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ORGINAL ARTICLE

FAS and FAS-L expression by tumor cells and lymphocytes in breast carcinomas and their lymph node metastases ☆

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Abstract

FAS receptor (FAS, CD95) and FAS ligand (FAS-L, CD95-L) are complementary members of a particular apoptotic pathway that plays a major role in immune regulation. The activation of FAS-L may trigger cytotoxic mechanisms leading to the death of FAS-expressing cells. Tumor cells and tumor-infiltrating lymphocytes (TIL) may express FAS and FAS-L in various proportions, and their interplay may affect tumor behavior.

In the present study, we explored the expression of FAS and FAS-L in 28 mammary carcinomas (19 ductal and 9 lobular) and in their lymph node metastases. The expression of these mediators in immunostained sections was graded and evaluated comparatively between normal and neoplastic mammary epithelium, between tumor cells and TILs, and between mammary carcinoma cells and their lymph node metastases. We demonstrated the coexpression of FAS and FAS-L by breast carcinoma cells and TIL, with FAS expressed more strongly by normal epithelial cells and TIL than tumor cells. FAS-L was better stained on tumor cells than on TIL. There was equal or greater expression of FAS and FAS-L in the primary tumors and their TIL than in the metastatic counterparts. Comparing the expression of FAS with that of FAS-L, we recorded FAS equal or stronger than FAS-L in the primary mammary tumors and the reversal of their expression, FAS-L greater than FAS in the lymph node metastases. These results are consistent with reports of studies with other tumors, suggesting that the upregulated FAS-L indicates an increased ability of tumor cells to induce apoptosis in TIL and in the normal tissues invaded. However, it is understood that the FAS/FAS-L system, although essential for apoptosis, is only a contributing factor to the complex process of tumor invasion and antitumor defense.

Keywords: FAS/FAS-L in breast carcinoma; FAS/FAS-L in lymph node metastases

Introduction

The major cause of morbidity and mortality of tumors is their capacity for metastasis. Although substantial progress has been achieved in early diagnosis and treatment of malignant tumors, the mechanism of their dissemination remains largely unknown. The metastasis of tumors is a complex phenomenon composed of a cascade of interdependent events controlled at the genetic level through the activation and deactivation of multiple genes. To a large extent, the capacity for metastasis represents an intrinsic quality of tumor cells that, in most cases, correlates inversely with the degree of cellular differentiation. During tumor progression owing to genomic instability and frequent mutations,

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new populations of tumor cells endowed with the ability to invade and disseminate may emerge [11,18,20,23].

On the other hand, there are a variety of defense mechanisms that can be activated in order to withstand the progress of tumors. Cytotoxic T-lymphocytes and natural killer (NK) cells are mediators of immune responses against tumor cells and, as tumor-infiltrating lymphocytes (TIL), they are constant companions of tumors [2]. The intricate interactions of tumor cells and TIL, studied mostly in in vitro systems, are not well understood. Even more obscure are the establishment and growth of tumor metastasis in lymph nodes. Both lymphocytes and tumor cells in the primary tumor, as well as in its metastasis, are engaged in a reciprocal induction of cell death. The complicated process of apoptosis comprises multiple systems that normally preserve the balance between cell proliferation and cell involution and, while under abnormal conditions, may favor neoplasia or necrobiosis [4,5,7–9,15,22]. FAS and its ligand FAS-L are one system within the process of apoptosis which may be effective in the interactions of tumor cells and TIL [3,10,11,26].

FAS, a member of the tumor necrosis factor family, is expressed by a wide variety of normal and abnormal cell types, including many tumor cells. FAS-L, its ligand, known initially to be present on the surface of activated T and NK cells, was subsequently noted to be also expressed by some non-lymphoid cells and by certain types of tumor cells ([1,6,13,14,16,17,19,24,25–28,30,31,33,34,37]).

The expression of FAS-L by tumor cells demonstrated in cell cultures and in tissue sections was interpreted by some authors as evidence for its role in the defense of tumors against cytolytic lymphocytes by triggering the apoptosis of the attacking cells [21,32,35]. Thus, functional FAS-L on tumor cells was seen as a mechanism of escape from immune destruction, a hypothesis supported by some work with tissue cultures of tumor cells and described under titles such as "the tumor cell strikes back" [19,35,37]. A study exploring the presence of apoptosis-related protein such as FAS, FAS-L, bcl-2, and p53 in different types of breast tumors found them in larger amounts in carcinomas with greater reactive lymphoid infiltrates [1], while another investigation showed a higher expression of FAS-L in BRCA 1-associated hereditary mammary carcinomas than in their sporadic counterparts [13].

The present study was initiated to examine the process of lymph node metastasis of breast tumors, to explore the presence of FAS/FAS-L in this context, and to gain some insights into the mechanisms of apoptosis by comparing the expression of these mediators on normal vs. neoplastic mammary epithelium, on tumor cells vs. TILs, and on mammary carcinoma cells vs. their lymph node metastases.

Material and methods

This study included 28 cases of breast carcinoma comprising 19 duct carcinomas, seven classic lobular carcinomas, and two atypical lobular carcinomas. The age of patients with duct carcinoma ranged between 37 and 82 years, with a median of 56 years; the range of patients with lobular carcinoma between 38 and 77 years, with a median of 50 years. All cases were initial mastectomies in order to avoid the secondary changes of healing post biopsy. They were randomly chosen from the mastectomies performed over a 3–4 year span in Lenox Hill Hospital. The only selection criterion used was the presence of lymph node metastases, which by itself was associated with tumors of intermediate or high histologic grades. In each case, sections of the primary mammary tumor and of axillary lymph nodes with metastatic tumor were examined. They were routinely fixed, embedded in paraffin, and stained with the trichrom HPS (Hematoxylin, Phloxine, Saffranin) technique. They were also stained with mucicarmine and reticulin.

Immunohistochemical stainings were performed with the streptavidin–biotin–horseradish peroxidase method (LS AB2) (Dako Corp. Carpinteria, CA) using the capillary-gap technology on a BioTekMate 1000 stainer (Santa Barbara, CA). The chromogen used was 3,3-diaminobenzidine counterstained with hematoxylin. Immunohistochemistry was performed on deparaffinized fixed tissue using the LSAB2 Streptavidin–Biotin immunoperoxidase system (Dako Corp., Carpinteria, CA). Slides stained with antibodies requiring target antigen retrieval were steamed for 30 min in Dako Target Retrieval Solution (0.01 M citrate buffer pH 6.1). The slides were then allowed to cool down for 20 min and subsequently mounted on a Dako autostainer instrument for immunohistochemical staining.

Antibodies monoclonal or polyclonal to CD3 and CD20 (prediluted; Dako Corp, Carpinteria, CA), CD79A (predil. — Dako Corp.), CD21 (predil. 1:50 — Dako Corp), CD68 (predil. — Dako Corp.), HAM-56 (predil. 1:50 Dako Corp.), cytokeratin wide spectrum (predil. Dako Corp), as well as to FAS (1:50 predil.) and FAS-L (1:100 predil.), Santa Cruz Biotechnology), were used for the immunostaining of all sections.

The expression of markers was graded by a semiquantitative scoring system from 1 to 3, taking into account both the intensity of staining and the amount of positive cells. Normal unaffected tissues in the breast and lymph node specimens were used as comparative built-in controls.

Results

Histopathology

The mammary duct carcinomas were of intermediate or high histologic grades (Scarff–Bloom–Richardson