Novel measurements of mammary stem cells in human umbilical cord blood as prospective predictors of breast cancer susceptibility in later life

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Background: The size of the breast stem-cell pool could underlie the intrauterine roots of breast cancer. We studied whether breast stem cells exist in umbilical cord blood and if they correlate with hematopoietic stem-cell measurements that have been positively associated with perinatal risk factors for breast cancer.

Subjects and methods: We isolated mononuclear cells from umbilical cord blood of 170 singleton full-term pregnancies and determined, by reverse transcription polymerase chain reaction, the presence of genes of putative breast epithelial stem-cell/progenitor markers [including epithelial cell adhesion molecule (EpCAM), CD49f (α6-integrin), CD117 (c-kit receptor), CD24, and CD29 (β1-integrin)]. By immunocytochemistry, we colocalized protein expressions of EpCAM$^+$CD49f$^+$, CD49f$^+$CD24$^+$, and CD24$^+$CD29$^+$. We correlated concentrations of putative breast stem-cell/progenitor subpopulations, quantified by flow cytometry, with concentrations of hematopoietic stem cells.

Results: Mammary stem-cell phenotypes were identified in umbilical cord blood. The measured EpCAM$^+$ subpopulation was positively correlated with concentrations of CD34$^+$ and CD34$^+$CD38$^-$ hematopoietic stem cells (both $P = 0.006$). Additionally, EpCAM$^+$CD49f$^+$ and CD49f$^+$CD24$^+$ subpopulations were positively correlated to the CD34$^+$ cells ($P = 0.03$ and 0.008, respectively).

Conclusion: The positive association between measurable breast and hematopoietic stem cells in human umbilical cord blood suggests plausible mechanisms for a prenatal influence on breast cancer risk.

Key words: epithelial cell adhesion molecule, flow cytometry, hematopoietic stem cell, integrins, in utero environment, prenatal origin

introduction

The hypothesis that a woman's risk for breast cancer in the adult life is influenced already in the *in utero* environment [1] has implicated a role of mitogens and stem cells [2, 3]. In a large cohort of normal singleton pregnancies, we showed that the concentration of hematopoietic stem cells in umbilical cord blood is positively correlated with perinatal levels of insulin-like growth factor-1 (IGF-1) and estrogens [4, 5] and with birth weight [6], an indicator of adult life breast cancer risk [3, 7, 8]. These results are consistent with a 'stem-cell burden and

susceptibility' hypothesis which predicts that levels of mitogens increase the number of stem cells ('burden'), and that such stem cells are targets for genetic and/or epigenetic alterations ('susceptibility') that might lead to malignant transformation [3, 9, 10]. In previous studies [4–6], we used hematopoietic stem cells (defined by the CD34$^+$, CD34$^+$CD38$^-$, and CD34$^+$CD117$^+$ cell surface markers) as a surrogate for the overall stem-cell levels. However, levels of epithelial breast stem or progenitor cells in the *in utero* environment would be a more biologically relevant indicator for future breast cancer risk.

Although umbilical cord blood contains hematopoietic [11] and endothelial [12, 13] stem/progenitor cells, it is challenging to assume that organ-specific breast stem cells are present in an *in utero* compartment far removed from the organ of interest. However, umbilical cord blood mononuclear cells (MNC) express embryonic [14] and neural [15, 16] stem-cell markers. Additionally, umbilical cord blood stem cells, possibly

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including mesenchymal stem cells, can be differentiated to
other cell types, such as osteoblasts, chondroblasts, and
adipocytes, indicating their pluripotent potential [17, 18].
Mammary phenotypes have however not been reported in
human umbilical cord blood.

Here, we analyzed MNC derived from human umbilical cord
blood for gene expressions of markers reported for putative
breast epithelial stem cells and progenitors [19] and quantified
such cell populations by flow cytometry. We report the
existence of putative breast stem/progenitor cell phenotypes in
umbilical cord blood and, more importantly, that the
concentrations of certain breast stem/progenitor cell
phenotypes found in umbilical cord blood correlate positively
with those of hematopoietic stem cells.

subjects and methods

The study protocol was approved by the institutional review boards of the
University of Massachusetts Medical School, Worcester, MA and Tufts
Medical Center, Boston, MA.

subject recruitment and umbilical cord blood processing
Study subjects were recruited from November 2006 to November 2010
among pregnant women who delivered at the Tufts Medical Center, who
were ≥18 years and human immunodeficiency virus and hepatitis B
negative, with a fetus free of anomalies by ultrasound examination. For this
analysis, we included only full-term (gestational age ≥37 weeks),
normotensive, and singleton pregnancies. Infants were delivered according
to standard obstetric practices. Umbilical cord blood was collected from the
umbilical vein into a sterile bag containing 35 ml of citrate phosphate
dextrose anticoagulant (Fenwal, Lake Zurich, IL). Samples were processed
for MNC using a Ficoll-Paque (STEMCELL Technologies, Vancouver,
Canada) density gradient within 24 h of birth as described previously [5, 6].

RT-PCR
PolyA$^+$ messenger RNA (mRNA) was isolated from MNC using the
QuickPrep Micro mRNA Purification Kit (Amersham Biosciences,
Piscataway, NJ) and reversed transcribed to complementary DNA (cDNA)
using the Superscript III First-Strand Synthesis System (Invitrogen,
Carlsbad, CA), according to manufacturer's instructions. Genes of
interest were detected using a set of two specific forward and reverse
primers for each gene by PCR: epithelial cell adhesion molecule
(EpCAM), forward 5′-TTGGTGATGAAGGCAGAAATGAATGG-3′
and reverse 5′-TGAACTAAAACACAAAGCAAGAGAAAAACCT-3′
giving a PCR product of 268 bp; CD49f (α6-integrin) [20], forward 5′-
CAAGATGGCTACCCAGATAT-3′ and reverse 5′-
CTGAATCTGAGAGGGAACCA-3′ giving a PCR product of 210 bp;
CD117 (c-kit receptor) [21], forward 5′-AACGACACGCTGGTCCGCTG-
3′ and reverse 5′-GTACACAGAACTAGACACATC-3′ giving a PCR
product of 341 bp; CD24 [22], forward 5′-TGCTCCTACCCACGCAGATT-
3′ and reverse 5′-GGCCAACCCAGAGTTGGAA-3′ giving a PCR product
of 88 bp; CD29 (β1-integrin) [23], forward 5′-
GTTACACGGCTGCTGGTCTT-3′ and reverse 5′-
CTACTGCTGACTTAGGGATC-3′ giving a PCR product of 264 bp; and
cyclophilin [24], forward 5′-CCACCGTGTTCTTCGACATC-3′ and reverse
5′-GGTCCAGCATTTGCCATGG-3′ giving a PCR product of 302 bp. PCR
was carried out using 0.4 μM of each primer, 200 μM deoxyribonucleotide
triphosphate, 1.5–3.0 mM MgCl$_2$, 5 U AmpliTaq DNA polymerase (Applied
Biosystems, Foster City, CA) and ~2 μg template cDNA in a 1X PCR buffer
(50 mM KCl, 10 mM Tris-HCl pH 8.3, 0.001% gelatin). Amplifications
were carried out in a thermocycler using 41 cycles of 95°C for 30 s, 55–60°C

for 30 s, 72°C for 1 min, followed by a final extension at 72°C for 10 min.
Amplification products were visualized under ultraviolet light after gel
electrophoresis on a 2% agarose gel and staining with GelGreen Nucleic
Acid Gel Stain (Botium, Hayward, CA) or ethidium bromide.

immunocytochemistry and confocal microscopy
Umbilical cord blood-derived MNC were spread and dried on to glass
slides. The cells were fixed in 4% paraformaldehyde for 10 min at room
temperature. After washing in 1X phosphate-buffered saline (PBS), the cells
were blocked with 10% donkey serum (Millipore, Billerica, MA) in PBS for
30 min at room temperature, followed by incubation in the following
primary antibodies in blocking solution overnight at 4°C: mouse anti-
EpCAM (Clone E144, 1 : 100 dilution; Abcam, Cambridge, MA) and rat
anti-CD49f (Clone NKI-GoH3, 1 : 200 dilution; Millipore); rat anti-CD49f
and mouse anti-CD24 (Clone SN3, 1:200 dilution; Millipore); and rabbit
anti-CD29 (Clone EP1041Y, 1 : 200 dilution; Millipore) and mouse anti-
CD24. The cells were then washed and incubated with the appropriate
secondary antibodies for 1 h at room temperature in the dark: Alexa Fluor
488 donkey antimouse, Alexa Fluor 488 donkey antirabbit, Alexa Fluor 594
donkey antirat, and/or Alexa Fluor 568 donkey antirabbit (all 1 : 200
dilution; Invitrogen/Molecular Probes, Eugene, OR). After washing, the
cells were stained in DRAQ5®, 1,5-bis{[2-(di-methylamino)ethyl]amino}-4,
8-dihydroxyanthracene-9,10-dione, (Cell Signaling Technology, Danvers,
MA) and coverslipped in Prolong anti fade reagent (Invitrogen/Molecular
Probes). Primary antibodies were omitted for negative controls. Cellular
colocalizations were examined with a True Confocal Scanning
Spectrophotometer microscope (Leica Microsystems, Deerfield, IL)
using excitation wavelengths 488 nm for Alexa Fluor 488, 568 nm for Alexa
Fluor 568 and Alexa Fluor 594, and 633 nm for DRAQ5. Optical
scanning was carried out every 0.5 μm of cell thickness by a sequential
scanning method.

flow cytometric analyses
Flow cytometric analyses were carried out as described previously [5].
Briefly, 1×10^6 umbilical cord blood-derived MNC were incubated for 30
min on ice in the dark with the following fluorochrome-conjugated
antibodies: anti-CD34 fluorescein isothiocyanate (FITC; Clone 581; BD
BioSciences Pharmingen, San Diego, CA), anti-CD38 phycoerythrin (PE;
Clone HIT2; BD Biosciences Pharmingen), anti-EpCAM FITC (Clone VU-
1D9; STEMCELL Technologies, Vancouver, Canada), anti-CD49f PE
(Clone GoH3; BD BioSciences Pharmingen), anti-CD117 allophycocyanin
(APC; Clone YB5.B8; BD BioSciences Pharmingen), anti-CD24 FITC
(Clone SN3; Antibodies-online, Aachen, Germany), anti-CD29 APC (Clone
MAR4; BD BioSciences Pharmingen), or the combination of anti-CD34
FITC and anti-CD38 PE, or the combination of anti-EpCAM FITC, anti-
CD49f PE, and anti-CD117 APC, or the combination of anti-CD24 FITC,
anti-CD49f PE, and anti-CD29 APC. Samples treated with no antibody
served as negative controls. Cells were washed, fixed with 4%
paraformaldehyde, and analyzed using a FACSCalibur flow cytometer (BD
Biosciences Immunocytometry Systems, San Jose, CA). Hematopoietic
(CD34$^+$, CD34$^+$CD38$^-$) and putative breast (EpCAM$^+$, EpCAM$^+$CD49f$^+$,
EpCAM$^+$CD49f$^+$CD117$^+$, CD49f$^+$CD24$^+$, CD24$^+$CD29$^+$,
CD49f$^+$CD24$^+$CD29$^+$) stem/progenitor cell subpopulations were quantified
from the gated MNC population (lymphocytes and monocytes based on
forward versus side light scatter) using the FlowJo software program (Tree
Star, Ashland, OR). The number of cells in these populations was
normalized to 10^3 MNC.

statistical analysis
Descriptive statistics on the characteristics of study population and
laboratory data were summarized. Spearman's rank correlation coefficients
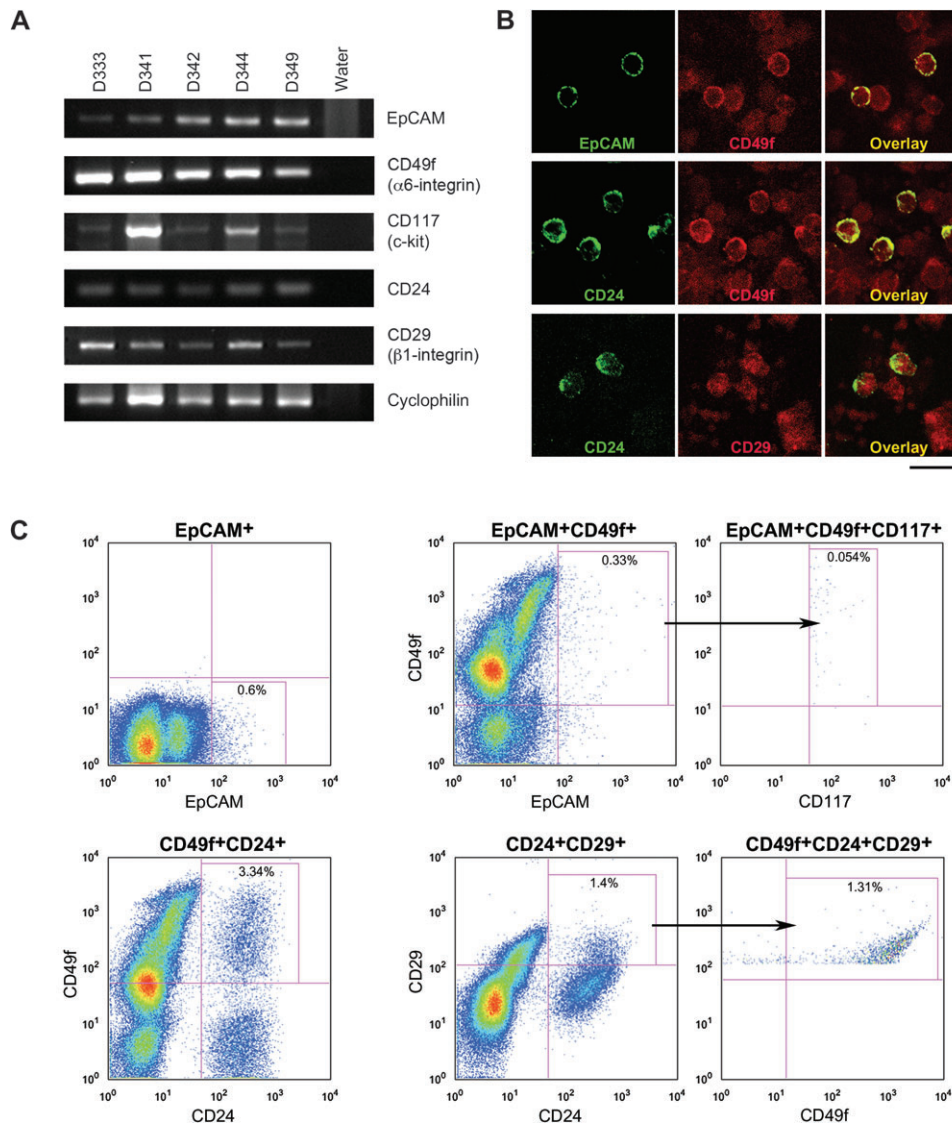were estimated for bivariate analyses between levels of different stem-cell

subpopulations. Statistical significance was set at 0.05 (two sided). STATA version 11 (StataCorp LP, College Station, TX) was used to conduct statistical analyses.

results

To determine whether putative breast stem/progenitor cell phenotypes were present in umbilical cord blood, we analyzed genes reported for breast stem-cell markers [19] in the MNC fraction of umbilical cord blood by RT-PCR. Because breast stem cells are considered epithelial in nature [3], we first detected the gene for EpCAM or epithelial-specific antigen (ESA), as a marker

for epithelial cells [25]. Additionally, genes of putative markers for breast stem/progenitor cells, i.e. CD49f (α6-integrin), CD117 (c-kit receptor), CD24, and CD29 (β1-integrin) were detected in umbilical cord blood-derived MNC (Figure 1A).

Second, we determined protein expressions by immunocytochemistry and observed colocalized staining of EpCAM+CD49f+, CD49f+CD24+, and CD24+CD29+ surface markers in umbilical cord blood-derived MNC by confocal microscopic analyses (Figure 1B). We further quantified the percentages of umbilical cord blood-derived MNC with putative markers of the different breast stem-cell

Figure 1. (A) Gel electrophoresis showing the detection of PCR products for epithelial cell adhesion molecule (EpCAM), CD49f (α6-integrin), CD117 (c-kit receptor), CD24, CD29 (β1-integrin), and the housekeeping gene cyclophilin from umbilical cord blood-derived mononuclear cells (MNC) from five umbilical cord blood samples (D333, D341, D342, D344, and D349). Water (last lane) was used as negative controls. (B) Double-labeled immunofluorescent confocal microscopy of umbilical cord blood-derived MNC from sample D321 showing colocalization in the overlay image of EpCAM (green) and CD49f (red) (top panel); CD24 (green) and CD49f (red) (middle panel); and CD24 (green) and CD29 (red) (bottom panel). Scale bar represents 20 μm. (C) Flow cytometric pseudocolor plots showing the detection of the EpCAM+, EpCAM+CD49f+, EpCAM+CD49f+CD117+, CD49f+CD24+, CD24+CD29+, and CD49f+CD24+CD29+ subpopulations (boxed, with percentage of cells indicated) from umbilical cord blood-derived MNC of sample N41. The arrows indicate that the triple positive population was derived from the double positive population as shown. The markers shown in the top panel have been reported in humans while the markers shown in the bottom panel have been reported in mice.

subpopulations (EpCAM$^+$CD49f$^+$, EpCAM$^+$CD49f$^+$CD117$^+$, CD49f$^+$CD24$^+$, CD24$^+$CD29$^+$, and CD49f$^+$CD24$^+$CD29$^+$) in addition to EpCAM by flow cytometry (Figure 1C). Data analyses using the FlowJo software program showed that the EpCAM$^+$ subpopulation ranged from 0.19 to 19.8 cells/1000 MNC with a mean of 3.4 ± 4.0 cells; the EpCAM$^+$CD49f$^+$ subpopulation ranged from 0.049 to 9.7 cells/1000 MNC with a mean of 1.7 ± 1.8 cells; the EpCAM$^+$CD49f$^+$CD117$^+$ subpopulation ranged from 0.02 to 2.4 cells/1000 MNC with a mean of 0.48 ± 0.56 cells; the CD49f$^+$CD24$^+$ subpopulation ranged from 0 to 48.1 cells/1000 MNC with a mean of 14.7 ± 12.9 cells; the CD24$^+$CD29$^+$ subpopulation ranged from 0.11 to 46.2 cells/1000 MNC with a mean of 10.3 ± 9.8 cells; and the CD49f$^+$CD24$^+$CD29$^+$ subpopulation ranged from 0 to 44.4 cells/1000 MNC with a mean of 8.3 ± 8.8 cells (Table 1).

We also quantified the percentages of umbilical cord blood-derived MNC with hematopoietic stem-cell markers, i.e. CD34$^+$ and CD34$^+$CD38$^-$, and carried out a rank correlation analysis between concentrations of hematopoietic and breast stem/progenitor cell subpopulations. Levels of the EpCAM$^+$ subpopulation were positively correlated with concentrations of CD34$^+$ and CD34$^+$CD38$^-$ hematopoietic stem cells (both P = 0.006; Table 2). Except for the CD24$^+$CD29$^+$ cells, all putative

breast stem/progenitor cell subpopulations were positively associated with the hematopoietic stem-cell subpopulations. Notably, the EpCAM$^+$CD49f$^+$ and CD49f$^+$CD24$^+$ subpopulations were positively and significantly correlated to the CD34$^+$ cells (P = 0.03 and 0.008, respectively). These associations were clearer among female than among male newborns, in particular, the EpCAM$^+$ subpopulation (Table 2).

discussion

To our knowledge, this is the first report of measurable breast stem cells in human umbilical cord blood. Levels of these breast stem cells correlated significantly and positively with that of hematopoietic stem cells; concentrations of hematopoietic stem cells (as surrogate measurements of overall stem-cell levels in the intrauterine environment) are correlated with umbilical cord blood plasma levels of IGF-1 and with birth weight [4–6].

Because the procurement of human cord blood samples is unpredictable, we chose to assay for putative breast stem/progenitor cells by analyzing published surface markers instead of using live cell-based methods employing dyes such as Hoechst 33342 (e.g. Invitrogen/Molecular Probes) or ALDEFLUOR® (STEMCELL Technologies) [26, 27]. Since all cells within the mammary epithelium—except for the myoepithelial cells—express EpCAM, or ESA [28, 29] and breast stem cells are considered to be epithelial in nature [3, 30], we initially examined and found the gene for EpCAM—a marker of many epithelial cells [25]—in the MNC of umbilical cord blood. In fresh, uncultured umbilical cord blood-derived MNC, we also detected other putative genes related to breast stem/progenitor cells; these include CD49f (α6-integrin), CD117 (c-kit receptor), CD24, and CD29 (β1-integrin), the latter two markers originally reported in mouse tissues [19, 31–33].

By immunostaining umbilical cord blood-derived MNC, we demonstrated the coexpression of EpCAM and CD49f (α6-integrin), a major cellular phenotype of human breast stem/progenitor cells [28, 34, 35]. The coexpressions of the proteins for CD24 and CD49f, and CD24 and CD29, indicate that mouse and human do share common markers of putative breast stem/progenitor cells.

The detection of putative breast stem/progenitor cell phenotypes suggests that there is a 'mammary' compartment within the umbilical cord blood. These putative breast stem/progenitor cell phenotypes in the umbilical cord blood niche could be rare but detectable by flow cytometry (Figure 1C, Table 1). Our results indicate that, at the time of birth, intrauterine conditions may sustain certain subpopulations of breast stem/progenitor cell phenotypes. It is not clear, however, how these breast phenotypes come about. Possibly, umbilical cord blood cells with breast phenotypes were derived from embryonic-like stem cells [14, 36–40] due to exposures to specific hormones or growth factors *in utero*, or dedifferentiated from hematopoietic stem cells as a result of epigenetic reprogramming, as had been proposed for the neural phenotypes found in umbilical cord blood [41]. These mammary phenotypes might also be differentiated from

Table 1. Maternal and newborn characteristics and umbilical cord blood stem/progenitor cell counts

Subject characteristics	N	Mean ± standard deviation or %	Range
Mother's age (years)	169	30.1 ± 6.2	19–44
Parity			
First	52	30.8	
Second	49	29.0	
Third	24	14.2	
Fourth and above	44	26.0	
Gestation duration (weeks)	170	39.2 ± 1.2	37–41
Newborn gender			
Male	84	49.4	
Female	86	50.6	
Newborn birth weight (g)	170	3,363.7 ± 510.5	1,973–4,917
Umbilical cord blood volume (ml)[a]	169	98.4 ± 29.4	45–216
Umbilical cord blood stem/progenitor cell populations[b]			
CD34$^+$	169	8.5 ± 7.4	0.0–56.7
CD34$^+$CD38$^-$	167	2.4 ± 2.5	0.0–23.8
EpCAM$^+$	112	3.4 ± 4.0	0.19–19.8
EpCAM$^+$CD49f$^+$	109	1.7 ± 1.8	0.049–9.7
EpCAM$^+$CD49f$^+$CD117$^+$	32	0.48 ± 0.56	0.02–2.4
CD49f$^+$CD24$^+$	56	14.7 ± 12.9	0.0–48.1
CD24$^+$CD29$^+$	55	10.3 ± 9.8	0.11–46.2
CD49f$^+$CD24$^+$CD29$^+$	55	8.3 ± 8.8	0.0–44.4

[a]Includes 35 ml of citrate phosphate dextrose anticoagulant.
[b]Cell counts per 10^3 mononuclear cells.
EpCAM, epithelial cell adhesion molecule.

Table 2. Spearman's correlation coefficients (P values in parentheses) between umbilical cord blood hematopoietic and breast stem/progenitor cell populations

Hematopoietic stem-cell subpopulations	Breast stem/progenitor cell subpopulations					
	EpCAM$^+$	EpCAM$^+$CD49f$^+$	EpCAM$^+$CD49f$^+$CD117$^+$	CD49f$^+$CD24$^+$	CD24$^+$CD29$^+$	CD49f$^+$CD24$^+$CD29$^+$
All subjects (N)	112	109	32	56	55	55
CD34$^+$	0.26 (**0.006**)	0.21 (**0.03**)	0.24 (0.20)	0.35 (**0.008**)	0.14 (0.32)	0.17 (0.21)
CD34$^+$CD38$^-$	0.26 (**0.006**)	0.15 (0.12)	0.30 (0.09)	0.24 (0.07)	0.01 (0.97)	0.18 (0.19)
Newborn gender						
Males (N)	53	53	14	25	25	25
CD34$^+$	0.23 (0.37)	0.22 (0.12)	0.41 (0.15)	0.33 (0.11)	0.07 (0.76)	0.28 (0.18)
CD34$^+$CD38$^-$	0.02 (0.90)	0.04 (0.77)	0.27 (0.36)	0.38 (0.06)	0.09 (0.67)	0.36 (0.08)
Females (N)	59	56	18	31	30	30
CD34$^+$	0.36 (**0.005**)	0.18 (0.19)	0.03 (0.90)	0.35 (**0.05**)	0.18 (0.34)	0.05 (0.78)
CD34$^+$CD38$^-$	0.37 (**0.004**)	0.19 (0.17)	0.18 (0.48)	0.19 (0.29)	-0.03 (0.88)	0.08 (0.68)

EpCAM, epithelial cell adhesion molecule. Statistically significant P values ($P < 0.05$) are shown in boldface.

multipotent mesenchymal stem cells present in umbilical cord blood [18].

The concentration of umbilical cord blood-derived MNC carrying the epithelial EpCAM antigen was positively associated with the concentrations of hematopoietic stem-cell populations identified by the CD34 surface marker [42, 43] and the subpopulation that was positive for CD34 but negative for CD38 [44]. Notably, the EpCAM$^+$CD49f$^+$ and CD49f$^+$CD24$^+$ breast stem/progenitor cell phenotypes showed a positive correlation with the CD34$^+$ hematopoietic stem cells. This finding supports the 'stem-cell burden' hypothesis [3] in predicting breast cancer risk. Nonsignificant associations might be due to the limited sample sizes for markers reported more recently and thus incorporated in later stages of our study. The inconsistent associations observed for the strictly 'mouse' CD24$^+$CD29$^+$ subpopulation suggest that such cells might not be functional mammary phenotypes in humans, although the CD49f$^+$CD24$^+$ subpopulation was originally reported also in mice [32]. These correlations should be reexamined in larger sample sizes, as should some of the significant associations observed in the analyses stratified by gender.

In summary, early-life exposures have been linked to risk of breast cancer in the offspring through a pathway hypothesized to involve the mammary stem-cell pool. The 'breast stem-cell burden and susceptibility' hypothesis is based on the assumption that breast cancer originates from mammary stem cells [3, 9, 10, 45]. The greater the number of breast stem cells, the higher the likelihood that one of these cells will undergo malignant transformation. Because mammary stem cells arise primarily during the fetal/perinatal period, the *in utero* environment becomes a major determinant of their number. Hence, a breast 'stem-cell potential'—a term proposed for measurable variables that reflect the effects of intrauterine and perinatal influences on stem-cell burden and susceptibility—in umbilical cord blood might predict subsequent risk of breast cancer in the adult life. Future research will determine whether these putative populations with breast stem/progenitor phenotypes in umbilical cord blood are indeed functional mammary cells. If so, we will have a model system to understand whether genetic and/or epigenetic alterations in breast stem/progenitor cells explain fetal programming of breast cancer risk in the adult life.

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disclosure

The authors declare no conflict of interest.

references

1. Trichopoulos D. Hypothesis: does breast cancer originate in utero? Lancet 1990; 335: 939–940.
2. Baik I, Becker PS, DeVito WJ et al. Stem cells and prenatal origin of breast cancer. Cancer Causes Control 2004; 15: 517–530.
3. Savarese TM, Low HP, Baik I et al. Normal breast stem cells, malignant breast stem cells and the perinatal origin of breast cancer. Stem Cell Rev 2006; 2: 103–110.
4. Baik I, DeVito WJ, Ballen K et al. Association of fetal hormone levels with stem cell potential: evidence for early life roots of human cancer. Cancer Res 2005; 65: 358–363.
5. Savarese TM, Strohsnitter WC, Low HP et al. Correlation of umbilical cord blood hormones and growth factors with stem cell potential: implications for the prenatal origin of breast cancer hypothesis. Breast Cancer Res 2007; 9: R29.
6. Strohsnitter WC, Savarese TM, Low HP et al. Correlation of umbilical cord blood haematopoietic stem and progenitor cell levels with birth weight: implications for prenatal influence on cancer risk. Br J Cancer 2008; 98: 660–663.
7. Michels KB, Xue F. Role of birthweight in the etiology of breast cancer. Int J Cancer 2006; 119: 1007–1025.

8. Park SK, Kang D, McGlynn KA et al. Intrauterine environments and breast cancer risk: meta-analysis and systematic review. Breast Cancer Res 2008; 10: R8.

9. Trichopoulos D, Lagiou P, Adami HO. Towards an integrated model for breast cancer etiology: the crucial role of the number of mammary tissue-specific stem cells. Breast Cancer Res 2005; 7: 13–17.

10. Trichopoulos D, Adami HO, Ekbom A et al. Early life events and conditions and breast cancer risk: from epidemiology to etiology. Int J Cancer 2008; 122: 481–485.

11. Mayani H, Lansdorp PM. Biology of human umbilical cord blood-derived hematopoietic stem/progenitor cells. Stem Cells 1998; 16: 153–165.

12. Hill JM, Zalos G, Halcox JPJ et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 2003; 348: 593–600.

13. Ingram DA, Mead LE, Tanaka H et al. Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. Blood 2004; 104: 2752–2760.

14. Zuba-Surma EK, Klich I, Greco N et al. Optimization of isolation and further characterization of umbilical cord blood-derived very small embryonic/epiblast-like stem cells (VSELs). Eur J Haematol 2009; 84: 34–46.

15. Ha Y, Lee JE, Kim KN et al. Intermediate filament nestin expressions in human cord blood monocytes (HCMNCs). Acta Neurochir 2003; 145: 483–487.

16. Zangiacomi V, Balon N, Maddens S et al. Human cord blood-derived hematopoietic and neural-like stem/progenitor cells are attracted by the neurotransmitter GABA. Stem Cells Dev 2009; 18: 1369–1377.

17. Kögler G, Sensken S, Airey JA et al. A new human somatic stem cell from placental cord blood with intrinsic pluripotent differentiation potential. J Exp Med 2004; 200: 123–135.

18. Wagner W, Wein F, Seckinger A et al. Comparative characteristics of mesenchymal stem cells from human bone marrow, adipose tissue, and umbilical cord blood. Exp Hematol 2005; 33: 1402–1416.

19. Visvader JE. Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. Genes Dev 2009; 23: 2563–2577.

20. Gaudreault M, Vigneault F, Gingras ME et al. Transcriptional regulation of the human α6 integrin gene by the transcription factor NFI during corneal wound healing. Invest Ophthalmol Vis Sci 2008; 49: 3758–3767.

21. Rassidakis GZ, Georgakis GV, Oyarzo M et al. Lack of c-kit (CD117) expression in CD30+ lymphomas and lymphomatoid papulosis. Mod Pathol 2004; 17: 946–953.

22. Kaipparettu BA, Malik S, Konduri SD et al. Estrogen-mediated downregulation of CD24 in breast cancer cells. Int J Cancer 2008; 123: 66–72.

23. Bouchet BY, Colón M, Polotsky A et al. Beta-1 integrin expression by human nasal chondrocytes in microcarrier spinner culture. J Biomed Mater Res 2000; 52: 716–724.

24. Mayer-Proschel M, Kalyani AJ, Mujtaba T, Rao MS. Isolation of lineage-restricted neuronal precursors from multipotent neuroepithelial stem cells. Neuron 1997; 19: 773–785.

25. Litvinov SV, Velders MP, Bakker HAM et al. Ep-CAM: a human epithelial antigen is a homophilic cell-cell adhesion molecule. J Cell Biol 1994; 125: 437–446.

26. Alvi AJ, Clayton H, Joshi C et al. Functional and molecular characterization of mammary side population cells. Breast Cancer Res 2003; 5: R1–R8.

27. Ginestier C, Hur MH, Charafe-Jauffret E 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: 555–567.

28. Stingl J, Eaves CJ, Zandieh I, Emerman JT. Characterizaton of bipotent mammary epithelial progenitor cells in normal adult human breast tissue. Breast Cancer Res Treat 2001; 67: 93–109.

29. Gudjonsson T, Villadsen R, Nielsen HL et al. Isolation, immortalization, and characterization of a human breast epithelial cell line with stem cell properties. Genes Dev 2002; 16: 693–706.

30. Smith GH, Chepko G. Mammary epithelial stem cells. Microsc Res Tech 2001; 52: 190–203.

31. Shackleton M, Vaillant F, Simpson KJ et al. Generation of a functional mammary gland from a single stem cell. Nature 2006; 439: 84–88.

32. Stingl J, Eirew P, Ricketson I et al. Purification and unique properties of mammary epithelial stem cells. Nature 2006; 439: 993–997.

33. Visvader JE, Lindeman GJ. Mammary stem cells and mammopoiesis. Cancer Res 2006; 66: 9798–9801.

34. Stingl J, Raouf A, Eirew P, Eaves CJ. Deciphering the mammary epithelial cell hierarchy. Cell Cycle 2006; 5: 1519–1522.

35. Villadsen R, Fridriksdottir AJ, Rønnov-Jessen L et al. Evidence for a stem cell hierarchy in the adult human breast. J Cell Biol 2007; 177: 87–101.

36. Baal N, Reisinger K, Jahr H et al. Expression of transcription factor Oct-4 and other embryonic genes in CD133 positive cells from human umbilical cord blood. Thromb Haemost 2004; 92: 767–775.

37. McGuckin CP, Forraz N, Baradez MO et al. Production of stem cells with embryonic characteristics from human umbilical cord blood. Cell Prolif 2005; 38: 245–255.

38. Habich A, Jurga M, Markiewicz I et al. Early appearance of stem/progenitor cells with neural-like characteristics in human cord blood mononuclear fraction cultured in vitro. Exp Hematol 2006; 34: 914–925.

39. Zhao Y, Wang H, Mazzone T. Identification of stem cells from human umbilical cord blood with embryonic and hematopoietic characteristics. Exp Cell Res 2006; 312: 2454–2464.

40. Halasa M, Baskiewicz-Masiuk M, Dabkowska E, Machalinski B. An efficient two-step method to purify very small embryonic-like (VSEL) stem cells from umbilical cord blood (UCB). Folia Histochem Cytobiol 2008; 46: 239–243.

41. Domanska-Janik K, Buzanska L, Lukomsja B. A novel, neural potential of non-hematopoietic human umbilical cord blood stem cells. Int J Dev Biol 2008; 52: 237–248.

42. Civin CI, Strauss LC, Brovall C et al. Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J Immunol 1984; 133: 157–165.

43. Sutherland DR, Stewart AK, Keating A. CD34 antigen: molecular features and potential clinical applications. Stem Cells 1993; 11: 50–57.

44. Xiao M, Dooley DC. Cellular and molecular aspects of human CD34$^+$CD38$^-$ precursors: analysis of a primitive hematopoietic population. Leuk Lymphoma 2000; 38: 489–497.

45. Ginestier C, Wicha MS. Mammary stem cell number as a determinate of breast cancer risk. Breast Cancer Res 2007; 9: 109–110.