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Profile of hMSH2 expression in breast tumors and lymph nodes: a preliminary study

D. LIRA[1], T.R. TEODORO[1], M.A. DA SILVA PINHAL[1], F.L. AFFONSO FONSECA[1,2], F. DE SOUSA GEHRKE[1], L.A. AZZALIS[2], V.B. DE CAMPOS JUNQUEIRA[2], B. DA COSTA AGUIAR ALVES[1]

[1]Department of Oncology, ABC Medical School, Santo André, SP, Brazil.
[2]Biological Science Department – UNIFESP, Diadema, SP, Brazil

Abstract. – OBJECTIVE: The mismatch repair (MMR) genes play a central role for the onset of cancer. One of these genes is hMSH2. A differential hMSH2 protein expression has been detected in the mononuclear fraction of peripheral blood of patients with breast cancer when compared to healthy women. This work aims to evaluate the expression of hMSH2 in patients diagnosed with breast cancer undergoing treatment at various stages of the disease to verify its potential use as a prognostic marker.

PATIENTS AND METHODS: Immunohistochemical expression of hMSH2 at different stages of breast cancer in 40 patients biopsy samples were analyzed.

RESULTS: hMSH2 has a considerable increased expression in all groups of patients with tumors, when compared to patients without tumors.

CONCLUSIONS: immunohistochemistry indeed can be a great tool for the diagnosis of breast cancer, as it is an easy and versatile technique.

Key Words:
hMSH2, Mismatch repair, Genomic instability, Neoplasia, Prognostic marker.

Introduction

Breast cancer is one of the most frequent cancers, with approximately 73 new cases per 100 thousand women[1]. The mismatch repair (MMR) plays a central role on the onset of cancer, both in development and in responses to therapy[2] and a dysfunction of this system can result in genetic instability that may cause tumors. Thus, genes involved in DNA repair are known to be guardians of the genome and are considered as tumor suppressors[3].

In breast cancer, changes in mismatch repair genes are associated with microsatellite instability (MSI)[4]. A high content of genomic instability linked to chromosome 2p was found in families containing at least four cases of breast cancer[5] before the age of 50. In this chromosomal region there are many breast cancer-related genes including the repair gene human mutS homolog 2 (hMSH2)[6]. A potential involvement of hMSH2 in DNA damage induced by ionizing radiation has already been described[7].

Each year approach to cancer diagnosis becomes more complex. With the availability of monoclonal antibodies, immunohistochemistry has been established and is widely used in cancer diagnosis. In a biopsy material obtained from a metastatic lesion, this technique is able to differentiate malignant tumors from different origins and locates the source of the primary tumor metastasis, through the detection of organ or tissue specific antigens[8].

A differential hMSH2 protein expression has been detected in the mononuclear fraction of peripheral blood of patients with breast cancer when compared to healthy women[9]. The present work aims to evaluate the expression of hMSH2 with immunohistochemical technique in patients diagnosed with breast cancer undergoing treatment at various stages of the disease to verify its potential use as a prognostic marker.

Patients and Methods

Patients diagnosed with breast cancer definitively established by pathologic examination were selected during their first consultations in oncology service at the Faculty of Medicine of ABC in the period from 01/01/2010 to 01/01/2011. The characteristics and purposes of the study were explained to all patients, and those who chose to

Corresponding Author: Beatriz da Costa Aguiar Alves, MD; e-mail: bcaalves@uol.com.br

participate have signed a consent form previously approved by the Ethics Committee and Institutional Research. This work was approved by FMABC Ethics Committee (384/2007).

There were 40 eligible patients in four different stages of the disease, 10 patients for each group according to their disease characteristics: patients presenting tumor with committed lymph node, patients with committed lymph node, patients presenting tumor without committed lymph node and patients without tumor and with healthy lymph node; 12 control women with healthy breast. To quantify the areas of the tumors, hematoxylin-eosin staining technique was used. The biopsy material was embedded in paraffin and sectioned with 3 μm, and immunohistochemical staining was performed according to standard technique of avidin biotin immunoperoxidase. The mouse monoclonal antibody hMSH2 (LabVision®, Fremont, CA, USA) was diluted in a ratio of 1:40 and used as primary antibody. The brownish color is considered as positive evidence to the hMSH2 expression in tumor cells. The histological slices of normal patients were used as controls.

The slides were analyzed using a microscope Nikon Eclipse TS100® to identify the areas that best represented the expression of hMSH2. We used two methods to quantify the expression: semi-quantitative analysis and digital analysis of the program. The images of 640 × 480 pixels were obtained from consecutive fields with a 400x magnification with a digital camera Nikon Coolpix 4300®, adjusted for these parameters. The images were analyzed and processed by using the program ImageLab® (Computer Softium®, São Paulo, Brazil)[10].

A semi-quantitative analysis described by Klein at al.[11] was obtained by calculating the rate of expression (EI_{sq}) that relates to the area fraction of labeled cells (ALC) with the intensity of expression (ITI_{sq}) obtained by qualitative visual observation.

Statistical Analysis

Correlations among categorical variables were analyzed using the χ^2 or Fisher's exact test. Statistical calculations were performed using GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA, USA). $p < 0.05$ was considered statistically significant.

Results

A total of 40 patients were divided in 4 four groups, according to their disease characteristics: tumor with committed lymph node, committed lymph node, tumor without committed lymph node and not committed lymph node; 12 control women with healthy breast were included. Mean age of patients and histological type are shown in Table I. Figure 1 shows the hMSH2 immunohistochemical expression profile of each group. All groups had hMSH2 expression analyzed and the results of each one are shown in Figure 2.

Discussion

We assessed the expression of hMSH2 using immunohistochemical technique at different stages of breast cancer. Results show that this gene has a considerable increased expression in all groups of patients who have tumors, when compared to patients without tumors in the groups "not committed lymph node" and control. These results can confirm the involvement of hMSH2 gene in oncogenic processes and that there is a difference of expression of hMSH2 not only in peripheral blood, but also at different stages of breast histology[9,12].

Changes in the expression of hMSH2 may be related changes in microsatellite instability (MSI), since these regions are prone to errors in replication. The repair proteins are initiated by

Table I. Average age of each group and the histological type used.

Groups	Average age	Histological type
Tumor without committed lymph node	53.1	idc*
Not committed lymph node	53.1	idc*
Tumor with committed lymph node	56.1	idc*
Committed lymph node	56.1	idc*
Healthy breast (control)	42.4	
Groups mean	52.15	

*idc: invasive ductal carcinoma

heterodimer complex hMSH2 – hMSH6 (hMutSα) and hMSH2 – hMSH3 (hMutSβ)[13]. Thus, a change in the expression of hMSH2 may also explain the distribution of mutations related to hereditary and acquired susceptibility to cancer[14]. Furthermore, hMSH2 gene is also related to another gene that plays a role in sporadic and in familial breast cancer, BRCA1. This gene encodes a tumor suppressor activity protein that regulates the repair of DNA damage, transcription, cell cycle arrest as well as apoptosis[15]. Mutations in BRCA1 – as well as in hMSH2 – are already known to be related to breast cancer[16] probably due to an increased rate of errors during DNA replication that occurs in proliferation of tumor cells.

Southey et al[17] also showed that breast cancer due to a Muir-Torre syndrome with hMSH2 mutation had all the characteristics of the dysfunction of mismatch repair including hMSH2, mea-

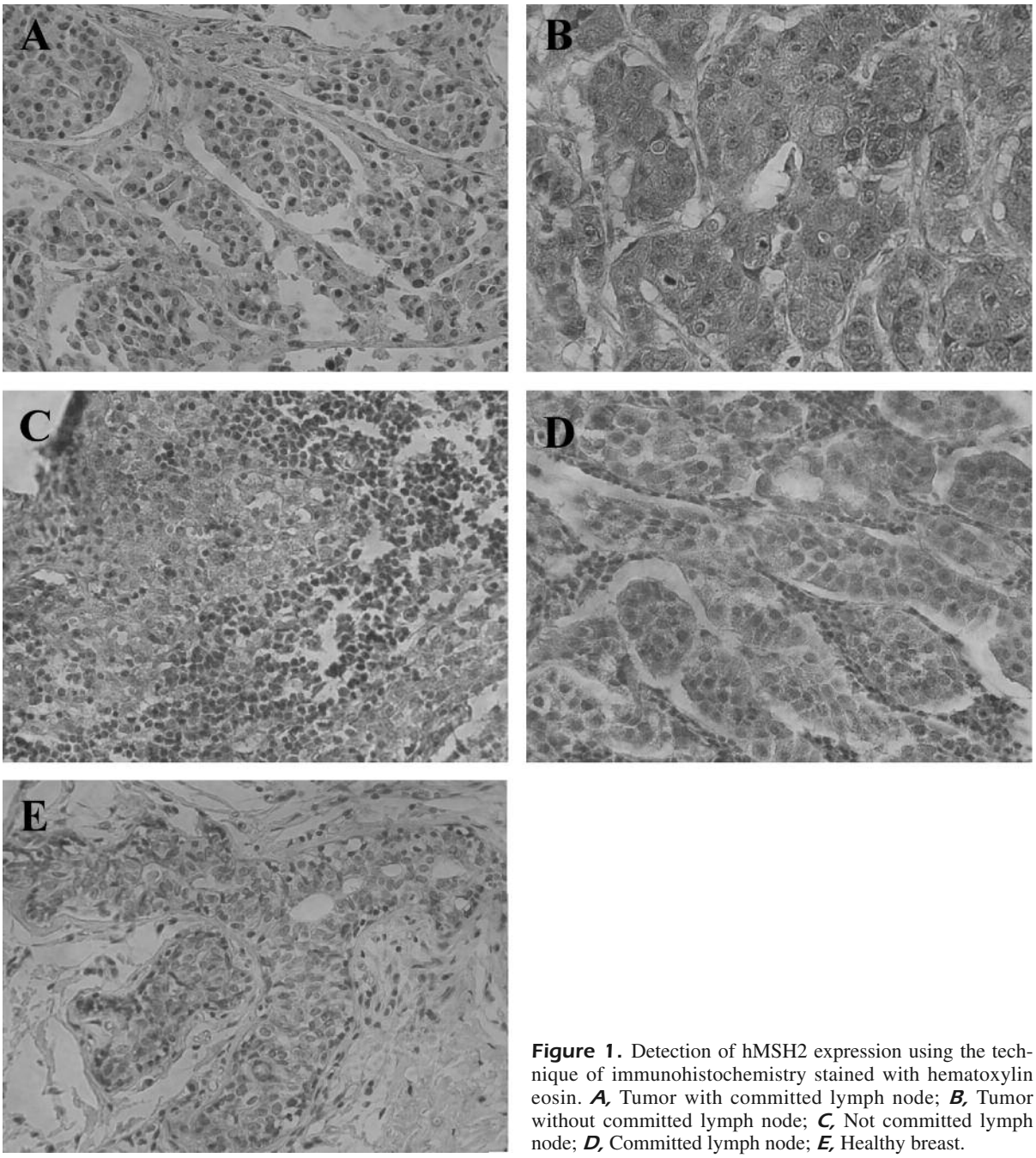

Figure 1. Detection of hMSH2 expression using the technique of immunohistochemistry stained with hematoxylin eosin. ***A,*** Tumor with committed lymph node; ***B,*** Tumor without committed lymph node; ***C,*** Not committed lymph node; ***D,*** Committed lymph node; ***E,*** Healthy breast.

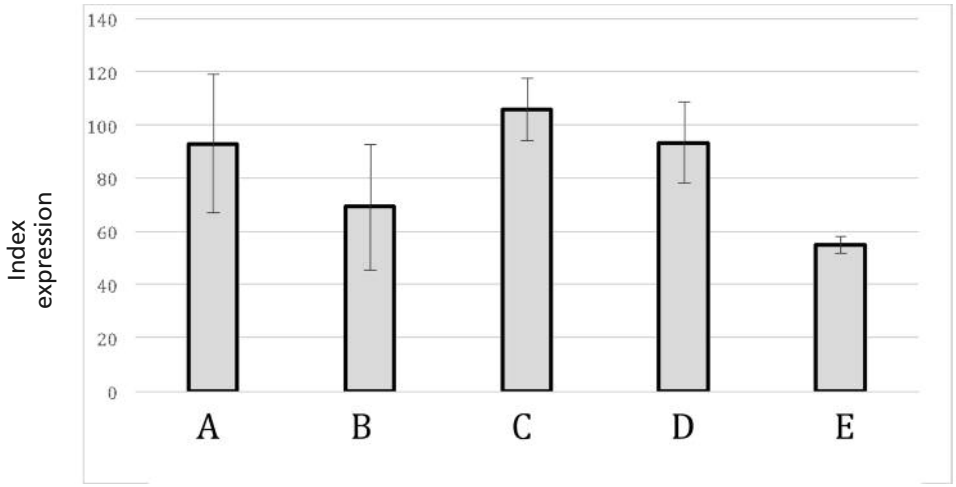

Figure 2. Average expression index and standard deviation; ***A,*** Tumor without committed lymph node; ***B,*** Not committed lymph node; ***C,*** Tumor with committed lymph node; ***D,*** Committed lymph node; ***E,*** healthy breast (control). $p < 0.0001$.

sured by immunohistochemistry and microsatellite instability (MSI). Martin et al[18] suggest that the MSH2 deficiency is associated with increased expression of the DNA polymerase β, so the immunohistochemical detection of this protein in biopsy can serve as potential biomarker. On the other hand, our group already related changes in genomic stability in others biological matrix (saliva samples)[19].

Conclusions

The technique of immunohistochemistry can be a great tool for the diagnosis of breast cancer, as it is an easy and versatile technique that indicates the presence of tumor, differentiating and finding the primary source. Our results also showed that there was an increased expression of hMSH2 in groups of patients with the presence of tumor comparing to control group (healthy breast), which suggests the potential use of this gene expression in the prognosis for breast cancer.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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