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Fas expression in primary breast cancer is related to neoplastic infiltration of perilymphatic fat

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ABSTRACT

Purpose: Various studies have revealed that both Fas and its ligand play an important role in cancer biology. The aim of our study was to determine if there is a relationship between the expression of Fas or Fas-ligand in breast cancer and the presence of malignant cells in perilymphatic fat.
Material/Methods: Tumor samples from 147 consecutive breast cancer patients, aged 35-81 (median, 59), were subjected to analysis. The expressions of Fas and Fas-ligand were determined immunohistochemically.
Results: The expression of Fas, but not Fas-ligand, was significantly less frequent in breast cancer patients in whom malignant cells infiltrated through the perilymphatic fat (p=0.042). The infiltration of paranodal fatty tissue occurred more often in cases of ductal carcinomas (p=0.008), larger primary tumors (pT≥2, p=0.030) and regional lymph node involvement (pN≥1, p=0.021). Univariate analysis revealed that perilymphatic fat infiltration shortened overall survivals in breast cancer patients (p=0.05), similarly to postmenopausal status (p=0.034), age >60 years (p=0.05) and regional lymph node involvement (p=0.05). None of the aforementioned factors, however, was revealed as an independent predictor of survival in multivariate analysis.
Conclusions: The study showed that lack of Fas in primary breast cancer is associated with perilymphatic fat infiltration. Consequently, both the absence of Fas in the primary tumor and the occurrence of neoplatic cells in paranodal fatty tissue should be considered in the prognosis, complementing existing conventional factors.

Key words: Fas, Fas-ligand, breast cancer, perilymphatic fat, prognosis

INTRODUCTION

Various studies have revealed that both Fas and its ligand play an important role in cancer biology. The significance of these molecule expressions in the primary tumor for the clinical outcome of cancer is, however, not fully understood [1-8].

Our previous studies had shown that the lack of Fas or Fas-ligand in the primary tumor was associated with known negative prognostic factors of breast cancer and determined its spread either to the regional lymph nodes or to the bones [9-10].

Although this approach has changed with time, perilymphatic fat infiltration is currently considered as an accessory prognostic factor in breast cancer patients [11-13].

The aim of our present study was to determine if there is a relationship between the expression of Fas or Fas-ligand in primary breast cancer and the presence of malignant cells in perilymphatic fat.

MATERIAL AND METHODS

Tumor samples from 147 consecutive breast cancer patients of the Regional Comprehensive Cancer Center, Wroclaw, Poland, treated radically at the local Surgical Department between 1998 and 2000 were studied. The median age of the patients was 59 (range, 35-81 years). Detailed characteristics of the group studied are given in *Tab. 1*. All patients had undergone surgery (radical mastectomy or breast conserving surgery with lymph node dissection) with adjuvant treatment conducted in accordance with obligatory standards. The follow-up period amounted to five years. Breast tumor specimens, freshly

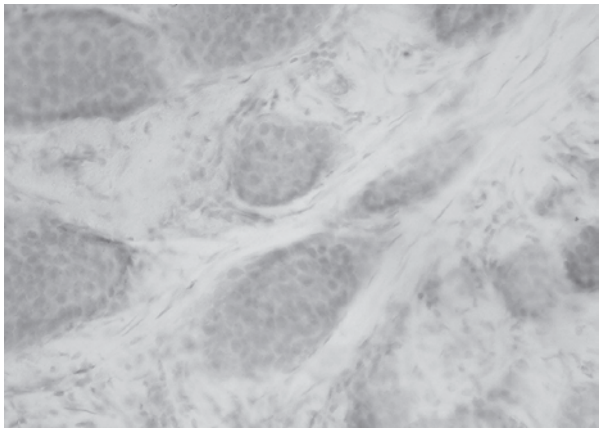
Table 1. **Patient and tumor characteristics in breast cancer women with (+) or without (-) perilymphtic fat inflitration (Mann-Whitney *U* test, $p \leq 0.05$)**

Characteristics	Total (n=147)	Perilymphatic fat (+) (n=52)	Perilymphatic fat (-) (n=95)	*p*-value
Age at diagnosis (years)				
≤40	7 (5%)	2 (4%)	5 (5%)	0.985
41-50	41 (28%)	15 (29%)	26 (27%)	
51-60	31 (21%)	10 (19%)	21 (22%)	
61-70	49 (33%)	18 (35%)	31 (33%)	
>70	19 (13%)	7 (13%)	12 (13%)	
Menopausal status				
premenopausal	48 (33%)	17 (33%)	31 (33%)	0.994
postmenopausal	99 (67%)	35 (67%)	64 (67%)	
Histological type				
ductal ca	108 (74%)	45 (87%)	63 (66%)	0.008
lobular ca	39 (26%)	7 (13%)	32 (34%)	
pT				
0+1	35 (24%)	7 (13%)	28 (29%)	0.030
2+3	112 (76%)	45 (87%)	67 (71%)	
pN				
0	49 (33%)	11 (21%)	38 (40%)	0.021
1+2	98 (67%)	41 (79%)	57 (60%)	
Histological grade				
G1+G2	103 (70%)	33 (63%)	70 (74%)	0.197
G3	44 (30%)	19 (37%)	25 (26%)	
Fas				
+	91 (62%)	26 (50%)	65 (68%)	0.042
−	56 (38%)	26 (50%)	30 (32%)	
Fas-ligand				
+	70 (48%)	21 (37%)	49 (52%)	0.124
−	77 (52%)	31 (63%)	46 (48%)	
Estrogen receptor				
+	79 (54%)	32 (65%)	47 (49%)	0.096
−	68 (46%)	20 (35%)	48 (51%)	
Progesterone receptor				
+	48 (33%)	17 (34%)	31 (33%)	0.875
−	99 (67%)	35 (66%)	64 (67%)	
p53				
+	82 (56%)	29 (52%)	53 (56%)	0.664
−	65 (44%)	23 (48%)	42 (44%)	

obtained at the time of surgery, were immediately placed in an RPMI1640 medium (Sigma, Poland), snap-frozen and stored at -80˚C. For purposes of standard histological studies (pTNM and perilymphatic fat infiltration), postoperative specimens were also fixed in 10% formalin and subsequently stained with hematoxilin and eosin.

The 4-6 μm cryostat sections were immunostained for Fas and FasL using the Biotin-Streptavidin-Peroxidase method. Air-dried (overnight) and fixed in cold acetone (10 min), the sections were incubated with a peroxidase-blocking solution (Dako A/S, Glostrup, Denmark) for 30 min. They were then incubated with anti-Fas or anti-Fas-ligand monoclonal antibodies (1:100, Santa Cruz Biotechnology, Inc. CA, USA) overnight at 4ºC. The sections were then incubated (37ºC) with a biotin-labeled secondary mouse anti-goat IgG antibody (1:50, Dako A/S) and streptavidin-biotin-peroxidase (1:50, Dako A/S) for 30 min each. Tissue was stained for five min with 0.05% 3,3'-diaminobenzidine tetrahydrochloride (DAB)

Figure 1. **Normal breast tissue (negative control).**



and then counterstained with haematoxylin, dehydrated and mounted. The expression of the molecules studied was analyzed under light microscopy. The localization and the intensity of the color reaction were compared with negative controls (normal breast tissue), *(Fig. 1)*. Samples were considered positive when at least 10% of breast cells were stained *(Fig. 2-3)*.

Moreover, ER and PR status were determined using the Dako A/S Cytomation kit (mouse anti-human estrogen receptor 1D5-clone and mouse anti-human progesterone-receptor clone PGR 636), and immunohisochemical staining for p53 status was performed using monoclonal anti-p53 protein (DO-7, Dako A/S).

The associations between Fas or Fas-ligand expression and perilymphatic fat infiltration and the relationship of the latter to other clinicopathological variables were tested using the Mann-Whitney *U* test. Overall survivals (OS) were obtained by the Kaplan-Meier method and compared with the log-rank test. The Cox proportional hazards regression model was used for multivariate analysis of survival. All variables with $p \leq 0.07$ in the univariate analysis were entered in the regression model. Calculations were performed using Statistica 5, Version 97 (StatSoft®, Poland) software, and statistical significance was defined as $p \leq 0.05$.

RESULTS

The occurrence of Fas in primary breast tumors was significantly less frequent in patients in whom malignant cells infiltrated throughout the perilymphatic fat (p=0.042). A similar relationship, however, was not observed in Fas-ligand expressions *(Tab. 1)*.

The infiltration of paranodal fatty tissue was also more frequent in cases of ductal carcinomas (p=0.008), larger primary tumors ($pT \geq 2$, p=0.030) and regional lymph node involvement ($pN \geq 1$, p=0.021), *(Tab. 1)*.

Univariate analysis revealed that the perilymphatic fat infiltration significantly shortened the overall survivals in breast

Figure 2. **Positive immunohistochemical staining of Fas in breast cancer tissue.**

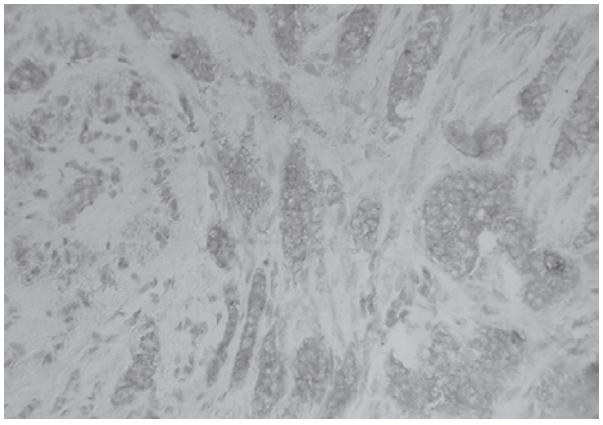
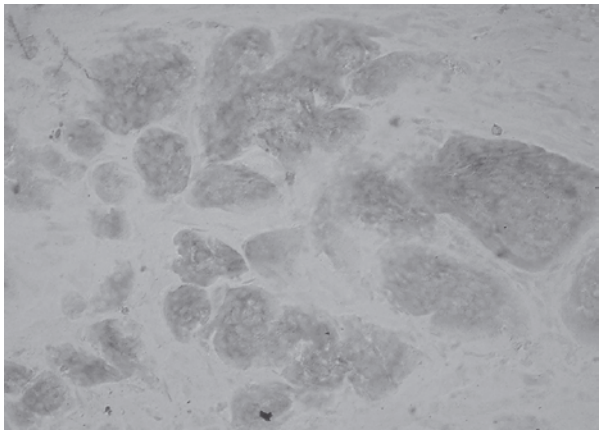


Figure 3. **Positive immunohistochemical staining of Fas-ligand in breast cancer tissue.**



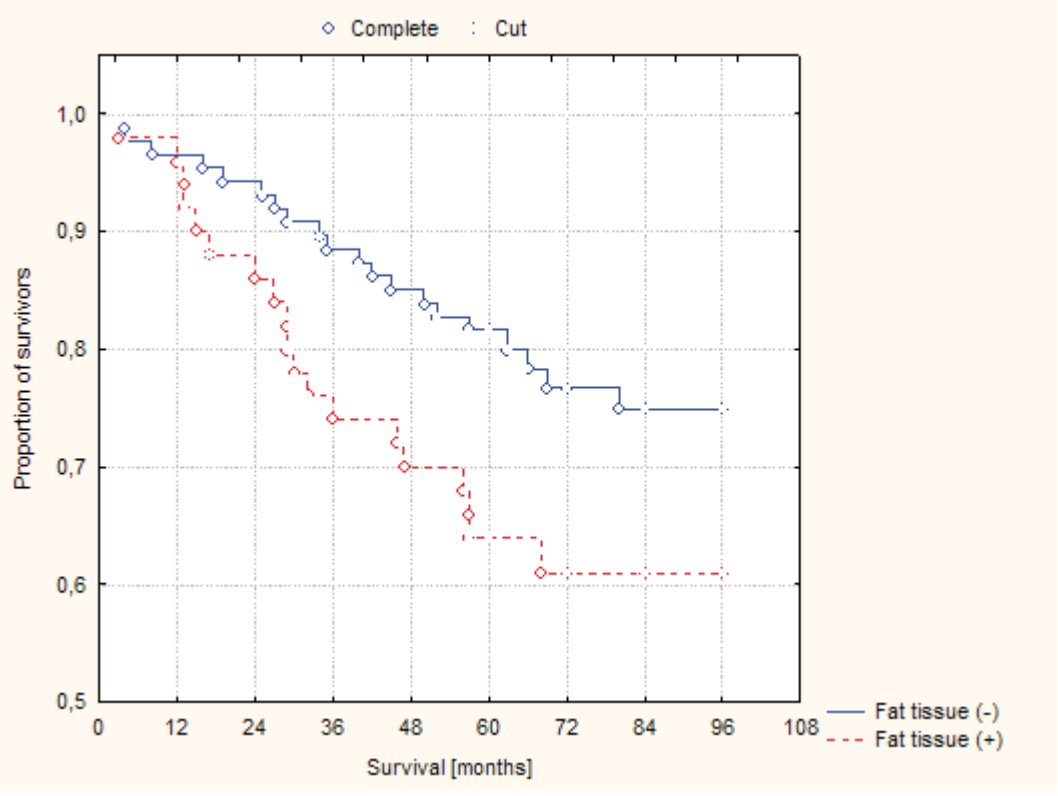
cancer patients (p=0.05), *(Fig. 4),* similar to postmenopausal status (p=0.034), age >60 years (p=0.05) and regional lymph node involvement (p=0.05). None of the aforementioned factors, however, was revealed as an independent predictor of survival on multivariate analysis.

DISCUSSION

Our study revealed that perilymphatic fat is more frequently infiltrated by malignant cells in Fas-negative breast cancers. Hence, we demonstrated another association between the lack of Fas in cells of a primary tumor and an unfavorable prognosis in breast cancer patients. Our previous studies had revealed an association between Fas deficiency in primary breast cancers and the frequency of recurrence, or the lymphatic and skeletal spread, of those cancers [9-10].

Also, a number of other authors claim that the absence of Fas is characteristic for more aggressive breast cancers and reflects the duration of disease-free survival. Mottolese et al. [14] revealed that disease-free survival was significantly longer in patients with Fas-positive tumors compared to those

Figure 4. **Survival curves for breast cancer patients with positive and negative perilymphatic fat (log-rank test, significant differences by p≤0.05).**



with Fas-negative breast cancer tissues. The aforementioned results were further confirmed by Reimer et al. [15] and Botti et al. [16], who found that the FasL : Fas ratio >1 was related to significantly shorter disease-free survival.

Our results agree with current knowledge according to which an apoptosis of breast cancer cells is induced and mediated through the activated Fas/Fas-ligand pathway. Activation of Fas by its ligand was proven to result in the oligomerization of its intracellular death domain and the activation of caspases, resulting in apoptotic cell death [17,18].

Nevertheless, we have not demonstrated an association between the lack of Fas-ligand in the primary tumor and perilymphtic fat infiltration. Hence, the perilymphatic spread of breast cancer did not seem to be correlated with the simultaneous absence of both components of the Fas/Fas-ligand system. We had previously shown that lymph node involvement was associated with the lack of Fas in primary breast tumors, while it was independent of the occurrence of Fas-ligand [9]. Consequently, the molecular background of the nodal and paranodal invasion of breast cancer seems to be similar in terms of Fas/Fas-ligand expressions.

Besides the expression of Fas, the ductal phenotype of breast cancer, the large size of the primary tumor and regional lymph node involvement were the other clinicopathological variables determining the infiltration of perilymphatic fat by neoplastic cells. All the aforementioned factors belong to the established negative prognostic factors of breast cancer [17]. Consequently, their association with the involvement of paranodal fatty tissue suggests that the latter also belong to the unfavorable prognostic factors of breast cancer. Moreover, the results of univariate analysis of survival proved the negative influence of perilymphatic fat infiltration on prognosis.

Concluding, the study showed that the lack of Fas in primary breast cancer is associated with perilymphatic fat infiltration. Consequently, both the absence of Fas in the primary tumor and the occurrence of neoplastic cells in paranodal fatty tissue should be considered in the prognosis, complementing existing conventional factors.

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