Maternal Embryonic Leucine Zipper Kinase Is Upregulated and Required in Mammary Tumor-Initiating Cells *In vivo*

Lionel W. Hebbard1, Jochen Maurer1, Amber Miller1, Jacqueline Lesperance1, John Hassell2, Robert G. Oshima1, and Alexey V. Terskikh1

Abstract

Maternal embryonic leucine zipper kinase (MELK) is expressed in several developing tissues, in the adult germ line, and in adult neural progenitors. MELK expression is elevated in aggressive undifferentiated tumors, correlating with poor patient outcome in human breast cancer. To investigate the role of MELK in mammary tumorigenesis *in vivo*, we used a MELK-green fluorescent protein (GFP) reporter mouse, which allows prospective isolation of MELK-expressing cells based on GFP fluorescence. We found that in the normal mammary gland, cells expressing high levels of MELK were enriched in proliferating cells that express markers of mammary progenitors. The isolation of cells with high levels of MELK in mammary tumors from MMTV-Wnt1/MELK-GFP bitransgenic mice resulted in a significant enrichment of tumorsphere formation in culture and tumor initiation after transplantation into mammary fat pads of syngeneic mice. Furthermore, using lentiviral delivery of MELK-specific shRNA and limiting dilution cell transplantsations, we showed that MELK function is required for mammary tumorigenesis *in vivo*. Our findings identify MELK as a potential target in breast tumor-initiating cells. *Cancer Res 70(21): 8863–73.*

Introduction

The cancer stem cell model originates from the observed heterogeneity of tumors and the presence of a minority population of tumor cells that are capable of both self-renewal and differentiation (1–5). Targeting cancer stem cells, also called tumor-initiating cells, critically depends on the identification of therapeutic targets. In breast cancer, cells with a CD44+CD24low/− phenotype isolated from human tumors show high tumor-forming capacity (6). ALDH1 is one of the few intracellular, potentially functional markers of human breast tumor-initiating cells (7). However, the paucity of current molecular targets warrants the search for new candidates that allow the development of therapeutic strategies to specifically target and eliminate tumor-initiating cells.

Maternal embryonic leucine zipper kinase (MELK; ref. 10) is a member of the Snf1/AMPK family of kinases but is unique in that it is not regulated by LKB1 kinase (11). In addition to a Ser/Thr kinase domain, MELK contains an unstructured COOH-terminal ubiquitin associated (UBA) domain that prevents ubiquitin-mediated degradation (12), a leucine zipper motif, and a COOH-terminal kinase–associated (KA1) domain. During development, it is expressed in tissues that contain normal progenitor cells (13) and in adult brain progenitor cells (14, 15). The MELK gene was first cloned from a human myeloid leukemic cell line (16) and subsequently shown to be commonly expressed in poorly differentiated aggressive cancers (17, 18). For example, MELK RNA is dramatically upregulated in glioblastomas and is correlated with malignancy grade in human astrocytomas (19, 20). We have previously shown *in vitro* that MELK siRNA inhibits the growth of primary glioblastoma cell lines (21). The *in vivo* function of MELK is currently unknown; however, CDC25B phosphatase, a critical G2–M checkpoint protein, and apoptosis signal-regulating kinase 1 (ASK1) were suggested as potential MELK targets (22, 23).

Elevated expression of MELK was found to be associated with poor prognosis of breast cancer patients (24) and glioblastoma patients (21). MELK was found to physically interact with and phosphorylate proapoptotic Bcl-G. The overexpression of wild-type (wt) MELK, but not a kinase-dead mutant, was reported to suppress Bcl-G–induced apoptosis, promoting mammary carcinogenesis (25). Based on...
the growth inhibition of several cancer cell lines in vitro. MELK was proposed to be a promising target for multiple cancer types (26). However, two critical questioned remained unanswered: First, do tumor-initiating cells express MELK? Second, is MELK required for mammary tumorigenesis in vivo? Here, we used the MMTV-Wnt1 mouse mammary tumor model to show that cells expressing elevated levels of MELK are enriched in tumor-initiating cells. Critically, we showed that MELK function is required for mammary tumor growth in vivo.

Materials and Methods

Tumorsphere cultures

For culture, the cells were resuspended in Mammary Stem Cell Medium (MSCM) composed of MEBM (Lonza), 20 ng/mL epidermal growth factor, 20 ng/mL basic fibroblast growth factor, 4 μg/mL heparin, 1× B27 (Invitrogen), and 2× penicillin/streptomycin. For sphere-forming assays, cells in MSCM were mixed (1:1) with growth factor–deficient Matrigel (BD Bioscience) and MSCM was overlaid each week; images were taken after 2 weeks with a Nikon microscope. Unless otherwise stated, all reagents were from Sigma-Aldrich.

Limiting dilution transplantation of Wnt1-induced tumor cells

Transplantation was performed as previously described (27). Briefly, primary tumors were dissociated into single cells. Cells were sorted by fluorescence-activated cell sorting (FACS) into GFPhigh (top 10%) and GFPlow (bottom 10%) fractions and injected into cleared mammary fat pads of 19-day-old FVB/N females at limiting dilutions of 1,000, 100, and 10 cells mixed with a 1:1 ratio of MSCM/Matrigel. Tumor development was checked by palpation three times a week. Mammary glands were removed and imaged with a LEICA fluorescent dissecting scope, after which they were fixed in Carnoy’s, stained with Carmine Alum, and mounted with Permount.

Inhibition of tumorsphere growth by MELK shRNA

Tumors were dissociated into single cells and cultivated for two passages (3 weeks per passage) in a mix of MSCM and Matrigel (1:1) in low-attachment dishes. MSCM (0.5 mL) was added every week to the culture. The resulting tumorspheres were separated from the Matrigel by dispase treatment and dissociated into single cells with Accutase. Cells (2 × 105) were treated with 10 μL of two different lentiviral constructs (multiplicity of infection ~10), one carrying a MELK shRNA construct and the other a control shRNA. Cells were incubated with the virus overnight at 37°C and plated in a mix of MSCM and Matrigel (1:1) in low-attachment dishes the following day.

Statistical analysis

The frequencies of tumor-initiating cells and 95% confidence intervals (95% CI) were calculated using Poisson statistics and maximum likelihood estimate as implemented in ELDA software http://bioinf.wehi.edu.au/software/elda/index.html (28). The ELDA software was specifically designed to handle the extreme situations when all transplanted pads are either negative or positive. The goodness of fit to a single-hit Poisson model was tested using χ² test as implemented in ELDA software.

Results

MELK is expressed in proliferating luminal cells in normal mammary epithelium

Mammary epithelial tissue consists of the ducts, alveoli, and terminal end buds. The ducts are organized as layers of internal luminal epithelial cells covered by the basally located myoepithelial cells (29, 30). First, we investigated which population of cells expresses MELK in the normal mammary gland. Because of the lack of antibodies that robustly detect MELK, we took advantage of the MELK-green fluorescent protein (GFP) promoter-reporter mouse, which we previously used to trace the expression of endogenous MELK in the neural stem/progenitor population (31). We found that MELK was selectively expressed in both the ducts and the terminal end buds of virgin mammary epithelial tissue. In whole mounts, GFP fluorescence was increased in the terminal end buds and alveolar expansion during pregnancy (Fig. 1A and B). Immunofluorescent detection of GFP expression in sectioned material and costaining with the epithelial marker CD44v6 (32) indicated enhanced expression in the luminal epithelium (Fig. 1C and D). To confirm that the MELK-GFP reporter truly represents the levels of endogenous MELK protein, we sorted GFPhigh (10% bottom) and GFPhigh (10% top) cells and immunostained both populations for the endogenous MELK protein (Fig. 1E and F). Quantification of the fluorescent signal showed an ∼7-fold increase in immunoactivity for the endogenous MELK protein in GFPhigh cells as compared with GFPlow cells (Fig. 1G), which correlated with an ∼5-fold increase in GFP fluorescence between the GFPhigh (median, 5.5 × 10³) and GFPlow (median, 1.1 × 10³) populations (Fig. 1H).

In addition, quantitative PCR analysis of endogenous MELK mRNA illustrated an ∼3-fold increase between the GFPhigh and GFPlow populations (Fig. 1I). Taken together, these results confirm that the GFP signal in the mammmary tissue of MELK-GFP mice correlates with the expression level of the endogenous MELK mRNA and MELK protein, and that MELK expression is predominantly localized to the luminal epithelium of the mammary gland.

We have previously shown selective expression of MELK in proliferative progenitors in the developing and neonatal nervous system (31) and in proliferative cancer cells in culture (33). To determine the cell cycle status of MELK-GFP cells in the normal mammary gland, we costained GFP-positive cells for proliferating cell nuclear antigen (PCNA). The GFP-positive luminal cells were positive for PCNA, suggesting that MELK was upregulated in proliferating luminal cells (Fig. 2A). We then determined the cell cycle status of various GFP fractions. The high, medium, low, and negative fractions were isolated by FACS and analyzed for DNA content. The GFPhigh cells had the highest proportion of cells (21%) in the S-G2-M phase (Fig. 2B). Together with
The PCNA staining data, these results indicate that MELK expression is associated with proliferating cells and that the GFP<sup>high</sup> fraction is enriched in cycling cells.

**Increased MELK expression in mammary cells with markers of progenitor cells**

Previous studies indicate that in the normal mammary gland, stem cell activity is found in the Lin<sup>−</sup> (CD45<sup>−</sup>CD31<sup>−</sup>TER119<sup>−</sup>) CD29<sup>high</sup>CD24<sup>+</sup> compartment, whereas progenitors are found in the Lin<sup>−</sup>CD29<sup>low</sup>CD24<sup>+</sup> compartment (34). In addition, the mammary stem cell compartment was positive for keratin 14 (K14), a basal epithelium marker, whereas progenitors were positive for K8, a luminal area marker (34). We used MELK-GFP transgenic mice to investigate the expression of MELK with respect to the previously defined stem cell and progenitor cell compartments in the normal mammary epithelium. We found a significantly higher expression of MELK in cells that contained progenitors compared with the population that included stem cells (Fig. 3A). These results are consistent with the immunofluorescent detection of MELK expression in situ (Fig. 1C and D).

Next, we examined the expression of the CD24/29 markers within the GFP<sup>high</sup> cells (top 10–15%) and GFP<sup>low</sup> cells (bottom 10–15%) freshly isolated from normal mammary glands. We found that a majority of the GFP<sup>high</sup> cells (77%) expressed levels of CD24 and CD29 similar to those previously found in a cell population enriched in mammary progenitors (34). The GFP<sup>low</sup> cells were broadly distributed with a minority (20%) clustered in a population with the levels of CD24 and CD29 typical for mammary stem cells (Fig. 3B). These results suggest that within the normal mammary glands of MELK-GFP mice, the top 10% to 15% of GFP-positive cells are within the population that is enriched in normal mammary progenitors (34).

Next, we isolated the GFP<sup>high</sup> cells (top 10%) and GFP<sup>low</sup> cells (bottom 10%) and immunostained these populations for keratins. The GFP<sup>low</sup> fraction predominantly expressed basal cell–associated K14 (∼55% K14<sup>+</sup> cells versus ∼10% K8<sup>+</sup> cells) whereas GFP<sup>high</sup> cells were enriched in luminal cell–associated K8 (25% K14<sup>−</sup> versus 45% K8<sup>+</sup>; Fig. 3C and D). The increased proportion of K8-expressing cells in the GFP<sup>high</sup> fraction corresponds with previous reports showing that luminal progenitor enrichment is associated with K8/K18 expression and intermediate/low levels of K14 (34).
Taken together, these results suggest that MELK is upregulated in normal proliferating mammary progenitors and that isolated GFP<sup>high</sup> cells are enriched in such progenitors. The presence of both K8- and K14-positive cells suggests that GFP<sup>high</sup> cells may contain both luminal and basal epithelial proliferating progenitors.

**Tumor-initiating cells in MMTV-Wnt1 tumors express high levels of MELK**

Mammary tumors induced by the Wnt1 gene under the influence of the MMTV enhancer are heterogeneous, containing both luminal and basal epithelial cells (Fig. 4A; ref. 35), and are suggested to originate from progenitor-like cells (36). We crossed the MELK-GFP mice with MMTV-Wnt1 mice and analyzed MELK expression in these tumors. Whole mounts of mammary fat pads of MMTV-Wnt1/MELK-GFP bitransgenic mice consistently revealed GFP expression within tumors (Fig. 4B). We isolated GFP<sup>low</sup> and GFP<sup>high</sup> cells (top 10% and bottom 10% of the GFP-positive cells) from MMTV-Wnt1/MELK-GFP bitransgenic mice using flow cytometry and determined the expression of K8 and K14 (Fig. 4C). The GFP<sup>low</sup> fraction predominantly expressed K14 (30% K14<sup>+</sup> and 10% K8<sup>+</sup>), whereas the GFP<sup>high</sup> cells were significantly enriched (5-fold) for K8 (10% K14<sup>+</sup> and 50% K8<sup>+</sup>; Fig. 4D). These results parallel the MELK expression in the normal mammary gland. We also examined the expression of CD29, CD24, CD49f, and CD61 surface markers in MMTV-Wnt1/MELK-GFP bitransgenic tumors. We found elevated expression of CD29, CD24, and CD49f in the GFP<sup>high</sup> population (Fig. 4E), consistent with the concept that GFP<sup>high</sup> cells contain luminal progenitors in Wnt1 tumors (30, 34, 37). The marker CD61 was recently suggested to identify cancer stem cells in preneoplastic cells in the MMTV-Wnt1 model (38). Indeed, the CD61 expression in GFP<sup>high</sup> cells (median fluorescence, 134) was higher than that in the GFP<sup>low</sup> population (median fluorescence, 39). However, a population of CD61<sup>+</sup> cells in the GFP-negative fraction was also identified (Fig. 4F). Collectively, these results suggest that although MELK expression is not strictly restricted to one type of tumor cell, the GFP<sup>high</sup> fraction in MMTV-Wnt1 tumors is enriched in cells expressing luminal epithelial markers.

Next, we investigated the proliferative capacity of GFP<sup>low</sup> and GFP<sup>high</sup> cells in culture using a tumorsphere assay (39) modified to include Matrigel to decrease anoikis, increase colony formation efficiency, and eliminate cellular aggregation during sphere formation (see Materials and Methods for details). Time-lapse observation of individual cells in Matrigel confirmed the clonal origins of the tumorspheres under these conditions (data not shown). We found that the GFP<sup>high</sup> population contained
the vast majority of tumorsphere-forming cells, providing 5- to 10-fold enrichment compared with the other populations at all tested cell densities (Fig. 5A). The spheres can be serially cultivated and maintained GFP expression (Fig. 5B and C). However, despite the linear increase in cumulative total number (Fig. 5D), the sphere-forming capacity progressively decreased with passage (Fig. 5E), suggesting that under these conditions, the sphere-initiating cells undergo progressive differentiation (Fig. 5F).

To investigate the in vivo tumorigenicity of primary MMTV-Wnt1/MELK-GFP bitransgenic tumors, we performed limiting-dilution transplantation into cleared mammary fat pads. Based on the in vitro results, we chose to compare the GFP<sup>high</sup> and GFP<sup>low</sup> populations. In the first tumor analyzed (WMG49), we found that GFP<sup>high</sup> cells are enriched ∼6.5-fold in tumorigenic cells compared with GFP<sup>low</sup> cells (Table 1A). In this experiment, the calculated frequency of cells with tumor-initiating activity for GFP<sup>high</sup> cells was 1/55 cells (95% CI,
1/112–1/27), compared with 1/351 (95% CI, 1/1443–1/85) for GFPlow cells (~6.4-fold enrichment; $\chi^2 = 7.53, P = 0.0061$).

In a separate experiment, the GFPhigh cells from another primary tumor (WMG300) were isolated and cultured as spheres for 5 passages; then, GFPhigh/low fractions were isolated and transplanted to determine the frequency of tumor-initiating cells (Table 1B). The calculated frequency of cells with tumor-initiating activity for GFPhigh cells was 1/14 cells (95% CI, 1/55–1/4) compared with 1/276 (95% CI, 1/894–1/85) for GFPlow cells (~20-fold enrichment, $\chi^2 = 20.3, P = 0.0013$). When transplanted into a cleared fat pad, as few as 10 GFPhigh cells formed neoplastic outgrowths (WMG49; Fig. 5G; analyzed after 20 days) or solid tumor mass (WMG300; Fig. 5H and I; analyzed after tumors reached 1 cm in diameter). These results show that mammary tumor cells expressing the highest levels of MELK show tumorsphere-forming activity in culture and tumor-initiating activity on transplantation.

Figure 4. Heterogeneous expression of GFP in MMTV-Wnt1/MELK-GFP bitransgenic tumors. A, immunostaining of K8 and K14 in a wt MMTV-Wnt1 tumor. Bar, 50 μm. B, whole-mount bright-field and green fluorescent images of a bitransgenic tumor. Bar, 50 μm. C and D, immunostaining (bar, 50 μm) and quantification of K8 and K14 expression in GFPhigh and GFPlow fractions of GFP Wnt1 tumors. GFPhigh and GFPlow cells express significantly different levels of K8 and K14 ($P = 0.003$ and $P = 0.0215$, respectively; $n = 3$). E, flow cytometry analysis of stem/progenitor marker expression in GFPnegative (gray area), GFPhigh (blue line), and GFPlow (green line) cells from a MMTV-Wnt1/MELK-GFP bitransgenic tumor. GFPhigh cells express elevated CD24, CD49f, and CD61 as compared with GFPlow cells. F, flow cytometry analysis (colored dot plot, 5% probability with outliers) of CD61 versus GFP expression in MMTV-Wnt1/MELK-GFP bitransgenic tumor. Semi-transparent shaded areas correspond to the GFPhigh (green), GFPlow (blue), and GFPnegative (gray) populations. Horizontal line, level of background fluorescence (CD61-negative cells).
MELK is required for the development of MMTV-Wnt1 tumors in vivo

Finally, we investigated whether MELK is required for the initiation and/or propagation of Wnt1-induced mammary tumors in vitro and in vivo. We used lentiviral delivery of MELK shRNA, which was previously documented to specifically downregulate MELK expression in primary cells and several cell lines (21, 31). First, we evaluated the ability of lentivirus-coded MELK shRNA to affect the formation of tumorspheres in a three-dimensional (3D) Matrigel tumor-sphere-forming assay (modified from ref. 39). MELK shRNA (directed against the 3′ untranslated region of endogenous MELK transcript), but not control shRNA, dramatically reduced the number of spheres (∼10-fold) formed by the GFP<sup>high</sup> cells from a primary Wnt1 tumor (Fig. 6A). To verify that the reduction in frequency of tumorspheres occurred specifically as a result of MELK down-regulation, we performed a rescue experiment. We transduced MELK shRNA–treated mammary cells with a MELK cDNA–expressing lentivirus and observed a complete rescue of tumorsphere formation (Fig. 6B). These results confirm that the inhibitory function of the MELK shRNA on tumorsphere formation shRNA is specific for MELK.

To investigate if MELK shRNA can inhibit mammary tumorigenesis in vivo, we transduced WMG300 MMTV-Wnt1 tumor cells with lentiviruses expressing MELK shRNA or control shRNA followed by transplantation into cleared mammary fat pads. We observed a significant decrease in the number of tumors formed when the tumor cells were transduced with MELK shRNA virus as compared with
control shRNA (Table 1C). Statistically, the tumor initiation frequency was decreased \( \sim 4 \)-fold from 1/72 to 1/294 (95% CI, 1/791–1/109 to 1/174–1/30, \( \chi^2 = 4.6, P = 0.032 \)).

Nevertheless, tumors arose from shRNA MELK–transduced cells, suggesting that some cells had escaped the action of the shRNA MELK lentivirus. To investigate this, we undertook additional experiments using lentiviral vectors coexpressing mCherry in addition to the shRNAs. This permitted the evaluation of the efficiency of viral transduction and the fate of the tumors cells through evaluation of mCherry expression. Similar to the earlier experiments, we observed a significant reduction in the number of tumors arising from MELK shRNA–transduced MMTV-Wnt1 cells as compared with the control vector (Table 1D). The tumor initiation frequency was decreased \( \sim 6 \)-fold from 1/75 to 1/428 (95% CI, 1/878–1/209 to 1/140–1/40, \( \chi^2 = 14.2, P = 0.0001 \)). Moreover, the tumors emerging from MELK shRNA–transduced cells contained significantly fewer cells with mCherry expression as compared with control lentivirus expressing scrambled siRNA and mCherry (unpaired

**Table 1. Role of MELK in Wnt1 tumor initiation**

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NOTE: A, WMG49 tumor; \( \chi^2 = 7.53, P = 0.006 \). B, WMG300 tumor; \( \chi^2 = 10, P = 0.0013 \). C, WMG300 tumor, transduced with MELK shRNA or control shRNA (\( \chi^2 = 4.6, P = 0.032 \)). D, same as in C, but MELK shRNA virus that coexpresses mCherry instead of GFP was used (\( \chi^2 = 14.2, P = 0.0001 \)).

*The 1,000-cell point was censored from the calculation to satisfy the goodness of fit to a single-hit Poisson model.
In fact, we have previously documented MELK expression in the progenitor rather than the stem cell compartment in neural tissue, where MELK is required in neural progenitors both during development and in adult life (31). In addition, MELK is expressed in human colon cancer cell lines and in the middle and basal parts of normal colonic crypt epithelia, which is consistent with MELK expression in the proliferative progenitor compartment (26). Similarly, MELK expression in the testis is mainly localized to α6+ c-kit+ progenitor cells directly contacting the basal membrane (13). The high proliferation activity of progenitors compared with more quiescent stem cells is consistent with the suggested involvement of MELK in the cell cycle, particularly in the G2-M phase (22, 42).

Some cancer targets may be expressed and be functionally important in the normal stem cells of a tissue (e.g., Bmi1 is expressed in leukemia and normal hematopoietic stem cells; ref. 43). Targeting genes that are commonly expressed in the tumor and normal stem cells may eliminate normal stem cells, resulting in tissue collapse. On the other hand, if the tumor target is expressed in progenitor cells, then the aggressive targeting will likely eliminate both tumor cells and normal progenitors. The advantage of such a target is that the stem cell compartment is preserved, allowing progenitor division and tissue regeneration after therapy withdrawal. In this respect, MELK could be uniquely positioned, representing a promising therapeutic target that is preferentially expressed in progenitors.

The expression of MELK in both normal progenitors and tumor-initiating cells may reflect the cell cycle regulation of MELK (26, 33). The MMTV-Wnt1 oncogene results in an expanded stem/progenitor cell compartment before solid tumors arise because of secondary mutations such activated Ras (44). However, not all proliferating cells express MELK. For example, nontransformed proliferating cells such as IMR90 and WI-38 human fibroblasts do not express detectable

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3 A.V. Terskikh, unpublished observations.
levels of MELK (26). As expected, IMR90 cells continue to proliferate after transduction with MELK shRNA.~

The frequency of tumorigenic cells in MMTV-Wnt1 tumors has been reported to be from 1/177 (38) to 1/7,496 cells (8). The variability of cancer stem cell abundance may be due to technical issues of transplant location, the use of Matrigel, and the requirement for a secondary oncogenic event, such as mutated Ras to convert the MMTV-Wnt1–induced ductal hyperplasia to solid tumors. Thus, each tumor is likely to be genetically distinct and therefore have a variable abundance of tumor-initiating cells (44, 45). It is difficult to judge the utility of a given marker to enrich for tumor-initiating cells between different MMTV-Wnt1 tumors because of the expected oncogenic heterogeneity. However, within the cells of an individual tumor, the GFPhigh fraction of cells was enriched in tumor-initiating cells 5- to 10-fold greater than all other fractions (i.e., low and medium) in a 3D Matrigel tumorsphere-forming assay. Previously, using primary pleural effusions from breast cancer patients, the tumorsphere-forming capacity was directly correlated with in vivo tumorigenicity on transplantation into severe combined immunodeficient mice (46). In vivo, the GFPhigh cells were enriched 6- to 20-fold in tumor-initiating cells compared with GFPlow cells from the same tumor. However, the GFPhigh fraction was heterogeneous, as reflected by the presence of both K8- and K14-positive cells in the GFPhigh fraction. It will be important to determine if the tumor-initiating cells reside in the K8-positive or K14-positive fraction or in cells that express both keratins.

The CD61/β3 integrin is a suggested marker of mammary luminal progenitor cells and cancer stem cells in MMTV-Wnt1 mammary tumors (38). GFPhigh cells express higher levels of CD61 than GFPlow cells. However, a low-tumorigenicity AFPnegative fraction also expresses CD61, suggesting that GFPhigh cells contain only a fraction of CD61+ cells. Our enrichment for tumorsogenic cells based on MELK-GFP seems to be comparable or higher than the use of CD61 or the combination of CD24 and Thy1 (8).

The expression analysis of human breast cancers identified hundreds of gene candidates (18). Here, we provide evidence that MELK is a promising target for therapeutic intervention in breast cancer, particularly due to its expression in tumor-initiating cells. Given the success of small-molecule inhibitors of kinase catalytic activity for other cancer types (e.g., Gleevec, Iressa, Tarceva), the catalytic domain of MELK is an intriguing candidate target for drug development.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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