DETAILED DESCRIPTION OF RESEARCH PROPOSAL

Scientific Field: Laboratory Histology – Histopathology.
Keywords: Breast cancer, Bcl-2 oncogene, immunohistochemistry, prognostic and predictive markers.
University/Department/Division/Laboratory: University of West Attica, Department of Biomedical Sciences, Division of Medical Laboratories, Pathology Laboratory, Metaxa Cancer Hospital of Piraeus.
Language: Greek.

TITLE OF PROPOSED DOCTORAL DISSERTATION:
The study of Bcl-2 in primary infiltrating breast carcinoma and its association with the classic morphological and immunophenotypic prognostic and predictive markers.

Detailed description of research proposal

Introduction

Breast carcinoma is the most common type of carcinoma and the first most common cause of death due to malignancy in women, on a global level. It belongs to the three more common types worldwide, including lung cancer and colon cancer, regardless of gender (1). Around 1 million new breast carcinoma cases are diagnosed annually, and half of the patients and approximately 60% of deaths are found in the developing countries. Furthermore, a huge difference is observed in the survival rates following breast carcinoma diagnosis among different countries, with the developed ones being 80% more likely to achieve a 5-year survival compared to the developing ones, where this percentage is just 40%. (2).

In the era of personalized medicine, there has been significant progress in terms of the molecular analysis of breast cancer subtypes (3). The main classification schemes that are based on gene expression profiling are often referred to as intrinsic subtypes and these include the following:

- **Luminal type A:** ER positive (+) and/or PR positive (+), HER2 Negative (-)
- **Luminal type B:** ER positive (+) and/or PR positive (+), HER2 Negative (-) or Positive (+)
- **HER2+:** ER Negative (-) and PR Negative (-), HER2 Positive (+) (4) (5)
- **Triple-negative breast cancer (TNBC):** ER Negative (-) and PR Negative (-), HER2 Negative (-) (6) (7).

Unlike normal cells that grow, are divided, and die in a controlled manner, cancer cells proliferate uncontrollably. This results is the development of a mass of neoplastic cells called tumor. Characteristics of cancer cells is their resistance to the process of programmed cell death, the so-called apoptosis. Apoptosis is a highly regulated process of cell death. Unlike necrosis, which is a traumatic version of cell death, apoptosis is a conscious and active decision made to sacrifice specific cells for the greater benefits of the organism. It is a normal physiologic process routinely carried out in multicellular organisms (8).

The apoptotic process is controlled by a variety of genes, including the activation of cellular proto-oncogenes and the inactivation of tumor suppressor genes, such as genes Bcl-2 and p53 (9). Mutations of these genes cause changes in the quantity and quality of their proteins and contribute to the pathogenesis of certain types of cancer, including breast carcinoma. These genes are also involved in controlling the growth of apoptotic pathways, which seem to play a key role in tumor progression and the response to antineoplastic agents, affecting the rate of apoptosis or modifying the proliferation of cancer cells (10) (11).

Bcl-2 (B-cell lymphoma 2) is considered an oncogene that is involved in various types of malignancies, especially leukemia and lymphoma, constituting a key factor in the regulation of cell apoptosis (12).

Bcl-2 is detected in chromosome 18q21 and encodes an integrated protein in the outer mitochondrial membrane 26 kDa, which blocks the apoptotic death of cells. The overexpression of Bcl-2 protein inhibits the apoptotic cell death and activates cell proliferation and tumor progression.

The role of Bcl-2 in breast carcinoma has been investigated in various studies. Given its anti-apoptotic effect, it is expected to be associated with adverse prognosis. Nevertheless, previous studies have shown conflicting results. Some of them report an adverse effect on the survival of patients with breast carcinoma, while most studies suggest a favorable prognostic outcome. In addition, certain studies have not demonstrated the association between the expression of Bcl-2 and survival (12). The findings above are yet to answer the question of the extent to which Bcl-2 expression constitutes a significant prognostic marker.

What is also noted is the interaction between Bcl-2 and the status of estrogen receptor (ER) resulting in Bcl-2 expression differing depending on the molecular subtypes (13) (14).

Purpose

Despite the improvements in early diagnosis and treatment, the survival rate in breast carcinoma is moderate. Therefore, the evaluation of prognostic biological markers is still an important aim.

At times Bcl-2 has been suggested as a prospective prognostic marker for breast carcinoma. However, it is still not perfectly acceptable overall, given that the results of its association with patients' survival are conflicting.

The purpose of the study is to investigate the role of Bcl-2 as a potential prognostic marker in patients with primary breast carcinoma. Moreover, its relation to other molecular parameters, of significant prognostic and predictive value, will be studied: the status of hormone receptors, ER (estrogen receptor) and PR (progesterone receptor), HER2 (human epidermal growth factor 2) and the levels of the marker for cell proliferation Ki67 (MIB-1).

The aim is to control the expression of Bcl-2 in terms of its validity and reliability as an independent and powerful prognostic protein marker.

Materials and methods

In the Pathology laboratory of Metaxa Cancer Hospital of Piraeus, where Ms. Olympia Tzaida is the Director and Head of Division, n=100 cases of women with primary breast carcinoma will be recorded. In tumor sections the expression of Bcl-2 will be studied with the method of immunohistochemistry.

The following variables will also be considered:

- Age
- Tumor size
- Histological type
- Grade of histological differentiation
- Lymph node status and number of metastatic infiltrated lymph nodes
- Expression of key molecular markers:
    i. ER (estrogen receptor) and PR (progesterone receptor)
    ii. HER2 (human epidermal growth factor receptor 2) oncogene
    iii. Marker for cell proliferation Ki67 (MIB-1).

The primary tumor size dimensions, the lymph node status, and the grade of histological differentiation (according to the Scarff-Bloom-Richardson grading system) will be collected from the histopathology report.

The results of the expression of the estrogen and progesterone receptors (ER and PR respectively), the marker for cell proliferation Ki67 (MIB-1) and the oncogene of the human epidermal growth factor 2 (HER2) will be provided by the record of the Pathology Laboratory of Metaxa Cancer Hospital of Piraeus. (It should be noted that the ER and PR are considered positive when ≥ 1% of neoplastic cells exhibits nuclear immunostaining, and HER2 is considered positive when ≥10% of neoplastic cells exhibit strong complete membrane immunostaining, a result recorded as 3+).

The investigation of Bcl2 will be carried out with the use of an immunohistochemical technique in paraffin sections from tumor sample fixed in 10% neutral buffered formalin, following the international guidelines on fixation, embedding and sectioning.

Before their use, all the reagents and controls will reach room temperature (20^{o}-25^{o}C) and all the incubations alike will take place at similar temperatures. Suitable positive and negative controls will be used in all cases. The method that will be used is the streptavidin-biotin-peroxidase method.

The technical procedure will be described in detail in the technical section of the assignment.

The data collected will help us create a statistics protocol with a SPSS program.

The study is expected to be completed within a period of 36 months.

Expected results/Conclusions

Based on the results, the present study will investigate the potential prognostic role of Bcl-2 and its impact on the survival of patients with breast carcinoma. Furthermore, it will evaluate its association with the classic morphological and significant prognostic and predictive molecular markers in breast carcinoma to determine its validity and reliability as an independent and powerful prognostic marker in patients with breast carcinoma.

Literature

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