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Clinicopathological and prognostic significance of vascular endothelial growth factors (VEGF)-C and -D and VEGF receptor 3 in invasive breast carcinoma

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Abstract

Aims: Vascular endothelial growth factors C and D (VEGF-C and VEGF-D) play a major role in lymphangiogenesis and activate VEGF receptor 3 (VEGFR-3). Our purpose was to study the clinicopathologic and clinical value of VEGF-C, VEGF-D and VEGFR-3 in invasive breast carcinoma.
Material and methods: Immunohistochemistry was performed in paraffin-embedded tissue specimens from 177 invasive breast carcinomas to detect the proteins VEGF-C, VEGF-D, VEGFR-3, p53, Ki67, c-erbB-2, topoIIα and ER/PR. The results were statistically processed.
Results: VEGF-C, VEGF-D and VEGFR-3 were found to be predominantly expressed in the cytoplasm of the malignant cells. VEGF-C occasionally showed a submembranous intensification. VEGF-D and VEGFR-3 were also immunodetected in the nuclei of the malignant cells. Nuclear VEGF-D was positively correlated to p53, Ki67 and topoIIα proteins' expression ($p = 0.003$, $p = 0.009$ and $p = 0.017$ respectively) and nuclear VEGFR-3 to topoIIα ($p = 0.034$). Cytoplasmic expression of VEGF-C and its submembranous intensification were found to be independent indicators of patients' overall and disease-free survival, respectively ($p = 0.003$ and $p = 0.044$ respectively). The group with high expression of both cytoplasmic VEGF-C and stromal VEGFR-3 showed poor overall survival ($p = 0.024$) and the group with both submembranous VEGF-C and stromal VEGFR-3 immunostaining showed poor both disease-free and overall survival ($p = 0.012$ and $p = 0.038$ respectively).
Conclusion: VEGF-D and VEGFR-3 seem to exert proliferative activity in invasive breast carcinomas. VEGF-C was found to be an independent indicator of patient's poor prognosis and the simultaneous expression of tumor VEGF-C and stromal VEGFR-3 yielded additional prognostic information.

Keywords: Breast cancer; Immunohistochemistry; VEGF-C; VEGF-D; VEGFR-3; Proliferation

Introduction

Angiogenesis and lymphangiogenesis are of particular significance in the growth and metastasis of tumors.[1,2] Vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) play an important role in the formation of the vascular network. The VEGF family comprises five members (VEGF-A, -B, -C, -D and -E) with distinct binding patterns to the three different tyrosin kinase receptors: VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1) and VEGFR-3 (Flt-4).[3] Among them, VEGF-C and VEGF-D are structurally closely related and are considered to be lymphangiogenic.[3] Both stimulate the migration and proliferation of endothelial cells, in vitro.[4,5] In addition, they have been shown to induce lymphangiogenesis in transgenic mice and in other in vivo models.[6] Moreover, disruption of the VEGF-C gene demonstrates that the growth factor is indispensable for embryonic lymphangiogenesis.[7] VEGF-C and VEGF-D signal, primarily, through VEGFR-3[5,8] which is mainly expressed by lymphatic endothelia.[9]

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VEGF-C and VEGF-D bind also and activate VEGFR-2.[4,10] Except for the endothelial cells, VEGF-C, VEGF-D and their receptor have been found to be expressed in the cancerous cells themselves, raising the possibility that VEGFs may promote cancer growth not only by stimulating angiogenesis, but also by directly acting on receptors on the cancer cells.[11]

Since various types of cancer tend to metastize via the lymphatic system, the production of lymphangiogenic growth factors could stimulate lymphatic vessel development in the tumor, enabling cancer cells to gain assess to the lymphatics.[3] Indeed, studies in which VEGF-C or VEGF-D were expressed in transplanted tumor cells or transgenic tumor models have demonstrated that these factors promote tumor lymphangiogenesis and lymphatic metastasis.[12,13] Moreover, clinicopathological studies of these growth factors in various cancer types, including esophageal, colorectal, pancreatic, endometrial and ovarian carcinomas, reveal that, sometimes, the expression of VEGF-C or VEGF-D does correlate with the presence of lymph node metastasis,[14–18] affecting negatively the patients' survival.[14–16,19]

However, in breast cancer, the role of the lymphangiogenic growth factors VEGF-C and VEGF-D has not yet been elucidated. Some studies correlate either VEGF-C or VEGF-D expression with lymph node metastasis[20–23] and patients' poor survival,[20] while some others not.[21,22,24–26]

In the present study, we examined the expression pattern of VEGF-C, VEGF-D and their receptor VEGFR-3 in invasive breast carcinoma in relation to the well known clinicopathological parameters, as well as other biological indicators of tumor behavior, such as p53, c-erbB-2, Ki67 and topoisomerase IIα (topoIIα). We also investigated the potential contribution of the studied markers to the poor survival of the patients.

Material and methods

Patients and samples studied

One hundred and seventy seven paraffin blocks with tumor samples were available from patients with resectable breast cancer and had undergone surgery. We only selected women with histologically proven, clearly invasive breast carcinomas, regardless of their initial stage, in whom axillary lymph node dissection had been performed. The patients were aged from 25 to 86 years (mean age 56.89 years). None of them had received radiation or chemotherapy preoperatively. Lastly, an informed consent was obtained from patients in order the material derived from them to be used in research.

Routine histological examination was performed with heamatoxylin-eosin staining. All carcinomas were classified according to the criteria of the World Health Organization[27] and were recorded as invasive ductal or invasive lobular. The combined histological grade (1, 2 and 3) of infiltrating ductal carcinoma was obtained according to a modified Scarff-Bloom-Richardson histologic grading system with guidelines as suggested by Nottingham City Hospital pathologists.[28] Nuclear grading was based on nuclear polymorphism and mitotic activity. Staging at the time of diagnosis was based on the TNM system.[29] Tumor size (<2 cm, 2–5 cm, >5 cm) and lymph node status were evaluated separately. The clinicopathological characteristics of the series are shown in Table 1. During the immunohistochemical procedure some specimens were destroyed, while some other was considered to have too small tissue to be evaluated. Therefore, the samples which were finally included in the statistic evaluation were 169, 161 and 169 for VEGF-C, VEGF-D and VEGFR-3, respectively.

Follow up was available for 164 patients. Mean survival time was 96.7 months (range 5 to 135 months). Patient outcome was defined as disease-free and overall survival.

Immunohistochemistry

Immunohistochemical staining for VEGF-C, VEGF-D and VEGFR-3 was performed on 4 μm thick formalin-fixed paraffin sections, using an avidin-biotin immunoperoxidase technique as previously described.[30]

Polyclonal antibodies against VEGF-C (goat/C-2, sc:1881), VEGF-D (rabbit/H-144, sc:13085) and VEGFR-3 (rabbit/C-20, sc:321) (Santa Cruz Biotechnology Inc, CA, USA) were used at a dilution of 1:80, 1:120 and 1:180, respectively. The immunostaining of ER, PR, p53, c-erbB-2, Ki67 and topoIIα was performed as previously described.[30–32]

Evaluation of immunohistochemistry

The evaluation of the immunohistochemical staining was performed by two pathologists, independently, through light microscopic observation, without knowledge of the clinical data of each patient. Cases of disagreement were reviewed jointly to arrive at a consensus score. The score resulted as the average of 10 distinct high-power fields observed under ×400 magnification. As positive controls, formalin-fixed, paraffin-embedded sections from normal human placenta were stained for VEGF-C and VEGF-D, and sections of normal human umbilical cord were stained for VEGFR-3, using the aforementioned procedure. Negative controls had the primary antibody omitted and replaced by TBS.

Staining intensity and the number of stained cells were taken into consideration all through the evaluation process. Samples were considered to be positive for VEGF-C, VEGF-D and VEGFR-3 in the cytoplasm or the nucleus of the tumor cells when the proportion of immunoreactive cells was at least 10%, as previously described.[21,23] VEGFR-3 was also detected in endothelial cells and its staining was considered positive when, at least, 5% of