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Circulating Colony Stimulating Factor-1 (CSF1) and Breast Cancer Risk

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Abstract

Colony stimulating factor-1 (CSF1) and its receptor (CSF1-R) are important in mammary gland development and have been implicated in breast carcinogenesis. In a nested case-control study in the Nurses' Heath Study of 726 breast cancer cases diagnosed between June 1, 1992, and June 1, 1998, and 734 matched controls, we prospectively evaluated whether circulating levels of CSF1 (assessed in 1989–1990) are associated with breast cancer risk. The association varied by menopausal status ($P_{heterogeneity}$=0.009). CSF1 levels in the highest quartile (versus lowest) were associated with an 85% reduced risk of premenopausal breast cancer (RR=0.15, 95%CI 0.03–0.85, P_{trend}=0.02). In contrast, CSF1 levels in the highest quartile conferred a 33% increased risk of postmenopausal breast cancer (RR=1.33, 95%CI 0.96–1.86; P_{trend} =0.11), with greatest risk for invasive (RR=1.45, 95%CI 1.02–2.07, P_{trend}=0.06) and ER+/PR+ tumors (RR=1.72, 95%CI 1.11–2.66; P_{trend} =0.04). Thus, the association of circulating CSF1 levels and breast cancer varies by menopausal status.

Keywords

Colony stimulating factor 1; breast cancer; CSF1

Introduction

Macrophage colony-stimulating factor (CSF1), originally identified as a hematopoietic growth factor, stimulates proliferation, differentiation, and survival of monocytes and macrophages (1). More recently, CSF1 and its receptor (CSF1R) have exhibited an important role in mammary gland development (2,3) and are implicated in breast and ovarian carcinogenesis (3). Many cell types, including epithelial and mesenchymal cells and osteoblasts, produce CSF1 (4). While expressed at low levels in normal resting breast epithelium (5), CSF1 is expressed at high levels in the mammary gland during pregnancy and lactation and in breast tumors (6–8).

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CSF1's proliferative effects are mediated by its binding to CSF1-R and the induction of signal transduction pathways. Autocrine CSF1R activation induces hyperproliferation and loss of basement membrane integrity in human mammary epithelial cells (9). Levels of circulating CSF1 are higher in patients with ovarian, breast, and endometrial cancer than in healthy individuals (8,10). CSF1 expression in breast tumors also correlates with grade and progression (3,8). No studies of prospectively measured levels of circulating CSF1 and subsequent risk of breast cancer have been published. Thus, it is unclear whether circulating CSF1 is a tumor marker for breast cancer or predictive of breast cancer risk. We conducted a study within the Nurses' Health Study to determine whether circulating levels of CSF1 predict breast cancer risk.

Materials and Methods

Study Population

The Nurses' Health Study began in 1976, when 121,700 US registered nurses age 30–55 returned a questionnaire. Information on body mass index (BMI), reproductive history, age at menopause, postmenopausal hormone (PMH) use, and diagnosis of cancer and other diseases is updated every two years through questionnaires. During 1989 and 1990, blood samples were collected from 32,826 women (11), with 99% follow-up through 1998.

In a nested case-control study among women who provided blood samples, we included 726 reporting breast cancer diagnosis between June 1, 1994 and June 1, 1998 and 734 matched controls with no cancer history. Median time from blood draw to breast cancer diagnosis was 5.5 years (interquartile range: 4–7.1 years). Breast cancer cases were confirmed by medical record review; estrogen receptor (ER) and progesterone receptor (PR) status were obtained from pathology reports. This study was approved by the Committee on the Use of Human Subjects in Research at Brigham and Women's Hospital.

CSF1 Measurements

CSF1 was measured by ELISA in Dr. Nader Rifai's laboratory at Children's Hospital, Boston, MA. The assay coefficient of variation was 8.2%. A reproducibility study among 51 participants who provided three blood samples over 3 years demonstrated that one blood measure is well correlated with longer-term measures (intraclass correlation coefficient=0.65, 95%CI 0.51–0.76). Using the extreme Studentized deviate Many-Outlier procedure to determine outlying CSF1 values (12), one control was excluded with a CSF1 value of 2654.1 pg/ml.

Statistical Analysis

We used conditional and unconditional logistic regression models to estimate the relative risk (RR) of breast cancer, with 95% confidence intervals (CI) adjusted for known risk factors. Quartiles of circulating CSF1 were based on the distribution among controls. In analyses stratified by menopausal status, categories were based on premenopausal and postmenopausal distributions separately. The simple conditional model was based on 725 matched case-control pairs with circulating CSF1 data. Multivariate models were adjusted for prior benign breast disease (yes/no), BMI at age 18 (continuous), weight gain (<5, 5−<20, 20+, missing), parity/age at first birth(nulliparous,1–2 children first birth le 24, 1–2 children parous first birth after 24, 3+ children first birth le 24, 3+ children parous first birth after 24), alcohol (none, <3, 3–6, 7–13 drinks/week), family history of breast cancer (yes/no), age at menarche (<12, 12, 13, >13), age at menopause (<=45, 46–50, 51+), duration of PMH use (premenopausal, never, past <60, past60+, current <60, current 60+). Menopausal status and use of postmenopausal hormones at blood collection were assessed through a supplemental questionnaire administered at that time. All other covariates were assessed from biennial questionnaires. Tests for trend

were based on the Wald test when the log transformed continuous measure of circulating CSF1 was included as an independent variable. Polytomous logistic regression (13) was used to test for differences in trend across CSF1 levels according to ER+/PR+ to ER−/PR− tumors. There were too few cases of ER−/PR+ breast cancers for these groups to be considered separately.

Results and Discussion

Mean circulating levels of CSF1 were similar for cases (584.7 pg/ml, range 163.1–2170.5 pg/ml) and controls (583.7 pg/ml, range 169.7–2078.7 pg/ml). Among controls, circulating CSF1 was positively associated with BMI and weight gain since age 18 and inversely associated with alcohol consumption (Table 1). Women with the highest levels of circulating CSF1 were more likely to be postmenopausal, have a family history of breast cancer, and be parous than women with lower levels.

Overall, circulating CSF1 levels and risk of breast cancer (P_{trend} =0.37; Table 2) were not associated. However, the association varied by menopausal status ($P_{heterogeneity}$ =0.009). Women with CSF1 levels in the highest (vs lowest) quartile had an 85% reduced risk of premenopausal breast cancer (RR=0.15, 95%CI 0.03–0.85, P_{trend} =0.02) and a contrasting nonsignificant 33% increased risk of postmenopausal breast cancer (RR=1.33, 95%CI 0.96–1.86; P_{trend} =0.11). The association between CSF1 and postmenopausal breast cancer appeared stronger when limited to invasive cancers (RR=1.45, 95%CI 1.02–2.07; P_{trend} =0.06; Table 3). CSF1 was associated with ER+/PR+ breast cancers (RR=1.72, 95%CI 1.11–2.66; Table 3), but not ER−/PR− tumors (RR=0.70, 95%CI 0.34–1.44; $P_{heterogeneity}$=0.03; Table 3).

Because we observed a strong positive association between circulating CSF1 with both weight gain since age 18 and current BMI (Table 1), and adiposity is a risk factor for breast cancer we also ran multivariate models adjusted for both weight gain since age 18 and current BMI (continuous). The results were essentially unchanged from those presented in Tables 2 and 3.

To understand the divergence of associations by menopausal status, we conducted stratified analyses among postmenopausal women with higher estrogen environments. However, the CSF1-breast cancer association was not modified by postmenopausal hormone use ($P_{heterogeneity}$=0.68) or BMI ($P_{heterogeneity}$=0.29).

To our knowledge, this is the first prospective study to examine circulating levels of CSF1 and subsequent risk of breast cancer. CSF1 was inversely related to premenopausal and positively associated with postmenopausal breast cancer. Although *a priori* we would not have predicted differential effects for CSF1 by menopausal status, the association of other exposures (e.g., BMI, circulating IGF1 levels) with breast cancer depends on menopausal status. The mechanism by which CSF1 may differentially influence premenopausal and postmenopausal breast cancer risk is unclear. Additional studies in premenopausal women are necessary to confirm these results.

One study suggests that CSF1 can influence breast cell proliferation either positively or negatively depending on the estrogen environment (14). In ER+ cell lines, estradiol induced a three- to fivefold increase in growth, while CSF1 alone did not (14). However, in combination, CSF1 inhibited the proliferative effects of estradiol by inducing G1 arrest. In contrast, earlier work in ER− cell lines showed that CSF1 induced proliferation (15,16). These data suggest that in a high-estrogen environment CSF1 inhibits proliferative effects of mitogens such as estradiol.

Accumulating evidence supports a role for CSF1 in breast carcinogenesis. Activation of CSF1R causes uncontrolled growth (9), increases the invasive potential of epithelial cells (17), and promotes angiogenesis (18). In animal models, blockade of CSF1 through anti-sense

oligonucleotides or neutralizing anti-CSF1 antibodies suppressed tumor growth and prolonged long-term survival (19,20). Thus, CSF1R may be a target for breast cancer chemoprevention.

This initial prospective study of circulating CSF1 levels suggests that CSF1 is a biomarker of subsequent postmenopausal breast cancer risk. Additional studies will be necessary to replicate these findings. This study was limited by the number of premenopausal breast cancer cases; it remains to be seen whether CSF1 has disparate effects in premenopausal and postmenopausal women.

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Table 1

Age and age-adjusted characteristics among controls (N=734) according to CSF1 levels, Nurses' Health Study (1992–1998).

	Q1 (N=184)	Q2 (N=183)	Q3 (N=184)	Q4 (N=183)
Median (pg/ml)	359.5	487.9	602.9	832.2
Range (pg/ml)	(169.7–424.8)	(425.1–540.5)	(540.9–689.4)	(692.6–2078.7)
Means				
Age, y	56.3	56.9	58.3	56.2
Age at menarche, y	12.6	12.8	12.6	12.6
Age at first birth[1], y	25.0	24.8	25.1	24.6
Age at menopause[2], y	48.2	47.7	46.8	47.1
Parity[1]	2.8	3.1	2.9	3.0
BMI, kg/m^2	24.6	24.8	25.5	26.4
BMI at 18, kg/m^2	21.2	21.1	21.5	21.4
Weight gain since 18, kg	8.9	10.0	11.1	13.5
Alcohol, g	6.4	5.8	4.7	3.6
Frequency, %				
Premenopausal	24.5	23.2	22.0	20.7
Family history of BC	13.8	14.5	15.2	15.7
Prior benign breast disease	56.6	51.2	51.2	53.9
Nulliparous	10.1	6.7	9.1	4.1
Current PMH users[2]	59.5	53.2	59.1	61.1
Not current PMH users[2]	40.5	46.8	40.9	38.9
Never PMH users[2]	28.7	29.0	29.2	23.0

[1] Among parous women only.

[2] Among postmenopausal women only.

Table 2

Relative risk (RR) and 95% confidence interval of breast cancer associated with quartiles of circulating CSF1 in the Nurses' Health Study (1992–1998).

	Q1	Q2	Q3	Q4	P-trend[1]	P-Het
All Women						
Median (pg/ml)[2]	359.4	487.2	602.9	833.3		
Cases/controls	183/181	173/180	174/182	195/182		
Conditional[3]	1.0(Ref)	0.95(0.71–1.28)	0.95(0.70–1.28)	1.06(0.79–1.43)	0.61	
MV[4] Conditional RR	1.0(Ref)	1.00(0.73–1.38)	0.96(0.69–1.33)	1.15(0.82–1.60)	0.37	
Premenopausal						0.009
Median (pg/ml)[2]	365.8	467.7	648.6	882.7		
Cases/controls	18/15	20/15	16/15	4/14		
Matching factors	1.0(Ref)	0.96(0.34–2.67)	0.80(0.27–2.39)	0.17(0.04–0.69)	0.03	
MV[5] RR	1.0(Ref)	0.97(0.27–3.56)	0.55(0.12–2.44)	0.15(0.03–0.85)	0.02	
Postmenopausal						
Median (pg/ml)[2]	356.3	488.3	595.0	817.2		
Cases/controls	156/155	141/155	138/157	185/155		
Matching factors	1.0(Ref)	0.93(0.67–1.28)	0.90(0.65–1.24)	1.21(0.89–1.66)	0.20	
MV[6] RR	1.0(Ref)	0.98(0.70–1.36)	0.90(0.65–1.26)	1.33(0.96–1.86)	0.11	

[1] P for trend based on the log transformed continuous measure of circulating CSF1.

[2] Median of the quartiles is based on the distribution among controls.

[3] Simple conditional model based on 725 matched case-control pairs with circulating CSF1 data.

[4] Conditional logistic model adjusted for: Benign breast disease, BMI at age 18, weight gain , parity/age at first birth, alcohol, family history of breast cancer, age at menarche, age at menopause, duration of PMH use

[5] Unconditional model adjusted for matching factors and benign breast disease, BMI at age 18, weight gain, parity/age at first birth, alcohol, family history of breast cancer, age at menarche.

[6] Unconditional model adjusted for matching factors and benign breast disease, BMI at age 18, weight gain, parity/age at first birth, alcohol, family history of breast cancer, age at menarche, age at menopause, duration of PMH use.

Table 3

Relative risk (RR) and 95% confidence interval of postmenopausal breast cancer associated with quartiles of circulating CSF1 in the Nurses' Health Study (1992–1998) according to tumor characteristics.

	Q1	Q2	Q3	Q4	P-trend[1]	P-Het
Postmenopausal only						
In Situ						
Cases/controls	33/155	25/155	20/157	28/155		
Matching factors	1.0(Ref)	0.75(0.42–1.34)	0.65(0.35–1.20)	0.88(0.50–1.55)	0.86	
MV[2] RR	1.0(Ref)	0.79(0.43–1.45)	0.67(0.35–1.28)	1.05(0.56–1.97)	0.82	
Invasive						
Cases/controls	117/155	111/155	109/157	151/155		
Matching factors	1.0(Ref)	0.97(0.69–1.37)	0.94(0.66–1.33)	1.31(0.94–1.83)	0.12	
MV[2] RR	1.0(Ref)	1.02(0.71–1.47)	0.96(0.67–1.38)	1.45(1.02–2.07)	0.06	
ER+/PR+						0.03
Cases/controls	60/155	64/155	63/157	90/155		
Matching factors	1.0(Ref)	1.10(0.72–1.68)	1.15(0.76–1.74)	1.45(0.96–2.18)	0.10	
MV[2] RR	1.0(Ref)	1.21(0.78–1.88)	1.21(0.78–1.87)	1.72(1.11–2.66)	0.04	
ER+/PR−						
Cases/controls	8/155	15/155	9/157	11/155		
Matching factors	1.0(Ref)	1.85(0.75–4.58)	1.24(0.45–3.40)	1.38(0.53–3.60)	0.85	
MV[2] RR	1.0(Ref)	2.17(0.83–5.72)	1.48(0.51–4.28)	1.52(0.53–4.34)	0.97	
ER−/PR−						
Cases/controls	25/155	18/155	11/157	21/155		
Matching factors	1.0(Ref)	0.83(0.43–1.58)	0.56(0.27–1.16)	0.81(0.42–1.56)	0.32	
MV[2] RR	1.0(Ref)	0.80(0.41–1.58)	0.50(0.24–1.06)	0.70(0.34–1.44)	0.16	

[1] P for trend based on the log transformed continuous measure of circulating CSF1.

[2] Unconditional model adjusted for matching factors and benign breast disease, BMI at age 18, weight gain, parity/age at first birth, alcohol, family history of breast cancer, age at menarche, age at menopause, duration of PMH use.