Original article

# FasL is expressed in human breast cancer endothelia. Who benefits?

# FasL este exprimat in endoteliul vaselor asociate carcinoamelor mamare umane. Cine beneficiaza?

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# Abstract

Background: Fas ligand (FasL) expression is one of the mechanisms responsible for the "immune privilege" of tissues such as testis and eye. Many solid tumors express FasL as an immune escape strategy, aiming to induce apoptosis in Fas-expressing leukocytes, and thus to prevent the accumulation of effector cells. Materials and Methods: We investigated by immunohistochemistry the FasL expression in human breast carcinomas vasculature (blood vessels versus lymphatics), endothelial activation status and markers associated with tumor aggressiveness. Results: We bring evidence of a heterogeneous FasL expression in the human breast carcinomas associated blood vessels, but not lymphatics. The endothelial FasL expression was significantly correlated with the tumor FasL expression, tumor size, the presence of rich vascular networks, and axillary lymph nodes metastases; however, it was not correlated with low tumor invading leukocytes density. Conclusion: Breast carcinomas are among tumors in which the tumor-host interaction is correlated with the clinical outcome, hence understanding the significance of endothelial FasL expression is of outmost importance.

Keywords: FasL, breast carcinoma, endothelium, immune privilege, tolerance.

# Rezumat

Expresia Fas ligand (FasL) reprezintă unul din mecanismele responsabile pentru asigurarea statusului de situs imun privilegiat la nivelul unor țesuturi precum ochiul și testicolul. Multe tumori solide exprimă FasL ca strategie de eludare a răspunsului imun, celulele maligne inducând apoptoza leucocitelor Fas-pozitive și prevenind, în această manieră, acumularea celulelor efector la nivelul țesuturilor neoplazice. Materiale si metode. Am investigat prin imunohistochimie expresia FasL la nivelul vaselor asociate carcinoamelor mamare (vase sanguine versus limfatice), statusul de activare al endoteliului și o serie de markeri asociați cu potențialul agresiv tumoral. Rezultate. Am putut evidenția expresia heterogenă a FasL în endoteliul vaselor sanguine, dar nu și în vasele limfatice. Expresia endotelială a FasL s-a corelat cu expresia tumorală a moleculei, cu dimensiunea și vascularizația abundentă ale tumorii primare, cu prezența metastazelor ganglionare axilare, dar nu și cu densitatea infiltratului leucocitar. Concluzii. Evoluția carcinoamelor mamare este influențată de interacțiunile tumoră-gazdă, de aceea, înțelegerea semnificației expresiei endoteliale a FasL este extrem de importantă.

Cuvinte cheie: FasL, carcinom mamar, endoteliu, privilegiu imun, toleranță

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# Introduction

In 1948, Medawar has defined the immune privileged site as an anatomical location that allowed allograft or xenograft survival for longer periods compared with other areas (1). It is well known that FasL (CD95 ligand) (2) is able to induce cell death in Fas-bearing cells (3). The Fas/FasL interaction plays an essential role in the immune system homeostasis (4) and in the preservation of immune privilege status (2).

Using similar mechanisms, many tumors are probably able to escape immunologic rejection by suppressing the immune response. For example, melanomas (5), liver, esophageal (6), gastric (7), breast (8-10), cervical (11), colon (12), and pulmonary cancers (13) choriocarcinomas (14, 15), angiosarcomas (16) express FasL, which acts as a mediator of immune privilege (2). Several studies have shown that, in vitro, FasL-positive tumor cells kill activated Fas-bearing lymphocytes (17, 18), whereas in vivo, the expression of FasL by human esophageal carcinomas is associated with apoptotic depletion of tumor-infiltrating lymphocytes (TILs) (19).

In some cancers, such as gliomas and astrocytomas (20, 21), or in ovarian epithelial tumors (22), FasL was found to be expressed both by the tumor cells and by the tumor-associated vessels. Endothelial FasL expression was high in aggressive intracranial malignancies compared to more indolent neoplasms and also, it was associated with a significant reduction in T-cell infiltration (20, 21).

In the present study, our main aim was to investigate the expression of FasL in breast carcinomas associated vessels in order to better understand the potential implications of this apoptosis inducing mechanism upon several features influencing tumor aggressiveness. We were interested in identifying the blood or lymphatic nature of the FasL-positive vessels, but also to find possible associations between FasL presence on the vasculature and the expression of endothelial cells activation markers. We were also trying to correlate this expression with already known prognostic factors such as the expression of estrogen receptors (ER), progesterone receptors (PR), activated form of HER2/neu (HER-pY), tumor expression of Fas and FasL, tumor proliferation potential (PCNA), vascular density or the inflammatory infiltrate. We took a particular interest in trying to identify a possible association between FasL and ER in the carcinomas associated vessels, an aspect that might suggest the estrogen's involvement in the FasL expression control (23, 24).

# **Materials and Methods**

# Specimens

We have investigated 44 patients from "St. Spiridon" Hospital in Iasi (Romania) with histologically confirmed breast carcinomas. None of the patients had received any therapy prior to resection. The clinical and pathological features of the cases are presented in Table 1. The ductal invasive carcinomas group includes all the NOS (not otherwise specified) invasive ductal carcinoma, the pleiomorphic neoplasms and the mixed carcinomas, represented by ductal type associated with tubular, mucinous and cribriform lessions. All the mixed, ductal and lobular invasive carcinomas were included in a second group and the pure tubular carcinoma and invasive cribriform carcinoma were investigated as separate groups. Normal breast tissue from a human cadaver (harvested within 24 h since the time of death) was included in our study as control. All the specimens were fixed in neutral, buffered 10% formalin (Sigma-Aldrich; St Louis, MO) and embedded in paraffin.

#### *Immunohistochemistry*

Tissue sections (4  $\mu$ m) were deparaffinized in xylene and rehydrated in a series of graded ethanol solutions. Antigen retrieval was performed by heating the sections for 20 minutes in Antigen Retrieval Solution (Dako; Glostrup, Denmark), pH 6.1, at 98°C, in a water-bath, followed by 3% hydrogen peroxide/distilled H<sub>2</sub>O, 5 minutes pre-incubation to quench endogenous peroxidase. The slides were incubated overnight at 4°C with the following primary

| Parameter             |   | No. of cases |
|-----------------------|---|--------------|
| age                   | <50 years                                     | 11           |
|                       | >50 years                                     | 33           |
| tumor size (T)        | $T_{1-2}$ (<5 cm)                             | 37           |
|                       | T <sub>3-4</sub> (>5 cm)                      | 7            |
| lymph node status (N) | $\mathbf{N}_0$                                | 18           |
|                       | $\mathbf{N}_1$                                | 26           |
| histological type     | ductal invasive carcinoma                     | 29           |
|                       | lobular invasive carcinoma                    | 8            |
|                       | mixed (ductal and lobular) invasive carcinoma | 5            |
|                       | tubular invasive carcinoma                    | 1            |
|                       | cribriform invasive carcinoma                 | 1            |
| gradding (G)          | $G_1$   | 9            |
|                       | G <sub>2-3</sub>                              | 350          |

| Table 1. Distribution of th | e cases investigated i | n our study |
|-----------------------------|------------------------|-------------|
|-----------------------------|------------------------|-------------|

#### Table 2. Primary antibodies

| Molecule           |                                  | Clone    | Dilution     |
|--------------------|----------------------------------|----------|--------------|
| mo a hu FasL       | Santa Cruz Biotechnology; CA USA | WW10     | 1:100        |
| mo a hu Fas        | Dako; Glostrup, Denmark          | DX2      | 1:5          |
| mo a hu ER         | Dako; Glostrup, Denmark          | 1D5      | ready to use |
| mo a hu PR         | Dako; Glostrup, Denmark          | PgR 636  | ready to use |
| mo a hu Her2-pY    | Dako; Glostrup, Denmark          | PN2A     | 1:10         |
| mo a hu PCNA       | Dako; Glostrup, Denmark          | PC10     | 1:100        |
| mo a hu CD34 II    | Dako; Glostrup, Denmark          | QBEnd 10 | 1:50         |
| mo a hu D2-40      | Dako; Glostrup, Denmark          | D2-40    | 1:100        |
| mo a hu ICAM-1     | Santa Cruz Biotechnology; CA USA | 15.2     | 1:50         |
| mo a hu VCAM-1     | Santa Cruz Biotechnology; CA USA | E-10     | 1:50         |
| mo a hu endoglin   | Santa Cruz Biotechnology; CA USA | SN6h     | 1:5          |
| go a hu P-selectin | Santa Cruz Biotechnology; CA USA | G-1      | 1:50         |

antibodies (*Table 2*). The expression of the above mentioned molecules, except P-selectin, was detected using EnVision+ Dual Link System-HRP (Dako; Glostrup, Denmark) in a two-step staining procedure.

We incubated the slides with goat anti-rabbit and anti-mouse immunoglobulins conjugated to a peroxidase-labeled polymer, 20 minutes, at room temperature (RT). The second step consisted in 20 minutes incubation with DAB/H<sub>2</sub>O<sub>2</sub>, RT, followed by counterstaining in Mayer's haematoxylin (Dako; Glostrup, Denmark). For the detection of P-selectin, we used a swine anti-goat secondary biotinylated antibody and the streptavidin-peroxidase complex (Dako; Glostrup, Denmark), followed by DAB/ $H_2O_2$ (Dako; Glostrup, Denmark) incubation. The same protocol, except for the primary antibodies incubation step, was applied for negative controls.

# Stainings evaluation

Positive reactions were evidenced with a Nikon Eclipse TE300 optical microscope as brown precipitates with localization patterns corresponding to the investigated molecules distribution: nuc-



Figure 1. In normal mammary gland, FasL is weakly expressed by the epithelial and mioepithelial cells (HRP/DAB, x200)



Figure 2. FasL-positive vessels represent a minority of the entire vasculature associated with the breast carcinomas (HRP/DAB, x200).



Figure 3. FasL-positive vessels are distributed mainly in the abundant intratumoral stroma (A) and in peritumoral stroma (B), surrounded by leukocytes (HRP/DAB, x 100).

lear for ER, PR and PCNA and membrane/cytoplasmic for Fas, FasL, Her2-pY, CD34 II, D2-40, ICAM-1, VCAM-1, endoglin and P-selectin. For ER and PR, according to the most recent standardizations (25), we considered 1% positive cells as the limit between positive and negative breast tumors, while for PCNA, Fas, tumoral FasL, HER2pY, the limit was 10% positive cells.

According to the endothelial FasL expression, we have divided our specimens in two groups: a) negative, having no positive vessels at all and b) posit-

ive, irrespective to the number of stained vessels. Vascular density was assessed on sections stained for CD34 II as an average between the maximal and the minimal densities. The vessels were identified by examination at low power (x400 magnification) in the areas displaying the highest vascular density (five fields) and in the most poorly vascularized areas (five fields). We counted as vessels all clusters or single cells stained for CD34 II. The cases were divided in two groups, considering 10 vessels/field as threshold. The expression of D2-40,

| parameter              |  | cases | FasL+ vessels<br>(30 cases) | FasL- vessels (14 cases) | р             |
|------------------------|--|-------|-----------------------------|--------------------------|---------------|
| age                    | <50 years  | 11    | 9 (81.8%)                   | 2 (18.2%)                |               |
|                        | >50 years  | 33    | 21 (63.6%)                  | 12 (36.4%)               | p<0.2         |
| primary tumor size (T) | $T_{1-2}$ (<5 cm)                                  | 37    | 28 (75.7%)                  | 9 (24.3%)                | p<0,02        |
|                        | $T_{3-4}$ (>5 cm)                                  | 7     | 2 (28.6%)                   | 5 (71.4%)                |               |
| lymph nodes status(N)  | N <sub>0</sub>                                     | 18    | 5 (27.8%)                   | 13 (72.2)                | 0.001         |
|                        | $N_1$  | 26    | 25 (96.2%)                  | 1 (3.8%)                 | p<0,001       |
|                        | invasive ductal carcinoma                          | 28    | 19 (67.9%)                  | 9 (32.1%)                |               |
|                        | invasive lobular carcinoma                         | 8     | 6 (75%)                     | 2 (25%)                  |               |
| histological type      | mixed (ductal and lobular) in-<br>vasive carcinoma | 6     | 4 (66.7%)                   | 2 (33.3%)                | p<0,9         |
|                        | tubular invasive carcinoma                         | 1     | 0                           | 1                        |               |
|                        | cribriform invasive carcinoma                      | 1     | 1                           | 0                        |               |
| 11: (C)                | G <sub>1</sub>                                     | 9     | 6 (66.7%)                   | 3 (33.3%)                | p<0,9         |
| gradding (G)           | G <sub>2-3</sub>                                   | 35    | 24 (68.6%)                  | 11 (31.4%)               |               |
| ED evenession          | ER+  | 37    | 24 (64.9%)                  | 13 (35.1%)               | p<0,3         |
| EK expression          | ER-  | 7     | 6 (85.7%)                   | 1 (14.3%)                |               |
| PR expression          | PR+  | 34    | 24 (70.6%)                  | 10 (29.4%)               | p<0,8         |
|                        | PR-  | 10    | 6 (60%)                     | 4 (40%)                  |               |
|                        | PCNA+  | 30    | 22 (73.3%)                  | 8 (26.7%)                | n < 0.3       |
| tunior FCNA expression | PCNA-  | 14    | 8 (57.1%)                   | 6 (42.9%)                | p<0,5         |
|                        | Her2-pY+   | 13    | 9 (69.2%)                   | 4 (30.8%)                | n<0.0         |
| Herz-p1 expression     | Her2-pY-   | 31    | 21 (67.7%)                  | 10 (32.3%)               | p<0,9         |
| Eag avaragion          | Fas+   | 23    | 15 (52.2%)                  | 8 (47.8%)                | <b>n</b> ∠0.0 |
| Pas expression         | Fas-   | 21    | 15 (71.4%)                  | 6 (28.6%)                | p<0,9         |
| tumor Fact avaragion   | FasL+  | 22    | 20 (90.9%)                  | 2 (9.1%)                 | n <0.001      |
| unior rash expression  | FasL-  | 22    | 10 (45.5%)                  | 12 (54.5%)               | p<0,001       |
| vascular density       | <10 vessels/field (x400)                           | 18    | 8 (44.4%)                   | 10 (55.6%)               | p < 0.01      |
|                        | >10 vessels/field (x400)                           | 26    | 22 (84.6%)                  | 4 (15.4%)                | p<0,01        |
| leukocytes infiltrate  | 0  | 35    | 23 (65.7%)                  | 12 (34.3%)               | n<0.5         |
|                        | 1  | 9     | 7 (77.8%)                   | 2 (22.2%)                | p<0,5         |

 Table 3. Distribution of cases according to their clinico-pathological parameters and the expression of FasL by the tumor-associated vessels.

a molecule associated with the lymphatic vessels, and of molecules associated with the endothelium activation status, like P-selectin, ICAM-1, VCAM-1 and endoglin was compared, on serial sections, with the endothelial FasL in order to identify potential associations. The inflammatory infiltrate was assessed on haematoxylin-eosin sections and its intensity was graded in four categories: absent, mild (<10 cells/microscopic field, x200 magnification), moderate (10-50 cells/microscopic field, x200 magnification) and marked (>50 cells/microscopic field, x200 magnification). The cases were distributed in two categories: the first one, named 0, included all the tumors with absent and mild infiltrate and the second one, named 1, included all the tumors with moderate and marked infiltrate.

#### Statistical analysis

The statistical analysis was performed using the  $\chi^2$  test and the significance threshold was set at 0.05.

# **Results**

In normal breast tissues, FasL labeling generated only a weak signal in acinar and ductal epithelial cells or mioepithelial cells (*Figure 1*). The normal mammary gland vasculature is dominated by CD34 II positive blood vessels, but none of them were FasL-positive. Furthermore, no activated endothelia, positive for ICAM-1, VCAM-1, endoglin and P-selectin were found (data not shown). However, endothelial cells stained for FasL in 30 out of the 44 carcinomas included in our study, with percentages of positive vessels ranging from 1% to 20%, of the CD34 II positive endothelia (*Figure 2*).

*Table 3* presents the tumors parameters and molecules distribution for the investigated cases. Statistical analysis revealed significant correlations between the vascular expression of FasL and the small size of the tumors (p<0.02), the pres-



Figure 4. The vessels between the tumor cells cords and islands (within the fine intratumoral stroma) are rather FasL-negative (HRP/DAB, x100).



ence of axillary lymph node metastases (p<0.001), rich vascular networks – more than 10 vessels/field - (p<0.01), and the FasL expression in the tumor cells (p<0.001). Even though not statistically significant, other associations drew our attention: age under 50 years (81.8%), PCNA-positive tumors (73.3%), ER-negative tumors (85.75%), Fas-negative tumors (71.4%), and, last but not least, high density leukocyte infiltrate (77.8%).

Considering tumor FasL expression as a factor associated with the aggressive potential, it came as no surprise to identify the molecule in the endothelium of 67.9% of invasive ductal carcinomas or in 66.7% of invasive mixed, ductal and lobular, carcinomas. However, it was rather unexpected to evidence that 75% of invasive lobular carcinomas, a less aggressive histological type, associated endothelial FasL expression.

In most cases, the FasL positive vessels were identified within the abundant intratumoral stroma (*Figure 3A*) and within the peritumoral stroma (*Figure 3B*), surrounded by leukocytes, while vessels found between the tumor cells (within the fine intratumoral stroma) were rather FasL negative (*Figure 4*).

We investigated the possibility that the heterogeneous expression of FasL in endothelial cells might be associated with the nature of the vessels (blood or lymphatic) or with the endothelium activa-



B.

Figure 5. Lymphatic vessels, positive for D2-40 (A), are FasL-negative (B). FasL is present only in the blood vessels endothelial cells (B) (HRP/DAB, x200).



Figure 6. Serial sections identified a CD34 II-positive vessel (A), weakly positive for FasL (B), negative for P-selectin (C) and VCAM-1-positive (D) (HRP/DAB, x100).

tion status. Sequential sections labeled for FasL and D2-40 showed no lymphatic vessels positive for FasL (*Figures 5A and 5B*). Out of the markers of endothelial activation mentioned above, ICAM-1 was found to be expressed in only 10 cases (in a low percentage) by the tumor cells, but not by the endothelial cells (data not shown). VCAM-1 and P-selectin labeled a variable number of vessels, but also tumor cells in 12 and 7 cases, respectively (data not shown). The number of VCAM-1 positive vessels was always higher than the P-selectin positive vessels, however no correlation with the FasL expression could be noticed (*Figures 6A-6D*). VCAM-1 and P-selectin expression seemed to be mutually ex-

clusive (*Figures 7A and 7B*), and both molecules could be rather evidenced in the vasculature at tumor periphery. As expected, these vessels were surrounded by leukocytes (*Figures 6B and 6D*).

Endoglin was present in the neoformation proliferative vessels, but no association with the endothelial FasL expression could be evidenced (*Figures 8A and 8B*).

# Discussion

Apoptosis, also known as programmed cell death, is the major type of physiological cell death. FasL-mediated apoptosis of activated, Fas-positive, lymphocytes contributes to immune down-regulation and thus tolerance acquisition (26), T-cell activation-induced cell death (3) and immune response termination (27). However, many studies demonstrated so far that FasL is also present in other normal tissues, including breast (28) suggesting its involvement in maintaining the homeostasis, normal development, regeneration and functionality of cells and tissues beside the immune system effector cells. FasL endothelial expression in normal tissues such as cornea (29), testis, or fetal placenta (29-31) tells about the very important role played by this molecule in the generation and preservation of the immune privileged sites (32). However, Kokkonen et al. demonstrated the presence of FasL in reactive lymph nodes high endothelium venules, and this gives us a better understanding of the Fas-FasL mechanism in physiological regulation and control of lymphocyte traffic and access in other sites than immune privileged sites (33).

Many tumors, including carcinomas, express FasL and there is increasing evidence, although controversial, that FasL-expressing tumor cells become able in vivo to counterattack activated, Fas-bearing T cells, establishing tumor privileged sites (8, 34, 35). Furthermore, it has been shown that in some neoplasms, not only the tumor cells express FasL, but also the vasculature is positive for this apoptosis-inducing molecule. In some brain tumors such as gliomas and astrocytomas, FasL expression in the tumor-associated vessels is correlated with the decrease of T cells accumulation (20) and poor prognosis (21).

In normal breast tissues, FasL displayed only a weak expression in some epithelial and/or mioepithelial cells. However, this poor and heterogeneous expression might reflect a minimal but sufficient epithelial protection mechanism against infiltrating leukocytes or, even more probably, a mechanism involved in cellular homeostasis, since breast is a tissue with a rapid turnover throughout each menstrual cycle. We did not find any FasL- positive vessels in normal breast, but these results should be interpreted with caution since the molecule expression could be under the regulation of different factors during the menstrual cycle, the estrogen influence upon FasL expression being well documented (36-38). Endothelial activation markers were absent in the normal mammary gland, and this probably explains the extremely low number of leukocytes present in this tissue.

On the contrary, the development of a mammary tumor is a pathological condition accompanied by a local inflammatory response, demonstrated by the endothelial activation and, as a consequence, by the leukocyte accumulation within the tumor tissue (39).

In most of the cases we have investigated, we have noticed alterations in the status and distribution of blood and lymphatic vessels. The blood vessels between the tumor cells cords and islets expressed proliferation markers like endoglin, suggesting they are neoformation vessels involved in tumor cells nutrition and support (40), while the vasculature localized at tumor periphery displayed a highly variable expression of P-selectin and VCAM-1, accompanied by surrounding leukocytes, a feature that suggests the involvement of these activated endothelia in leukocytes diapedesis. In most of the tumors without a leukocyte infiltrate both P-selectin and VCAM-1 were missing, but, if present, such tumors displayed either one or the other adhesion molecule. Surprisingly, no ICAM-1 positive vessels could be evidenced; however, in a small number of cases -10 - we were able to demonstrate the expression of this molecule on the tumor cells.

It came as no surprise to find FasL expressed by the tumor cells in 22/44 cases, since this expression is associated with poor prognosis in ductal breast carcinomas (41), but the endothelial cells positivity for this molecule in 30/44 cases was rather surprising and, to the best of our knowledge, this is the first study to evidence such a particular FasL expression pattern in human breast carcinomas. The simultaneous expression of this molecule



Figure 7. The expressions of P-selectin (A) and VCAM-1 (B) are mutually exclusive (HRP/DAB, x100).



Figure 8. Most of the endoglin-positive vessels (A) are negative for FasL (B) (HRP/DAB, x100).

by tumor and endothelial cells in 90.9% of the cases might argue in favor of a conjugated immune escaping strategy on behalf of the tumor cells, aiming both at blocking effector cells to cross from the blood as well as to accumulate within the tumor microenvironment. However, our findings rather argued against this hypothesis, since, on the contrary, we found a high density leukocyte infiltrate in 77.8% of the FasL endothelial positive cases. The fact that each tumor exhibits also FasL-negative vessels might offer an explanation for the leukocyte "leakage" into the malignant tissue, as it is equally possible that the molecule is not functional, or that the invading leukocytes are not expressing Fas. No particular distribution pattern could be evidenced for the FasL positive vessels. The expression of FasL could not be associated with any markers of endothelial activation or vascular proliferation potential, even though we could identify on sequential sections double positive endothelia for FasL and P-selectin, FasL and VCAM-1 or FasL and endoglin. Furthermore, besides identifying very few ER positive vessels, no such endothelia were FasL positive. However, this does not exclude the possibility of estrogen involvement in FasL expression modulation, by acting upon other molecules than the classical estrogen receptors. One such molecule might be GPR30 (or GPER), a G protein coupled seven transmembrane receptor, demonstrated to mediate estrogen effects in many tissues, normal and malignant, especially on endothelial cells (42).

Serial sections labeled for D2-40 antigen and FasL showed no lymphatic vessel positive for both molecules, allowing us to conclude that only blood vessels expressed the FasL protein.

The correlation between the FasL expression and the small size (less than 5 cm) of the tumors (T1-T2) could be coincidental, considering that most cases in our study fall in this category. On the other hand, these tumors are evolving ones and the PCNA expression in 30/44 cases shows that the malignant cells are in a full process of proliferation, so that, in order to support their increase in volume, they have to develop rich vascular networks.

No statistically significant correlations could be evidenced between endothelium FasL positivity and parameters such as menopausal status, histological type (we compared only invasive ductal versus invasive lobular carcinomas) and grading, or tumor expression of PCNA, ER, PR, Her-pY, and Fas.

One of the most challenging features evidenced in our study is the statistically significant association between the endothelial FasL expression and the presence of axillary lymph nodes metastases. Furthermore, even though our results showed no statistically significant correlation, we would like to stress that FasL positive vessels were present in 81.8% young patients, in 73.3% of the PCNA-positive tumors, in 85.75% ER-negative and 71.4% Fasnegative tumors, parameters known to be associated with a higher tumor aggressive potential and/or poor prognosis (43-46). On the other hand, 75% of the lobular invasive carcinomas, characterized by a lower aggressiveness as compared to ductal invasive carcinomas (47), expressed endothelial FasL.

In summary, even though our study was not performed on a large cohort, our results bring for the first time clear evidence that human breast cancer is accompanied by the FasL expression not only in tumor cells, but also in the endothelial cells. This raises the question if the endothelial expression of the molecule is directly induced by the malignant cells in order to escape immune surveillance or by the immune system as a protection mechanism against vascular dissemination (48). The endothelial FasL expression proved to be significantly correlated with that of the cancer cells, but also with the presence of metastases in the axillary lymph nodes, with the rich vascular networks, with the small size of the tumors and, in almost 78% of the tumors, with a high density leukocyte infiltrate. An increasing number of studies support the conclusion that the tumor associated inflammation is of major importance for the clinical evolution, and the breast carcinomas, in their vertical growth phase, are on the short list of malignancies that display a correlation between the tumor-host interaction and prognosis (49).

As some of these data are rather intriguing and raise several other questions regarding, for instance, FasL functionality or localization toward a certain cellular pole, this offers ground for further research aiming at understanding the mechanisms involved in tumor/host interaction.

# Acknowledgments

This work was supported by UMF Iasi 17080/2010.09.30 and CNCSIS 31GR./14.05.2007 grants.

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