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Original Article

Soluble vascular endothelial growth factor receptors 2 (sVEGFR-2) and 3 (sVEGFR-3) and breast cancer risk in the Swedish Mammography Cohort

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Abstract: Vascular endothelial growth factor (VEGF) is a signalling protein that has been established as a contributor to tumor angiogenesis, and expression of VEGF and its soluble receptors (sVEGFR2 and sVEGFR3) have been demonstrated in breast cancer cells. However, no prospective studies have examined the association between prediagnostic sVEGFR levels and breast cancer risk. We conducted a prospective case-control study nested within the Swedish Mammography Cohort examining the association between sVEGFR2 and 3 levels and breast cancer risk. The analysis included 69 incident breast cancer cases diagnosed after blood collection and 719 controls. Logistic regression models were used to calculate odds ratios and 95% confidence intervals. After adjustment for breast cancer risk factors, sVEGFR2 levels were associated with breast cancer risk (OR=1.28; 95% CI=1.06-1.56 per 1000 ng/L increase in concentration) while sVEGFR3 levels were not related to such risk (OR=1.00; 95% CI=0.93-1.07). Our results suggest that sVEGFR2 levels may be positively associated with breast cancer risk, however future studies with larger case groups are necessary to confirm this association.

Keywords: Breast cancer, vascular endothelial growth factor receptor, growth factors, tumor angiogenesis

Introduction

Vascular endothelial growth factor (VEGF) is a signalling protein that has been well established as a contributor to tumor angiogenesis [1]. sVEGFR2 and sVEGFR2, the soluble receptors of VEGF regulate formation of lymphatic and blood vessles. Among them sVEGFR2 is recognized as the primary marker of endothelial cell development which directly controls tumor angiogenesis. Expression of VEGF and its receptors has been demonstrated in breast cancer cells [2-5] and it has been observed that it enables the survival of tumor cells [6, 7]. Circulating VEGF also has been shown to be a prognostic indicator in breast cancer patients [8-14]. However, to our knowledge, no prospective studies have examined the association between sVEGF receptors 2 and 3 and breast cancer incidence despite of well-known presence of VEGF in breast tumor cells.

We conducted a prospective case-control study nested within the Swedish Mammography Cohort to investigate whether prediagnostic sVEGF receptor levels were associated with postmenopausal breast cancer risk.

Materials and methods

Study population

This study included participants from a case-control study nested within the Swedish Mammography Cohort (SMC). The Swedish Mammography Cohort is a population-based prospective cohort study comprised of women from Västmanland and Uppsala counties, Sweden. Recruitment and characteristics of this

Table 1. Characteristics of breast cancer cases and controls in the Swedish Mammography Cohort

	Cases (n=69)	Controls (n=719)
Age at blood draw	68.3 (6.4)	70.5 (7.1)
BMI (kg/m^2) at sample collection	26.6 (4.5)	26.3 (4.5)
Height (cm)	165.0 (5.4)	162.5 (5.9)
Ever OC use	49.3%	49.4%
Ever HRT use	59.4%	42.0%
Age at menarche		
<13 years	39.1%	26.8%
13 years	18.8%	23.3%
>13 years	36.2%	39.2%
Nulliparous	14.5%	10.6%
Number of children (among parous women)	1.4 (0.5)	1.4 (0.5)
Age at first birth		
<26 years	54.2%	54.6%
26-31 years	37.3%	33.0%
>31 years	6.8%	10.7%
Family history of breast cancer	11.6%	7.8%
History of benign breast disease	18.8%	15.7%
Physical activity (MET hours/day)	41.5 (4.0)	42.1 (4.6)
Alcohol intake among drinkers (grams/day)	4.9 (3.6)	5.8 (5.4)

Data represent mean (standard deviation) unless otherwise indicated. Percents may not add to 100 due to missing values.

cohort have been previously described [15, 16]. In brief, 66,651 women born between 1914 and 1948 were recruited between 1987 and 1990. Participants completed a baseline questionnaire with questions regarding diet, reproductive and other relevant factors. In 1997 a second questionnaire was extended to include dietary supplements, physical activity and smoking status, and was sent to participants who were still alive and residing in the study area; 39,227 (70%) women returned this questionnaire. Completion and return of the self-administered questionnaire was treated as informed consent of study participants. The study was approved by the ethics committee at the Karolinska Institutet.

The participants in the nested case-control study came from the SMC-Clinical, a subcohort of 5,022 women under the age of 85 and living in the city of Uppsala, that was established between 2003 and 2009. These women completed a questionnaire on diet and lifestyle factors and underwent a health examination. Blood, urine, and adipose tissue samples were collected at the health examination. Venous blood samples were collected after a 12-hour overnight fast. Samples were immediately centrifuged and separated in a dark room, and stored at -80°C.

Histologically confirmed incident invasive breast cancer cases were ascertained by linkage of the study cohort with Swedish Cancer registers. These registers have been estimated to provide almost 100% complete case ascertainment [17]. We defined our cases as participants who provided a blood sample and had a breast cancer diagnosis after blood collection and before December 31, 2011 (n=69). Controls were randomly selected from women without a breast cancer diagnosis who had provided a blood sample (n=719).

Laboratory measurements

Soluble VEGF receptors 2 and 3 were analyzed in peripheral plasma using commercially available enzyme-linked immunosorbent assay (ELISA) kit (DY357 [VEGF receptors 2] and DY349 [VEGF receptors 3], R&D Systems, Minneapolis, MN). The assays had a total coefficient of variation (CV) of approximately 6%.

Statistical analysis

Spearman correlation coefficients between levels of sVEGFR2 and sVEGFR3 were calculated separately for cases and controls. Tertiles of sVEGFR2 and sVEGFR3 were determined by the distribution among the controls. Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for the association between continuous measures and tertiles of these biomarkers and breast cancer. In the covariate-adjusted model we adjusted for the following *a priori* potential confounders: age at blood sampling (continuous), date at blood sampling (continuous), oral contraceptive use (ever, never), hormone replacement therapy use (ever, never), age at menarche (<13, 13, ≥13 years), age at menopause (<51, ≥51 years), parity/age at first birth (nulliparous, age at first birth <26/1-2 children, age at first birth 26-30

Table 2. Odds ratios (ORs) and 95% confidence intervals (CIs) of breast cancer by sVEGFR2 and sVEGFR3 levels

	Cases/Controls	Range (ng/L)	Adjusted OR (95% CI)[2]
sVEGFR2[1]			
Tertile 1	19/242	1900-4280	1.00 (ref)
Tertile 2	20/239	4300-5360	1.25 (0.62-2.50)
Tertile 3	30/238	5380-10540	1.75 (0.90-3.40)
P_{trend}[3]			0.09
Continuous (per 1000 ng/L)			1.28 (1.06-1.56)
sVEGFR3[1]			
Tertile 1	15/239	3300-8680	1.00 (ref)
Tertile 2	26/239	8681-11660	1.71 (0.84-3.49)
Tertile 3	28/241	11700-42792	1.54 (0.74-3.20)
P_{trend}[3]			0.28
Continuous (per 1000 ng/L)			1.00 (0.93-1.07)

[1]Tertiles were determined based on ln-transformed values among the controls. [2]Adjusted for age at visit, visit date, BMI at blood draw, height, education, OC use, HRT use, age at menarche, age at menopause, parity/age at first birth, family history of breast cancer, benign breast disease, physical activity, and alcohol intake. In addition each biomarker is adjusted for the other biomarker. [3]Determined using tertile medians.

years/1-2 children, age at first birth 31/1-2 children, age at first birth <26/3 children, age at first birth 26 years/3 children), family history of breast cancer (yes/no), history of benign breast disease (yes/no), body mass index (BMI) at blood sampling (continuous), height (continuous), highest education level (elementary, high school, college or greater), physical activity (<38.8, 38.8-<42.3, 42.3-<45.9, 45.9 MET-hours/day), and alcohol intake (continuous). To assess the association of each biomarker independent of the others we included both biomarkers (sVEGFR2 and sVEGFR3) together in each multivariable model. Tests for trend were performed using tertile medians. We also conducted analyses excluding cases diagnosed within one year of blood collection in order to ensure that the potential influence of sVEGFR2 or sVEGFR3 levels as a result of undiagnosed disease were minimized. All tests of statistical significance were two-sided, and statistical analyses were performed using SAS Version 9.2 (SAS Institute Inc., Cary, NC).

Results

Sixty-nine cases of breast cancer diagnosed after blood collection and 719 controls were included in the analyses. At the time of blood collection breast cancer cases were slightly younger and taller than controls (**Table 1**). Cases and controls were similar with regards to BMI and use of oral contraceptives. Cases were more likely to be nulliparous, to have a history of benign breast disease, a family history of breast cancer, and to have ever used hormone replacement therapy (**Table 1**). Plasma concentrations of sVEGFR2 varied from 1900 to 10540 ng/L and sVEGFR3 from 3300 to 42792 ng/L. Among controls the Spearman correlation between sVEGFR2 and sVEGFR3 was 0.23 ($p<0.0001$) while among cases the corresponding value was 0.16 ($p=0.18$).

Plasma concentration of sVEGFR2 was associated with an increased risk of breast cancer. After adjustment for covariates, sVEGFR2 was associated with an OR=1.28 (95% CI=1.06-1.56) per 1000 ng/L increase in concentration. When sVEGFR2 was categorized into tertiles, we observed an adjusted OR of 1.75 (95% CI=0.90-3.40; $p_{trend}=0.09$); however, this result was not statistically significant. No association was observed between sVEGFR3 and breast cancer when the association was examined continuously (OR=1.00; 95% CI=0.93-1.07) or by tertiles (OR=1.54; 95% CI=0.74-3.20; $p_{trend}=0.30$ comparing the top to bottom tertile) (**Table 2**).

To examine the potential influence of sVEGFR levels as a result of undiagnosed disease we also examined the association excluding those diagnosed within one year of blood collection (n=19). These results were not materially different when the associations were examined continuously sVEGFR2 (OR=1.28; 95% CI 1.02-1.60) and sVEGFR3 (OR=0.97; 95% CI 0.90-1.06). When examined by tertiles the associations were slightly attenuated with wider confidence intervals for sVEGFR2 (top tertile 1.46; 95% CI 0.70-3.08; $p_{trend}=0.30$) and sVEGFR3 (top tertile 1.18; 95% CI 0.53-2.66; $p_{trend}=0.63$).

Discussion

In this study of 69 breast cancer cases and 719 controls we observed a significant association between plasma concentrations of soluble vascular endothelial growth factor receptor-2 and

breast cancer. To our knowledge, this is the first study to examine the association between plasma sVEGFR2 and sVEGFR3 levels and breast cancer risk. VEGF is an established angiogenic factor [1], however its role in the earlier stages of breast cancer development is not clear.

sVEGFR2 [also known as KDR (kinase insert domain receptor) or Flk-1] and sVEGFR3 [also known as Flt-4] are members of tyrosine kinases (RTKs) transmembrane receptor family. Measurement of VEGFRs instead of VEGF in plasma samples is favourable in cancer risk determination as VEGFRs do not form artefactually like VEGF due to risk of platelet activation during serum sample preparation. VEGFR2 binds VEGF-A (VEGF121, VEGF165, VEGF189 and VEGF206 splice variants), VEGF-C and VEGF-D. VEGFR2 and VEGFR3 are preferentially expressed in the proliferating endothelium of solid tumor vessels [18]. The VEGF/VEGFR2 signalling pathway plays an important role in tumour angiogenesis and the inactivation of functional VEGFR2 with a specific antibody blocks angiogenesis and reduces tumour cell invasion [19, 20]. Different mechanisms regulate VEGFR2 concentrations and modulate its significance in angiogenesis [21]. It is also known that the VEGFR2 system generally works through an essential autocrine/paracrine loop on cancer cell proliferation and survival. In contrast to the significant association we observed with sVEGFR2 and breast cancer risk, we did not observe a significant association with sVEGFR3. This may be due to the different roles they play in breast cancer development. Cancer cells can circulate to other areas of the body through either the bloodstream or the lymph system to form tumor metastasis. The lymph nodes in the underarm are the first place breast cancer is likely to spread. VEGFR3 has been shown to play an important role in lymphangiogenesis, and is thought to be involved in the development of lymph node metastasis [22, 23]. The levels of sVEGFR3 could thus be related to the risk of lymph node spreading in breast cancer and correspondingly would be more likely to be associated with breast cancer progression and prognosis than initiation.

Participants with breast cancer may have elevated sVEGFR2 levels due to pre-clinical disease that was present at the time of blood collection. To assess this potential influence we conducted sensitivity analysis excluding those diagnosed within one year of blood collection and observed that the results were still significant for the continuous measure of sVEGFR2 but were attenuated when the association was examined by tertiles. This attenuation could be due to chance, however, some tumors may take years to become clinically detectable so there is the possibility that cancer present, but remain undiagnosed, at blood draw influenced the observed association.

Limitations of our study include having only a single plasma measurement which may not reflect long-term VEGFR levels. In addition, non-differential misclassification of the measurements due to laboratory error could have attenuated results. Strengths of our study include the measurement of sVEGF receptors rather than VEGF. VEGF levels are sensitive to preanalytical handling/variation while sVEGFR measurements are less influenced by preanalytical factors, and thus are easier to interpret when used in a clinical setting. Strengths also include blood samples collected prior to cancer diagnosis, nearly complete follow-up, and information on many important covariates including established breast cancer risk factors.

In conclusion, our findings suggest a positive association between plasma sVEGFR2 levels and breast cancer risk. Future studies with larger case groups are necessary to confirm this association.

Disclosure of conflict of interest

None.

Abbreviations

VEGF, Vascular endothelial growth factor; VEGFR, VEGF receptor; SMC, Swedish Mammography Cohort; CV, Coefficient of variation; OR, odds ratio; CI, Confidence interval; BMI, Body mass index; KDR, Kinase insert domain receptor; RTK, Receptor tyrosine kinase.

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