Expression of Flt-1 and Flk-1 Receptors for Vascular Endothelial Growth Factor on Tumor Cells as a New Prognostic Criterion for Locally Advanced Breast Cancer

L. G. Zhukova, N. V. Zhukov, and M. R. Lichinitser

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We studied expression of Flt-1 and Flk-1 receptors on tumor cells obtained from 83 patients with locally advanced breast cancer after neoadjuvant chemotherapy. The mean period of observations was 32.3 months. The median recurrence-free survival periods for Flt-1^+ and Flt-1^- patients were 55 and 32 months, respectively (p=0.0064). The overall survival periods for Flt-1^- and Flt-1^+ patients were 45 and 67.6 months, respectively (p=0.014). The mean recurrence-free survival periods for Flk-1^+ and Flk-1^- patients were 40.8 and 60.9 months, respectively (p=0.035). Expression of VEGF had no prognostic value. Our results show that overexpression of Flk-1 on breast cancer cells in patients receiving neoadjuvant chemotherapy is associated with a poor prognosis. By contrast, overexpression of Flt-1 improves survival.

Key Words: *breast cancer; VEGF, Flt-1, Flk-1*

Flt-1 (VEGFR-1) and Flk-1/KDR (VEGFR-2) are most-studied receptors for vascular endothelial growth factor (VEGF). Until recently the expression of these receptors was detected only on endothelial cells and some blood cells. Published data show that cells of epithelial tumors (breast cancer, BC), small-cell lung cancer, ovarian cancer, and prostate cancer express these receptors [1,2,7]. Flt-1 and Flk-1 receptors expressed on tumor cells cannot be directly involved in angiogenesis, and their functions and clinical value remain unclear [3,6].

Here we studied prognostic value of expression of VEGF, Flt-1, and Flk-1 on tumor cells from patients with locally advanced BC (LABC).

MATERIALS AND METHODS

Eighty-three patients with morphologically verified LABC (2 patients with stage IIb, 20 patients with stage IIIa, and 61 patients with stage IIIb) were examined in the period from 1994 to 2001. Complete morphological remission was not achieved after adequate neoadjuvant chemotherapy. The mean age of patients was 41.2 years (35-67 years, Table 1).

Before surgery the patients received empirically selected neoadjuvant chemotherapy (3-6 courses). After chemotherapy the patients underwent radical surgery. One patient refused surgery; after repeated biopsy she received radical radiotherapy. After local therapy the patients received therapy according to the standard protocols for the treatment of BC.

Expression of VEGF, Flt-1 and Flk-1 on tumor cells was studied after neoadjuvant chemotherapy. In 33 patients expression of these molecular markers on tumor cells was also studied before therapy. We used routine immunohistochemical method with polyclonal antibodies S-17 and monoclonal antibodies A-3 and C-1 for Flt-1, Flk-1, and VEGF, respectively (Santa Cruz Biotech). The tumor was considered to be Flt-1-positive or Flk-1-positive when more than 10% cells were stained with the corresponding antigens (independently on the intensity of staining). The tumor was considered to be VEGF-positive when more than 25%

N. N. Blokhin Russian Research Center for Oncology, Russian Academy of Medical Sciences, Moscow

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cells were moderately or strongly stained with the corresponding antigens.

The mean period of observations was 32.3 months (6-87 months). The recurrence-free (RFS) and overall survival periods (OS) were determined by EORTC criteria. The survival rates were compared with expression of molecular markers in residual tumors. The survival rate was evaluated by the method Kaplan-Meier. The differences were estimated by log-rank test. Nonparametric data were analyzed by exact Fischer's test or χ^2 test depending on the number of observations.

RESULTS

In 33 patients expression of molecular markers was studied before and after therapy. The percent of tumors expressing Flt-1 significantly increased after therapy (72 *vs.* 47.5% before therapy, $p<0.01$), while the percent of tumors expressing VEGF decreased (44 *vs*. 62.5% before therapy, $p<0.01$). The percent of tumors expressing Flk-1 remained practically unchanged (72 and 73%). In the whole group (83 patients) expression of VEGF, Flt-1, and Flk-1 after neoadjuvant chemotherapy was detected in 52, 70, and 67.5% residual tumors, respectively.

Of standard prognostic factors (age, stage of growth, receptor status, regimen of chemotherapy, degree of morphological remission, clinical remission, and count of affected lymph nodes) RFS and OS depended only on the number of affected lymph nodes (0, 1-4, >4). OS was associated with the number of receptors for estrogens ($p<0.05$). None of the studied molecular markers correlated with the number of affected lymph nodes and receptor status (Spearman coefficient <0.2, $p<0.05$).

The patients with Flt-1^+ tumors had better RFS compared to patients with Flt-1^- tumors ($p=0.0064$, Table 2, Fig. 1)

Flt-1 expression on tumor cells improved OS ($p=0.014$, Table 2, Fig. 2).

Flk-1 expression produced an opposite effect. Expression of this marker reduced patient's survival ($p=0.035$ for RFS, Table 3, Fig. 3).

When analyzing OS, the prognostic vector of this marker remained unchanged, but the differences became insignificant ($p=0.179$).

Published data show that extracellular domains of Flt-1 and Flk-1 have similar affinity for VEGF, but the intracellular tyrosine kinase domain involved in signal transduction is more active in Flk-1 compared to Flt-1 [5]. We hypothesized that Flt-1 act as a catcher for VEGF and reduces its content to tolerable values. However, our assumption contradicted the results of studies performed by C. Herold-Mende *et al*. These authors proposed a more adequate explanation for this phenomenon. VEGF dose-dependently suppressed proliferation of cultured tumor cells expressing Flt-1from patients with head and neck tumors [4]. Tumor cells cannot form vessels and therefore, Flt-1 and Flk-1 receptors expressed on tumor cells cannot be directly involved in angiogenesis. Probably, these well-studied

TABLE 1. Examined Patients

Characteristics		Number of patients, abs., %
Stage	IIb	2 (2.4)
	IIIa	20 (24.1)
	IIIb	61 (73.5)
Metastases in axillary lymph nodes		
	N^-	4 (5)
	N^+	79 (95)
Age	<50 years	49 (59.1)
	≥50 years	34 (40.9)
Menstrual cycle		37 (44.6)
	normal	10 (12)
	menopause	36 (43.4)
Histological type	DIC*	54 (65)
	LIC**	17 (20.5)
	others	12 (14.5)
Receptors for estrogen and progesterone***		
	RE^+ and/or RP^+	59 (71.1)
	RE^- and RP^-	18 (21.6)
	unknown	6 (7.3)

Note. *Ductal infiltrative cancer, **lobular infiltrative cancer, ***positive (>10 fmol/mg protein). Percent of patients is shown in brackets.

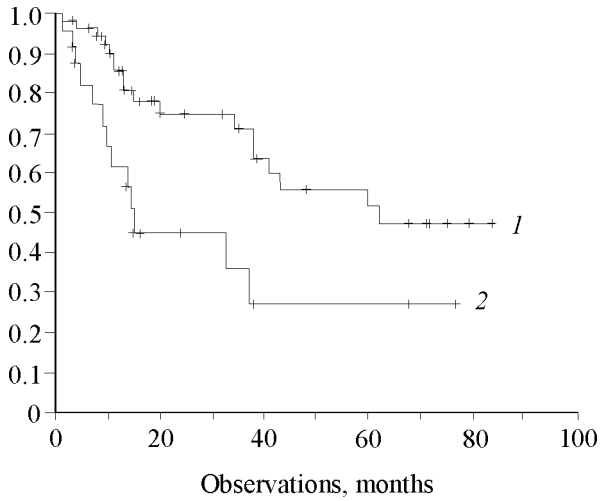

Fig. 1. Recurrence-free survival depending on Flt-1 expression. Ordinate: ratio of patients without recurrences. Here and in Fig. 2: patients with (*1*) and without Flt-1 overexpression in residual tumor tissue (*2*).

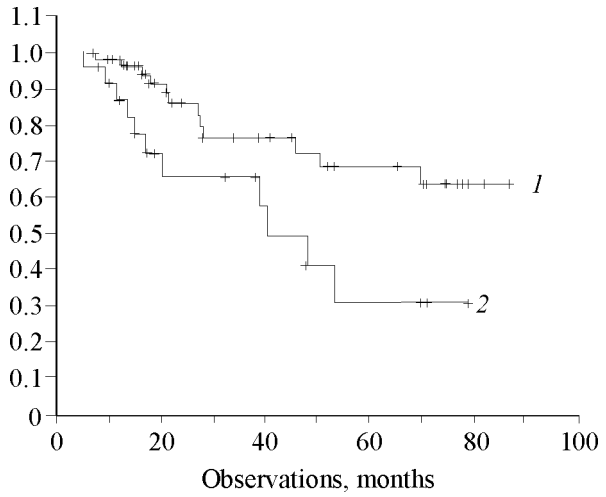

Fig. 2. Overall survival depending on Flt-1 expression on tumor cells. Ordinate: ratio of survived patients.

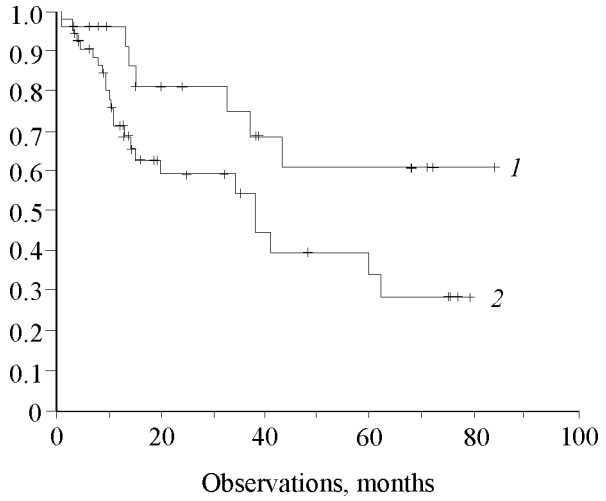

Fig. 3. Recurrence-free survival depending on Flk-1 expression on tumor cells. Ordinate: ratio of patients without recurrences. Here and in Fig. 2: patients without (*1*) and with Flk-1 overexpression in residual tumor tissue (*2*).

receptors play a non-angiogenic role in direct transduction of VEGF signal to tumor cells.

On the basis of these data we hypothesized that VEGF secreted by tumors in response to hypoxia interacts with Flk-1 receptors on endothelial cells and stimulates the formation of new blood vessels, which reduces the degree of tumor hypoxia. An alternative mechanism reducing hypoxia is inhibition of tumor growth under the influence of VEGF on Flt-1-positive tumor cells. The effects of Flk-1 expressed on tumor cells are poorly understood. We hypothesized that these receptors are involved in stimulation of tumor cell migration from the primary tumor node, *i.e.* from hypoxic conditions.

Despite numerous data on a negative effect of VEGF overexpression on survival, in our experiments VEGF expression on tumor cells had no prognostic value in relation to OS and RFS ($p>0.05$).

It should be emphasized that our results cannot be directly compared to published data. We studied expression of molecular markers in tumor tissues from patients receiving chemotherapy and not achieving complete morphological remission.

Expression of molecular markers was studied after neoadjuvant chemotherapy, since this treatment can modify tumor characteristics. Only certain cell clones survive, while cytostatics induce the appearance of clones with new biological properties. It is most likely that tumor cells survived after neoadjuvant chemotherapy in the primary node are similar to survived cells in micrometastases.

Our results indicate that expression of Flt-1 and Flk-1 on tumor cells after neoadjuvant chemotherapy determines survival of patients with LABC not achieving complete morphological remission. Expression of receptors on tumor cells has different prognostic significance. Flk-1 expression is associated with a poor prognosis, while Flt-1 improves the survival rate. This

TABLE 2. RFS and OS Depending on Flt-1 Expression on Tumor Cells

Parameter		Incidence	Mean survival, months	Median survival, months
RFS	Flt-1$^-$	24	32 (18-46)	15
	Flt-1$^+$	56	55 (45.0-65.3)	62
OS	Flt-1$^-$	25	45 (32.0-57.8)	40.5
	Flt-1$^+$	58	67.6 (58.3-76.9)	Not achieved

Note. Here and in Table 3: 95% confidence interval is shown in brackets.

TABLE 3. RFS Depending on Flk-1 Expression on Tumor Cells

Marker	Incidence	Mean survival, months	Median survival, months
Flk-1$^-$	26	60.9 (47.0-74.7)	Not achieved
Flk-1$^+$	54	40.8 (30.7-50.8)	38

is related to non-angiogenic functions of receptors expressed on tumor cells

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