

Effects of Humoral Immunity and Calreticulin Overexpression on Postoperative Course in Breast Cancer

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Abstract The aim was to investigate whether the humoral immunity and overexpression of calreticulin in tumor tissue determined before surgery, correlate with incidence of metastases in breast cancer patients within two years after operation. Before operation, their humoral immunity and overexpression of calreticulin and Her-2/neu in tumor tissue were analyzed by immunohistochemistry. In 23 patients with metastases in regionally lymph nodes, seven had Her-2/neu overexpression. Among those seven patients, three developed distant metastasis (two women one year and in one woman two years after surgery) and all of them showed the presence of stromal IgG immunoreactivity and overexpression of calreticulin in their tumors tissue. Preliminary data showed that serum IgG immunoreactivity to tumor stroma in combination with overexpression of calreticulin in tumor cells correlate with postoperative appearance of metastases, particularly in the group of patients with Her-2/neu overexpressed tumors and metastases in axillary lymph nodes.

Keywords Breast cancer · Calreticulin · Humoral immunity · Surgery

Introduction

The role of humoral immunity in tumor eradication is intensively investigated nowadays. It was identified more than 90 TAA (tumor-associated antigens) which react with

IgG from breast cancer patients sera. Her2/neu is one of the most characterized tumor antigens [1]. Besides, it was shown that tumor spread could be influenced by protein calreticulin (CRT), chaperone protein of the endoplasmic reticulum, which is overexpressed in some human tumors and also in some cancer cell lines [2, 3]. It is also present in cytotoxic granules of lymphocytes and NK (natural killer) cells [4]. CRT may induce tumor progression, because it was shown that this protein at concentration of 2.2×10^{-7} M completely blocks perforin-mediated lysis, by stabilizing membranes preventing polyperforin pore formation [5]. It is well known that for patients with operable breast cancer, the most significant prognostic sign is the degree of involvement of ipsilaterally lymph node. Extent of nodal involvement correlates significantly with the other prognostic characteristics such as: vascular invasion, stage or size of the tumor [6]. It is also well known that bad prognostic sign is the amplification of Her2/neu gene, or its overexpression which was found in almost 40% of breast cancer [7]. The aim of this work was to investigate whether the immunoreactivity of sera IgG, IgM and IgA immunoglobulines with membranous and/or intracellular tumor antigens or with antigens in tumor stroma, determined before surgery, and overexpression of calreticulin in tumor tissue correlate with incidence of metastases in breast cancer patients with positive axillary lymph nodes and overexpressed Her-2/neu antigen, within two years after surgery.

Patients and Methods

In this work 33 patients with breast carcinoma who have undergone surgery were included. Metastases in regionally lymph nodes (N+) had 23 patients and ten patients were without them (N0). All patients had histologically con-

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firmed invasive ductal or invasive lobular breast cancer. They underwent modified radical mastectomy and received adjuvant chemotherapy or/and hormonal therapy after operation.

Cryosections of tumor tissue were incubated with patients' autologous serum diluted with 1% bovine serum albumin (BSA) in phosphate buffer saline: 1:10 and 1:50 for 30 min. After washing, they were incubated with secondary rabbit anti-human anti-IgM, anti-IgG and anti-IgA antibodies. Visualization of formed complexes was done using Universal LSAB 2 kit and further processed and analyzed by immunohistochemistry (IHC) according to the manufacturer (DAKO) recommendation. Degree of immunoreactivity was estimated on the basis of the presence and intensity of reaction between immunoglobulines and tumor cellular or stromal antigens. Overexpression of Her2/neu and CRT was also determined on paraffin embedded tumor sections by IHC according to manufacturer recommendation.

Results and Discussion

From 33 tested patients' sera, 17 showed immunoreactivity to tumor antigens: 14 had IgM, six had IgG and 11 had IgM immunoreactivity. Immunoreactivity to tumor stroma was present in 26 patients' sera: 24 had IgM, nine had IgG and 16 had IgA immunoreactivity. Overexpression of CRT was observed in 22 patients. Both, stromal immunoreactivity and overexpression of CRT had 5/33 patients: three of them developed distant metastases, one woman two years and two women one year after operation. In the group of 23 patients with metastases in regionally lymph nodes, seven of them were Her2/neu positive. Among those seven patients, four did not develop distant metastases and three developed, depending on the presence of stromal IgG immunoreactivity and overexpression of CRT.

Preliminary data showed that serum IgG immunoreactivity to tumor stroma in combination with CRT overexpression correlate with postoperative appearance of distant metastases, particularly in group of Her2/neu positive patients and lymph node metastases. There are some data in literature about the possible indirect influence of IgG and IgA on tumor development. *In vitro* data indicate that immune complexes that contain tumor antigens and IgG strongly elicit stromal production of VEGF (vascular endothelial growth factor) [8]. Besides, immune

complexes of IgG and stromal proteins could activate urokinase/urokinase receptor system which could lead to subsequent destruction of stromal proteins and activate tumor cells spreading and metastasis [9].

In conclusion preliminary data support hypotheses exposed in recent studies that humoral immunity may have influence on tumor growth and metastases through the tumor stroma and that calreticulin in tumor cells may enhance malignant behavior. Obtained data indicate the need for further study including the larger number of breast cancer patients, to confirm the importance of IgG immunoreactivity with stromal proteins for faster development of distant metastases in Her2/neu and calreticulin positive breast cancer patients with metastases in axillary lymph nodes.

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