequency measurement depends on the system used.[13] This animal model presents several caveats at the practical level, such as dedicated expensive animal facilities, including maintaining a pathogen free area and trained pathologists and veterinarians; the number of animals that can be used in one experiment, usually limited to a few dozens, and the length of tumor formation extends over a period of months. Zebrafish embryos have been employed in several useful models for therapeutic drug research and preclinical studies, as well as high throughput screening[14] for several reasons: fish are inexpensive to maintain, breed in large numbers (100–300 embryos per week/couple), develop rapidly ex vivo, embryos are transparent, have short generational cycles (2–3 mo), are immunodeficient until day 11 post-fertilization (dpf) and require small amount of drugs per experiment. This model has become recently interesting for oncology research as a xenograft system for transplantation of malignant melanoma cells that showed that cells were not rejected, survived and showed motility.[15] In another study, the group of Haldi et al. established the conditions where human melanoma cells proliferate, migrate, produce melanin, form masses and stimulate angiogenesis in transplanted zebrafish embryos.[16]

In this work we investigated the use of zebrafish embryos as a xenograft model for CSCs isolated as mammospheres from breast carcinoma cell lines, as a proof of concept study for the use of this animal model for the in vivo evaluation of cancer stem cell phenotype. We have established a simple, fast, sensitive and cost effective model.

Results

Embryo xenograft assay set up. During early zebrafish development, the body plan expands from 0.5 mm at the 1 cell stage to 3 mm at 2-dpf.[17] During this period, cells present at this stage can be passively transported to distant sites during gastrulation. In order to prevent non-specific cell location effects, we chose the 2-dpf stage as cell migration after injection is likely to be a cell-intrinsic active process. Zebrafish embryos do not fully develop their immune system until day 11-pf,[18] allowing a window of observation of cell behavior for the lack of immune rejection. We tested transplantation into several sites (data not shown) and determined that the yolk sac was the optimum site for injection, as up to 500 cells from a single injection were retained in place and, at the same time, the nutrient rich environment provided by the yolk sac supported cell viability.

Zebrafish develop normally from fertilization through later stages at temperatures varying from 23 to 34°C[19] with a standard incubation temperature of 28°C. In contrast, the standard incubation temperature of human cell cultures is 37°C. In order to maximize cell viability during the time of the assay, embryos were incubated at 28°C for 2 h after cell injection and subsequently placed at 34°C for 7 days, without loss of fish viability.

A schematic representation of our assay set up is depicted in **Figure 1**.

Mammosphere-derived cells showed a significantly higher capacity to migrate to the tail and form masses than parental cells grown in monolayer. One of the properties of breast cancer stem cells and mammary stem/progenitor cells is their ability

to survive under serum-free anchorage independent conditions and generate mammospheres.[20] These spheres are composed of a small number of stem cells that self-renew, generate secondary spheres on serial passage and are capable of multilineage differentiation.[11] We examined the ability of breast carcinoma cells (from the cell line BT-474 and MCF7, as previously reported in ref. 21) to grow as mammospheres, measuring the capacity to form secondary mammospheres in serial passages (**Fig. 2A**) and to make clonal mammospheres from single cells in limiting dilution (data not shown), reflecting the stem cell-like properties of self-renewal and asymmetric division. On average, 40% of single cells rendered mammospheres in clonal assays. We then used single cells derived from mammosphere cultures and monolayer culture-derived controls, fluorescently labeled, to inject into the yolk sac of 2-dpf embryos (as schematically shown in **Fig. 1**) to assess their behavior as the embryo develops. A total of 100 embryos were used per condition. We observed that a fraction of the embryos injected with monolayer control cells showed the presence of cells in the tail 7 days after the injection (**Fig. 2B** shows results from the non-metastatic cell line BT-474; essentially the same results were obtained with the cell line MCF7, not shown). This migration could be observed in the embryos starting from day 4 after injection. The fraction of the embryos that showed tail migration corresponded to approximately 40% of the animals incubated both at 28°C and 34°C. When the embryos were injected with mammosphere-derived cells, cell migration to the tail could be observed in most animals, incubated both at 28°C (68% on average) and 34°C (80% on average). As a positive control for migration, cells from the metastatic cell line MDA-MB-435 were injected in parallel experiments and migration readily observed in 100% of the embryos (data not shown).

As a surrogate model for tumor formation, we observed the appearance of cell masses in embryos injected with BT-474 breast carcinoma cells. Interestingly, cell masses appeared early in the assay (clearly observed at day 3) though most of the embryos died before the 7-dpi allocated assay time. Therefore, cell masses were counted at day 3 post-injection (day 5 post-fertilization) to ensure that the results were not biased by premature embryo death. In these conditions, we observed that less than 10% of the embryos injected with both monolayer parental cells or sphere-derived cells formed masses when incubated at 28°C (**Fig. 2D**). Although we observed, on average, more embryos with masses in the group injected with sphere-derived cells, this different was not statistically significant. In order to favor mammalian cell viability, the experiments were repeated placing the injected embryos at 34°C. In these conditions, embryos injected with mammosphere-derived cells showed a significant increase in the frequency of cell masses, compared with embryos injected with parental monolayer cultured cells (23 vs. 10% on average, **Fig. 2D**).

Curcumin inhibits cell migration and cell mass formation in transplanted zebrafish embryos. Dietary polyphenols, such as Curcumin, have been demonstrated to modulate self-renewal of normal and malignant breast stem cells.[22] We determined the effects of varying concentrations of curcumin on BT-474 breast carcinoma cell line mammosphere formation assay. Consistently with previous reports, 10 μM curcumin inhibited tumorsphere formation by 40% while 20 μM curcumin inhibited by 70%[22] (**Fig. 3A and B**). This decrease in mammosphere formation was not due to non-specific toxicity, as cell viability, determined by MTT assay, was not affected (data not shown). As our results indicated that mammosphere-derived cells were differentially capable of showing migration and mass formation when injected into 2 dpf zebrafish embryos, we assessed the effect of curcumin on these phenotypes in the parental BT-474 population grown in monolayer. BT-474 monolayer cultures were treated with 10 μM curcumin for 48 h, then washed, harvested and single cell suspensions injected into 2 dpf embryos. Vehicle control (DMSO) monolayer cultured cells showed migration in approximately 40% of the embryos injected after 7 d, as well as mass formation in 10% of the embryos on average (**Fig. 3C**), consistent with our previous experiments. Curcumin-treated cultures significantly decreased the number of embryos showing these phenotypes, to 25% for tail migration and 5.7% on average for mass formation, consistent with the reduction on mammosphere formation observed in culture. These results suggested that the effects shown in monolayer parental controls on tail migration and mass formation were due to the mammosphere initiating cells in the parental monolayer cultures.

Discussion

Targeting CSCs, as the cell population responsible for tumor initiation and maintenance, represent a rational cancer therapeutical strategy. Indeed, targeting self-renewal of these cell populations may provide a less toxic approach than regular chemotherapeutic agents that target bulk, rapid proliferating tumor cells. Cancer self-renewal is commonly assessed using limiting dilution cell transplantation into immune-deficient rodent animal models and then scored for tumor engraftment. Recent work has shown that the level of immune suppression, site of cell injection (subcutaneous or orthotopic) and time point of end of experiment, may compromise the estimation of cancer stem cells.[23] The zebrafish model has been extensively used to understand human cancers, as zebrafish tumors are similar to human malignancies in a variety of tumor types.[24-27] The zebrafish system provides several advantages: large number of animals can be housed in a relatively small space and cost of breeding is inexpensive compared with mice; cell transplantation assays can be performed in hundreds of animals, unattainable using mice; fluorescence tracking of injected cells in live embryos is possible, even in adult fish as a zebrafish strain—named *casper*—was created lacking iridophores and melanocytes.[28] Several groups have shown transplantation of human tumor cell lines into zebrafish embryos to demonstrate migration, metastasis and vasculogenesis.[15,16,29-32] Two to 5 day old embryos can be used as recipients since full immune system is not yet developed.[16,18,30] This set up has the disadvantage that the fate of the transplanted cells cannot be followed beyond the 11th day mark, since the immune system develops and transplanted cells are rejected. In our hands, no cell could be detected in our transplanted animals at day 15 (data not shown). However, this assay provides a window where tail migration and mass formation can be measured, as demonstrated in our experiments, that

©2010 Landes Bioscience. Do not distribute

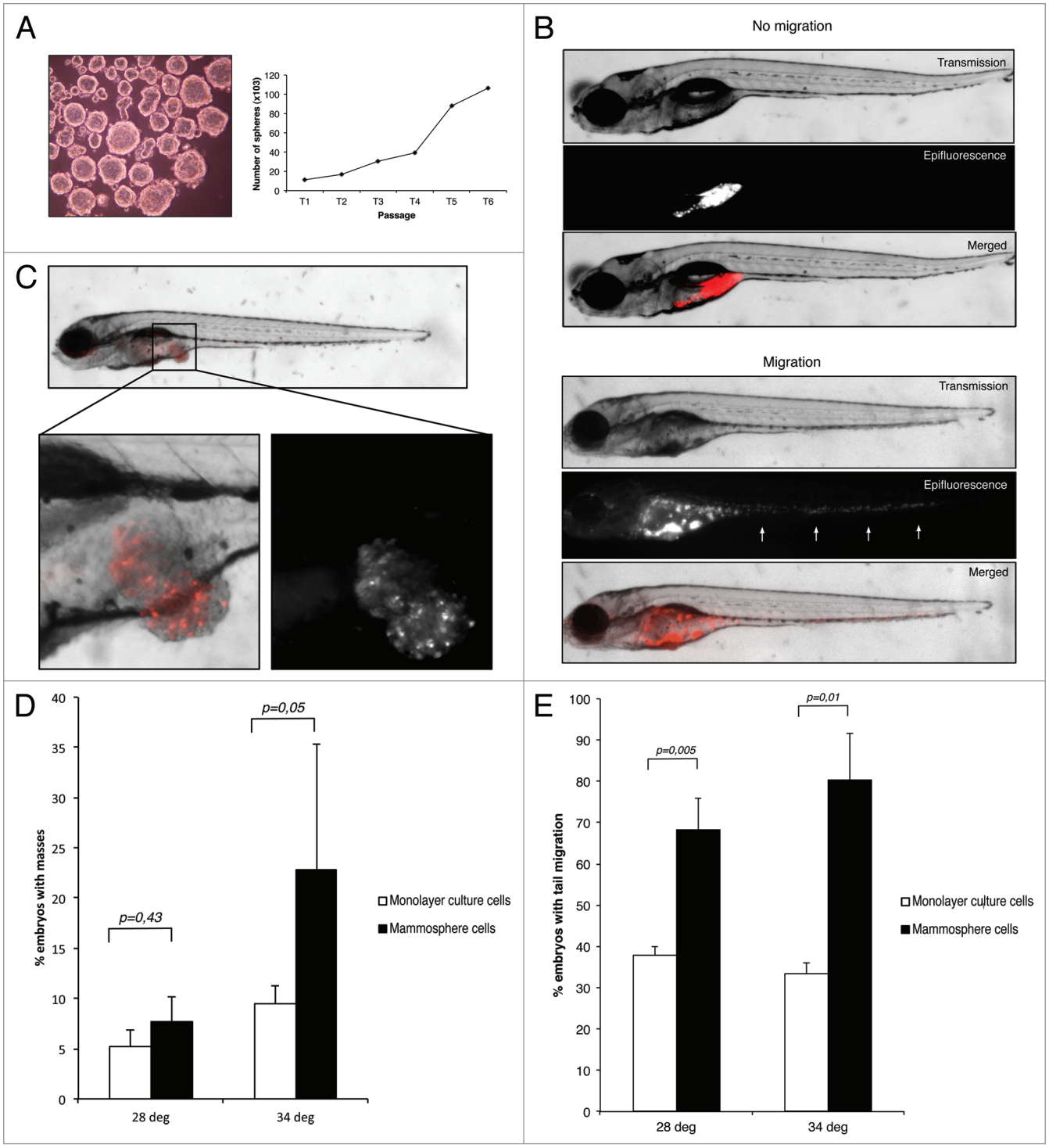

Figure 2. Mammosphere-derived cells migrate to the tail and form masses in zebrafish embryos. (A) Representative experiment of a 7-days mammosphere culture from BT-474 breast carcinoma cell line. Quantification of the proliferation of sphere cultures upon serial passage is represented. Sphere passages are named T to distinguish from regular monolayer culture passage (usually referred as P). (B) Representative image of an 9 dpf embryo with the injected cells retained in the yolk sac (upper part) or showing dissemination of the injected cells to the tail (lower part). (C) Representative image of a 5 dpf embryo showing a protruding cell mass in the yolk sac area. (D) Quatification of the number of embryos with evident mass formation 3 d post injection of the indicated cell populations, incubated at 28 or 34°C. The average of three experiments is shown. Statistical significance was assessed by Student t-test analysis and significance expressed as the value of p. (E) Quantification of the number of embryos with evident tail migration 7 d post injection of the indicated cell populations, incubated at 28 or 34°C. The average of three experiments is shown. Statistical significance was assessed by Student t-test analysis and significance expressed as the value of p.

may be suitable for functional phentoype assessment (as cancer stem-like cells) and drug screening. Other groups have used 30-day-old animals with continuous presence of dexamethasone to block immune responses, however, interpretation of the results is difficult due to lethal effects of the glucocorticoid on the animals and transplanted cells.[33]

Mammosphere formation assays may be a quantifiable functional assay to assess cancer stem-like cell-specific drugs. In the breast carcinoma cell line MCF7, the mammosphere assay has been demonstrated to enrich and propagate cells with enhanced tumor initiating ability[12] and as a functional in vitro assay for cancer stem-like specific drug screening.[34,35] In these culture conditions cancer stem cells are able to maintain stem-like properties such as tumor initiation, self-renewal and limited differentiation potential.[5,11] They are composed of a small number of stem cells and progenitor cells capable of self-renewing through serial passage. We chose to rely on this functional assay, instead of on a sorted population based on surface phenotype, such as the described $CD44^{+}CD24^{-}$ phenotype for breast tumor-derived cancer stem cells.[4] Breast carcinoma cell lines show a homogenous surface marker expression pattern, as opposed to fresh tumor cells, not evidencing the presence of particular subpopulations. When used to evaluate tumor initiation of common breast carcinoma cell lines, the percentage of $CD44^{+}CD24^{-}$ did not correlate to tumorigenicity;[36] it does, however, correlate strongly with rare basal and mesenchymal phenotype.[37] Therefore, we used the in vitro mammosphere assay for enrichment of breast cancer stem-like cells, to prevent marker bias, described for continuous cell lines.[36-38]

Both monolayer-derived cells and mammosphere-derived cells, presented the ability to migrate to the tail, with a significantly different frequency between populations. This migratory assay revealed that mammosphere-derived cells presented a significantly higher migratory capacity than monolayer-derived cells. Further analysis will be required to test how mammosphere-derived cells interact with the embryo vascular system, such as assessing VEGF-induced angiogenesis, demonstrated essential for the initial dissemination of tumor cells xenografted in zebrafish embryos.[39] Mammosphere forming cells are present in the parental monolayer population with a frequency of approximately 40% (assessed by limiting dilution and plating of single cells). When this frequency was reduced by the addition of curcumin, a dietary compound described to block breast cancer stem cell self renewal,[22] tail

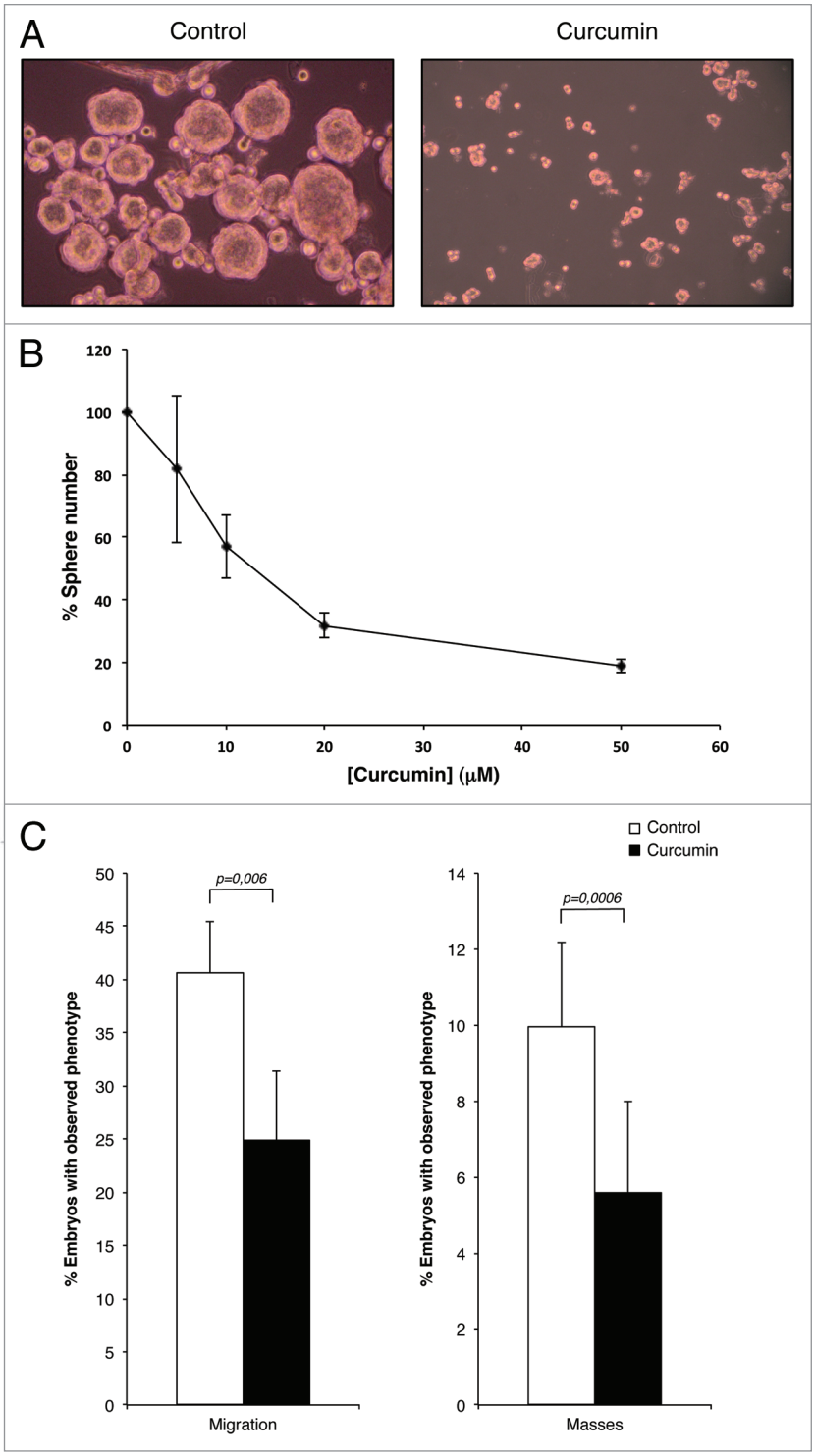

Figure 3. Curcumin treatment of monolayer cultures reduced tail migration and mass formation. (A and B) Dose dependent effect of Curcumin (Sigma) treatment on BT-474 mammosphere formation. Monolayer cultures were treated with varying doses of curcumin for 48 h, then washed and harvested. Single cells were plated on non-adherent tissue culture plates for sphere formation. No effect on cell viability was observed (data not shown). A representative micrograph of mammosphere cultures is presented in (A). (C) Treatment of monolayer cultures with 10 μM curcumin 48 h before injection into zebrafish embryos reduced significantly the number of embryos showing cell migration to the tail (7 dpi) and mass formation (3 dpi) incubated at 34°C. The average of three experiments is shown. Statistical significance was assessed by student t-test analysis and significance expressed as the value of p.

migration was reduced as well. Therefore our results are consistent with a breast cancer stem-like cell subpopulation being responsible for the migration phenotype observed. We observed the same phenotype at 28°C and at 34°C, although quantitatively reduced, suggesting that a component due to cell dispersion may not be ruled out. Nevertheless, cell dispersion alone does not explain the different outcome when mammosphere or monolayer-derived cells are injected. The frequency of embryos that showed mass formation was also significantly higher in cells derived from mammosphere cultures that derived from parental monolayer cultures. Interestingly, this effect was observed at 34°C rather than at the usual zebrafish growth temperature (28°C). Most of the masses are detected as extra-embryonary structures, although in some animals masses formed in the thoracic cavity and the head section (data not shown). This result indicates that, for mass formation, active cell proliferation and invasion is needed, while for tail migration the interaction with the process of embryo vasculogenesis may be more relevant.[39] Cancer cells have been described to undergo epithelial mesenchymal transition (EMT) as a necessary step for tumor dissemination,[40] and this process actively induce cancer stem cell features in transformed cells.[41] It is reasonable to speculate that, as mammosphere culture enriches for breast cancer stem-like cells, these cells may be more prone for invading other tissues, thus explaining the migratory and mass formation phenotypes we observe. Of notice, we used the BT-474 cell line, which is one of the few breast carcinoma cell lines derived from a primary lession, non-metastatic. Therefore, BT-474 cells have not undergone epithelial-mesenchymal transition, what may alter its migratory properties. As migration control, the mesenchymal-like cell line MD-MB-435 was used, and strong migration was readily observed (data not shown).

Our work demonstrates that transplantation of selected populations of cancer cells into 2 dpf zebrafish embryos can be used to functionally discern between cell populations based on cancer stem cell associated properties, thus suggests it may be used to uncover important pathways in cancer cell self-renewal, both as a basic discovery assay and drug screening. The unique attributes of the zebrafish model, over the commonly used rodent based models, may provide novel approaches for drug screening toward cancer stem cells.

Materials and Methods

Zebrafish handling and cell injection. Zebrafish (*Danio rerio*, wild-type AB) embryos were generated by natural mating of adult fish according to the Zebrafish Handbook.[17] In order to remove patches of surface pigmentation, we used PTU (N-Phenylthiourea, Sigma) at a 0.003% w/v concentration. Embryos were bred and maintained at 28°C prior to cell injection. For injection, 2 dpf zebrafish embryos were de-chorionized if necessary and anaesthetized with Tricaine methanesulfonate (MS-222) at 0.004% w/v and then placed in a Petri dish on their sides on a ramp made of 1% agarose (4 mm long). Immediately prior to injection, cells were labeled with the lipophilic fluorescent tracking dye CM-DiI (Invitrogen) according to manufacturer's instructions. Briefly, cells were incubated with CM-DiI dissolved in DMSO (final concentration 4.8 μg/ml) for 4 min at 37°C, followed by 15 min at 4°C. To remove unincorporated dye, cells were centrifuged and rinsed twice with HBSS. Cells were loaded into a pulled glass micropipette and then attached to an air driven microinjector (Eppendorf FentoJet Express). The tip of the needle was inserted into the yolk sac of the animals and cells were delivered in a single injection using 200–300 ms pulse time and 200 hPa positive pressure. In our conditions, we optimized the number of cells injected at ~500 cells per injection at a volume of approximately 20–50 nanoliters. After injection, embryos were maintained for 2 h at 28°C, examined for the presence of a fluorescent cell mass localized at the injection site in the yolk sac, and then transferred to fresh water and placed into an incubator at 28°C or 34°C for up to 7 d post injection (dpi), when the animals were euthanized by freezing.

Cell culture. Breast carcinoma cell lines BT-474, MCF7 and MDA-MB-435 were obtained directly from the ATCC (Manassas, VA) and grown in DMEM (Gibco) supplemented with 10% fetal bovine serum (FBS, Sigma) at 37°C in a 5% CO_2 incubator. For mammosphere formation, single cell suspensions were plated in 6-well tissue culture plates covered with poly-2-hydroxyethyl-methacrylate (Sigma) to prevent cell attachment, at a density of 1,000 cells/ml in serum-free DMEM supplemented with 1% L-glutamine, 1% penicillin/streptomycin, 30% F12 supplement (Sigma), 2% B27 (Invitrogen), 20 ng/ml EGF (Sigma) and 20 ng/ml FGFb (Invitrogen) medium. The medium was made semi-solid by the addition of 0.5% Methylcellulose (R&D Systems) to prevent cell aggregation. Mammospheres were collected by gentle centrifugation after 7 d and dissociated enzymatically (5 min in 1:1 trypsin/DMEM solution at 37°C) and mechanically by passing the suspension 10 times through a 25G needle. The resulting cell suspension was sieved through a 40 μm cell strainer to ensure a single cell suspension.

Imaging. Injections were performed using an Olympus SZX12 dissecting microscope. Xenotransplanted animals were examined using a Zeiss Lumar V12 (Carl Zeiss Microimaging Inc.) fluorescence microscope equipped to visualize Alexa 568 and/or CM-DiI (excitation 553 nm, emission 570 nm) labeled BT-474 cells. The brightfield and the Alexa 568 channel were simultaneously used to examine cells. Images were captured with a Zeiss high-speed monochrome camera (Carl Zeiss Microimaging Inc.) using Axiovision VS40 4.6.1.0 software (Carl Zeiss Microimaging Inc.).

Disclosure of Potential Conflicts of Interest

A.E., I.B., L.A., Y.S. and A.G.M. declare no competing financial interests in relation to the work described. C.C. and O.H. are employees of Biobide SL, a company focused on providing zebrafish-based assays.

Acknowledgments

The Regulation of Cell Growth Laboratory is supported by grants from Obra Social Kutxa, Fundación Médica Mutua Madrileña, Gobierno Vasco (Saiotek program and Consejería de Educación PI2010-25) and Instituto de Salud Carlos III Acción Estratégica

en Salud (PI2010-01035). Biobide and Inbiomed were funded by a grant from Ministerio de Industria IAP-580000-2008-59. We thank our colleagues at Inbiomed for helpful discussion. We thank Dr. C.I. Rodriguez and Dr. L. Vellon for critical reading of the manuscript.

References

1. Zhou BB, Zhang H, Damelin M, Geles KG, Grindley JC, Dirks PB. Tumour-initiating cells: challenges and opportunities for anticancer drug discovery. Nat Rev Drug Discov 2009; 8:806-23; PMID:19794444; DOI:10.1038/nrd2137.
2. Kemper K, Grandela C, Medema JP. Molecular identification and targeting of colorectal cancer stem cells. Oncotarget 2010; 1:387-95; PMID:21311095.
3. Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, et al. Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. Nat Med 2009; 15:907-13; PMID:19648928; DOI:10.1038/nm.2000.
4. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci USA 2003; 100:3983-8; PMID:12629218; DOI:10.1073/pnas.0530291100.
5. Ponti D, Costa A, Zaffaroni N, Pratesi G, Petrangolini G, Coradini D, et al. Isolation and in vitro propagation of tumorigenic breast cancer cells with stem/progenitor cell properties. Cancer Res 2005; 65:5506-11; PMID:15994920; DOI:10.1158/0008-5472.CAN-05-0626.
6. Ginestier C, Hur MH, Charafe-Jauffret E, Monville F, Dutcher J, Brown M, et al. ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 2007; 1:555-67; PMID:18371393; DOI:10.1016/j.stem.2007.08.014.
7. Li X, Lewis MT, Huang J, Gutierrez C, Osborne CK, Wu MF, et al. Intrinsic resistance of tumorigenic breast cancer cells to chemotherapy. J Natl Cancer Inst 2008; 100:672-9; PMID:18445819; DOI:10.1093/jnci/djn123.
8. Tanei T, Morimoto K, Shimazu K, Kim SJ, Tanji Y, Taguchi T, et al. Association of breast cancer stem cells identified by aldehyde dehydrogenase 1 expression with resistance to sequential paclitaxel and epirubicin-based chemotherapy for breast cancers. Clin Cancer Res 2009; 15:4234-41.
9. Curtin JC, Lorenzi MV. Drug discovery approaches to target Wnt signaling in cancer stem cells. Oncotarget 2010; 1:563-77; PMID:21317452.
10. Farnie G, Clarke RB, Spence K, Pinnock N, Brennan K, Anderson NG, et al. Novel cell culture technique for primary ductal carcinoma in situ: role of Notch and epidermal growth factor receptor signaling pathways. J Natl Cancer Inst 2007; 99:616-27; PMID:17440163; DOI:10.1093/jnci/djk133.
11. Dontu G, Abdallah WM, Foley JM, Jackson KW, Clarke MF, Kawamura MJ, et al. In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. Genes Dev 2003; 17:1253-70; PMID:12756227; DOI:10.1101/gad.1061803.
12. Ao A, Morrison BJ, Wang H, Lopez JA, Reynolds BA, Lu J. Response of estrogen receptor-positive breast cancer tumorspheres to antiestrogen treatments. PLoS ONE 2011; 6:18810; PMID:21533195; DOI:10.1371/journal.pone.0018810.
13. Heuser M, Humphries RK. Biologic and experimental variation of measured cancer stem cells. Cell Cycle 2010; 9:909-12; PMID:20348851; DOI:10.4161/cc.9.5.10852.
14. Zon LI, Peterson RT. In vivo drug discovery in the zebrafish. Nat Rev Drug Discov 2005; 4:35-44; PMID:15688071; DOI:10.1038/nrd1606.
15. Lee LM, Seftor EA, Bonde G, Cornell RA, Hendrix MJ. The fate of human malignant melanoma cells transplanted into zebrafish embryos: assessment of migration and cell division in the absence of tumor formation. Dev Dyn 2005; 233:1560-70; PMID:15968639; DOI:10.1002/dvdy.20471.
16. Haldi M, Ton C, Seng WL, McGrath P. Human melanoma cells transplanted into zebrafish proliferate, migrate, produce melanin, form masses and stimulate angiogenesis in zebrafish. Angiogenesis 2006; 9:139-51; PMID:17051341; DOI:10.1007/s10456-006-9040-2.
17. Westerfield M. The zebrafish book: a guide for the laboratory use of zebrafish. Eugene: The University of Oregon Press 1993.
18. Traver D, Herbomel P, Patton EE, Murphey RD, Yoder JA, Litman GW, et al. The zebrafish as a model organism to study development of the immune system. Adv Immunol 2003; 81:253-330; PMID:14711058; DOI:10.1016/S0065-2776(03)81007-6.
19. Detrich HW, 3rd, Westerfield M, Zon LI. Overview of the Zebrafish system. Methods Cell Biol 1999; 59:3-10; PMID:9891351; DOI:10.1016/S0091-679X(08)61816-6.
20. Liu S, Dontu G, Wicha MS. Mammary stem cells, self-renewal pathways and carcinogenesis. Breast Cancer Res 2005; 7:86-95; PMID:15987436; DOI:10.1186/bcr1021.
21. Leis O, Eguiara A, Lopez-Arribillaga E, Alberdi MJ, Hernandez-Garcia S, Elorriaga K, et al. Sox2 expression in breast tumours and activation in breast cancer stem cells. Oncogene 2011; In press; PMID: 21822303; DOI:10.1038/onc.2011.338.
22. Kakarala M, Brenner DE, Korkaya H, Cheng C, Tazi K, Ginestier C, et al. Targeting breast stem cells with the cancer preventive compounds curcumin and piperine. Breast Cancer Res Treat 2010; 122:777-85; PMID:19898931; DOI:10.1007/s10549-009-0612-x.
23. Quintana E, Shackleton M, Sabel MS, Fullen DR, Johnson TM, Morrison SJ. Efficient tumour formation by single human melanoma cells. Nature 2008; 456:593-8; PMID:19052619; DOI:10.1038/nature07567.
24. Park SW, Davison JM, Rhee J, Hruban RH, Maitra A, Leach SD. Oncogenic KRAS induces progenitor cell expansion and malignant transformation in zebrafish exocrine pancreas. Gastroenterology 2008; 134:2080-90; PMID:18549880; DOI:10.1053/j.gastro.2008.02.084.
25. Berghmans S, Murphey RD, Wienholds E, Neuberg D, Kutok JL, Fletcher CD, et al. tp53 mutant zebrafish develop malignant peripheral nerve sheath tumors. Proc Natl Acad Sci USA 2005; 102:407-12; PMID:15630097; DOI:10.1073/pnas.0406252102.
26. Haramis AP, Hurlstone A, van der Velden Y, Begthel H, van den Born M, Offerhaus GJ, et al. Adenomatous polyposis coli-deficient zebrafish are susceptible to digestive tract neoplasia. EMBO Rep 2006; 7:444-9; PMID:16439994.
27. Guo L, Chua J, Vijayakumar D, Lee KC, Lim K, Eng H, et al. Detection of the 113p53 protein isoform: a p53-induced protein that feeds back on the p53 pathway to modulate the p53 response in zebrafish. Cell Cycle 2010; 9:1998-2007; PMID:20505327; DOI:10.4161/cc.9.10.11732.
28. White RM, Sessa A, Burke C, Bowman T, LeBlanc J, Ceol C, et al. Transparent adult zebrafish as a tool for in vivo transplantation analysis. Cell Stem Cell 2008; 2:183-9; PMID:18371439; DOI:10.1016/j.stem.2007.11.002.
29. Nicoli S, Ribatti D, Cotelli F, Presta M. Mammalian tumor xenografts induce neovascularization in zebrafish embryos. Cancer Res 2007; 67:2927-31; PMID:17409396; DOI:10.1158/0008-5472.CAN-06-4268.
30. Marques IJ, Weiss FU, Vlecken DH, Nitsche C, Bakkers J, Lagendijk A, et al. Metastatic behaviour of primary human tumours in a zebrafish xenotransplantation model. BMC Cancer 2009; 9:128; PMID:19400945; DOI:10.1186/1471-2407-9-128.
31. Geiger GA, Fu W, Kao GD. Temozolomide-mediated radiosensitization of human glioma cells in a zebrafish embryonic system. Cancer Res 2008; 68:3396-404; PMID:18451167; DOI:10.1158/0008-5472.CAN-07-6396.
32. Langenau DM, Keefe MD, Storer NY, Guyon JR, Kutok JL, Le X, et al. Effects of RAS on the genesis of embryonal rhabdomyosarcoma. Genes Dev 2007; 21:1382-95; PMID:17510286; DOI:10.1101/gad.1545007.
33. Stoletov K, Montel V, Lester RD, Gonias SL, Klemke R. High-resolution imaging of the dynamic tumor cell vascular interface in transparent zebrafish. Proc Natl Acad Sci USA 2007; 104:17406-11; PMID:17954920; DOI:10.1073/pnas.0703446104.
34. Zhou J, Zhang H, Gu P, Bai J, Margolick JB, Zhang Y. NFkappaB pathway inhibitors preferentially inhibit breast cancer stem-like cells. Breast Cancer Res Treat 2008; 111:419-27.
35. Cioce M, Gherardi S, Viglietto G, Strano S, Blandino G, Muti P, et al. Mammosphere-forming cells from breast cancer cell lines as a tool for the identification of CSC-like- and early progenitor-targeting drugs. Cell Cycle 2010; 9:2878-87; PMID:20581442; DOI:10.4161/cc.9.14.12371.
36. Fillmore CM, Kuperwasser C. Human breast cancer cell lines contain stem-like cells that self-renew, give rise to phenotypically diverse progeny and survive chemotherapy. Breast Cancer Res 2008; 10:25; PMID:18366788; DOI:10.1186/bcr1982.
37. Keller PJ, Lin AF, Arendt LM, Klebba I, Jones AD, Rudnick JA, et al. Mapping the cellular and molecular heterogeneity of normal and malignant breast tissues and cultured cell lines. Breast Cancer Res 2010; 12:87; PMID:20964822; DOI:10.1186/bcr2755.
38. Blick T, Hugo H, Widodo E, Waltham M, Pinto C, Mani SA, et al. Epithelial mesenchymal transition traits in human breast cancer cell lines parallel the CD44(hi/)CD24 (lo/-) stem cell phenotype in human breast cancer. J Mammary Gland Biol Neoplasia 2010; 15:235-52; PMID:20521089; DOI:10.1007/s10911-010-9175-z.
39. Rouhi P, Lee SL, Cao Z, Hedlund EM, Jensen LD, Cao Y. Pathological angiogenesis facilitates tumor cell dissemination and metastasis. Cell Cycle 2010; 9:913-7; PMID:20160500; DOI:10.4161/cc.9.5.10853.
40. Creighton CJ, Chang JC, Rosen JM. Epithelial-mesenchymal transition (EMT) in tumor-initiating cells and its clinical implications in breast cancer. J Mammary Gland Biol Neoplasia 2010; 15:253-60; PMID:20354771; DOI:10.1007/s10911-010-9173-1.
41. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. Cell 2008; 133:704-15; PMID:18485877; DOI:10.1016/j.cell.2008.03.027.

©2011 Landes Bioscience. Do not distribute