FAS AND FASL EXPRESSION IN MAMMARY CARCINOMAS

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Abstract: Fas ligand (FasL) and its receptor (Fas, CD95, Apo-1) are a set of regulatory components in immune system. The aim of this study was to investigate the expression of Fas and FasL in the breast cancer. We were able to demonstrate that 90% of the invasive breast cancers expressed CD95L, with potential detrimental effects on the host organism. CD95 expression was lost (35%) in breast cancer, a mechanism probably involved in CD95L-mediated apoptosis resistance.

INTRODUCTION

Fas ligand (FasL) and its receptor (Fas, CD95, Apo-1) are a set of regulatory components of the immune system (Suda et al., 1993). Fas is a 45-kDa cell-surface receptor of the TNF/ nerve growth factor receptor family and is one of the most important death-domain receptors (Suda et al., 1993). Interaction of Fas with its ligand, FasL, induces receptor trimerization, which in turn results in the recruitment of the adapter protein FADD (Fas-associated death domain) and activation of caspases, which lead to irreversible cell damage and death (Nagata, 1995). FasL expression results in neoplastic cells becoming able to modify the immune response directed against them by affecting the tumor microenvironment in a way that favors the successful escape of the tumor from immune surveillance (Gutierrez et al., 1999).

AIMS OF THE STUDY

The aim of this study was to investigate the expression of apoptotic markers Fas and FasL in breast carcinomas. We were also interested in establishing a potential correlation between the expression of these two molecules and the clinical and biological parameters of the tumors.

MATERIALS AND METHODS

A number of 20 patients, diagnosed with mammary carcinoma and surgically treated in the III-rd Surgical Clinic of the "Sf. Spiridon" Hospital were taken into consideration. For each patient were investigated the following parameters: menopausal status, the histological type/gradding tumoral of the primary tumor and the lymph node status.

The neoplastic samples were harvested in sterile medium and incubated with 0.4% collagenase, at 37^0C, over night. The isolated cells were centrifuged on glass slides (cytospin) and fixed in ethanol absolut for 15 min. at room temperature. The slides were incubated with a primary antibody (anti-Fas and anti-FasL respectively) followed by a secondary biotinylated antibody and the streptavidin-peroxidase complex. The reaction was developed with diaminobenzidine (DAB) and the cells were counterstained with hematoxylin.

We have considered as positive those samples that expressed more than 5% Fas or FasL positive cells.

In order to identify possible correlations between the Fas and FasL expression and the other parameters we have considered, the χ^2 test was employed and the statistical significance threshold was established at <0.05.

RESULTS AND DISCUSSION

Our investigation has revealed the following features:

-FasL expression has been detected in 90% of the invasive breast carcinomas.

-Fas and FasL co-expression has been evidenced in 65% of the cases.

-Fas expression was partial lost in 35% of the cases.

-We were not able to demonstrate a statistically significant association between the Fas and FasL molecules expression and the clinical and biological parameters taken into consideration (tables I and II).

Table I. The expression of Fas associated with the clinical and biological parameters considered.

Parameter		*Number of cases(n=20)*	*Fas +Cells n%*	*Fas –Cells n%*
menopausal status	Pre-menopause	4	4 (100%)	0 (0%)
	Post-menopause	16	9(56,25%)	7(43,75%)
histological type/gradding tumoral	CDI-G2	13	9(69,24%)	4(30,76%)
	CDI-G3	5	3(60%)	2(40%)
	CLI	2	1(50%)	1(50%)
lymph node status	N0	5	3(60%)	2(40%)
	N1	15	10(66,7%)	5(33,3%)

CDI-G2= ductal invasive breast carcinoma-moderate differentiation; CDI-G3= ductal invasive breast carcinomas-poor differentiation; CLI=lobular invasive breast carcinomas; N0-node negative patients; N1-node positive patients

The consequence of FasL expression on the surface of a tumoral cell is the occurrence of an immuno-privileged site, tolerated by the immune system, hence it represents an escape mechanism from the cytotoxic activity of the T cells (Von Reyher et al., 1998)

Table II. The expression of FasL associated with the clinical and biological parameters considered.

Parameter		*Number of cases (n=20)*	*FasL+Cells n%*	*FasL–Cells n%*
menopausal status	Pre-menopause	4	4 (100%)	0 (0%)
	Post-menopause	16	14(87,5%)	2(12,5%)
histological type/gradding tumoral	CDI-G2	13	12(92,30%)	1(7,69%)
	CDI-G3	5	5(100%)	0(0%)
	CLI	2	1(50%)	1(50%)
lymph node status	N0	5	5(100%)	0(0%)
	N1	15	13(86,66%)	2(13,33%)

The co-expression of Fas and FasL in 65% of the investigated cases may suggest that the tumoral cells have been accumulated several defects in the signaling pathway in which FasL is involved (O'Conell et al., 1999).

Cells undergoing neoplastic transformation are characterized by the partial or complete loss of post-translational Fas expression, which leads to resistance to Fas/FasL-induced apoptosis (Lebel et al., 1996). In the present study, we showed that the tumor cells lost the Fas expression in 35 % of the cases, which is in accordance to the literature data.

The FasL and Fas molecules expression is shown in figure 1 and 2.

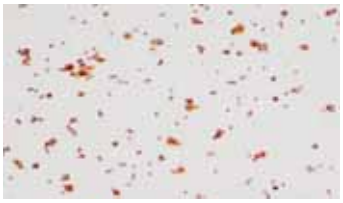

Fig.1. Ductal invasive breast. Cytospin of cells separated by enzymatic treatment with collagenase. Positive staining for FasL (x 400)

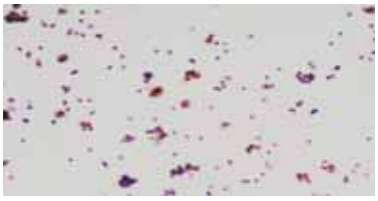

Fig.2.Ductal invasive breast. Cytospin of cells separated by enzymatic treatment with collagenase. Positive staining for Fas (x 400)

CONCLUSION

The results of our study have demonstrated that the tumor cells espress FasL *in vivo*, functioning as a potential inhibitor of the anti-tumoral immune response. Conversely, these cells partial lost the Fas expression, which in turn leads to resistance towards Fas/FasL - induced apoptosis

No correlation was found between the Fas and FasL expression and the other parameters (menopausal status, the histological type/gradding tumoral of the primary tumor and the lymph node status. taken into considerration in the present study.

The resistance to apoptosis and the concomitant ability to neutralize reactive T cells are currently considered as potential mechanisms used by tumor cells to evade immune recognition and response.

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