Original Paper

Bcl2 Gene Expression in Breast Tumors

SIMONA DANIELA NEAMTU[1], IULIA TUDORASCU[1], C. MARGARITESCU[1], C. TAISESCU[1] , C. TUDORASCU[2], MIHAELA NICULESCU[1]

[1] University of Medicine and Pharmacy of Craiova; [2] Hospital of Emergency Medicine Sf.Andrei Galati

ABSTRACT: Breast cancer is the most common form of woman cancer, it is a major problem for health, being the most common neoplasia with an increased incidence of morbidity and mortality. The overall growth of this form of malignancy of mammary gland disorder requires understanding the various mechanisms involved in tumorgenesis. The appearance of a very rich material and permanent concern of medicine on breast cancer incidence is justified by the occurrence of this type of cancer in young people, both women and men. The diversity of geographical spread of disease requires finding the means of limiting the expansion of therapeutic and aggravation of its evolution. In the aim of increasing life expectancy, genetic studies, the recognition of the intrinsic mechanisms intervening between cells and enter in triggering alteration cell populations involved in healing and therapeutic strategies aimed at discovering, in doing so, they gained a large share.

***KEYWORDS**: breast cancer, oxidative stress, bcl2, apoptosis*

Introduction

Breast cancer is the most common form of woman cancer, it is a major problem for health, being the most common neoplasia with an increased incidence of morbidity and mortality. The overall growth of this form of malignancy of mammary gland disorder requires understanding the various mechanisms involved in tumorgenesis. [1] There is significant data in the literature that highlights the fact that oxidative stress plays an important role in the development and promotion of various forms of cancer, including neoplasia in the breast.[2]

A few experimental and clinical studies indicate the involvement of free radicals in carcinogenicity. These, together with reactive oxygen species, interact with membranes of unsaturated bonds, obtaining nucleic acids and protein degrades [3]. The generation of reactive oxygen species leads to oxidative damage resulting from lipid peroxidation of biomolecule lipid metabolism and these are involved in neoplasia turning [2].

It is known from the international literature that there are 3 cell populations in the multicelular body: cells that are originary from the mitoza (under cell cycle); quiet cells (G0 slumbering) able to re-enter in the cell cycle when they are stimulated and cells capable to differentiate without multipling later.

Studies of cell biology, biochemistry and molecular genetics revealed common mechanisms for regulation of cell proliferation and death scheduled. Evan and Karen have proved that the traits that are evolving towards the mitosis cells, are similar to those directed to apoptosis. Today, it is accepted that the same parts of the cell cycle and gene can be used to adjust both apoptosis and proliferation. [4]

Unphysiological stimulus such as: oxidatings, ionizing radiation, cytotoxic drugs, vitamins and cancer induce apoptosis. A number of investigations have revealed that the disruption of homeostasis Ca^{++} determine the increases of Ca^{++} intracellular phenomenon which causes apoptosis.

A variety of genes in regulating apoptosis other than bcl have been identified. It is considered that apoptosis is related to the initial phase G1

- the barrier point -restriction of this phase linking the apoptosis of the cell cycle. The hypothesis is supported by the discovery that apoptosis is associated with genes like p 53, c-myc, Rb1, cyclin D1, E1a, c-fos and ckc2 and p34 and they are involved in the regulation of the cell cycle. [5]

Reactive oxygen species (ROS) are generated as the normal metabolism of the products and, once trained, they can act within the cell or can be released into the extracellular environment. Additional sources of ROS may be exogenous ones, for example, electromagnetic radiation, and ionising pollutants, cigarette smoke or it may result from the metabolism of certain drugs (anthracyclines-doxorubicina), pesticides and solventilor [2; 6].Under normal circumstances, a major source of cellular reactive oxygen species is represented by the "leakage" of electrons from mitochondrial and chains conveyor at the endoplasmic reticulum. In inflammation and

other pathological situations, polimorphonuclear and monocyte stimulated produce a large amount of H2O2 in the cellular respiratory chain; excessive amounts of H2O2 in tumor cells of human presence [7].

It has been calculated that under conditions of rest an adult uses 3.5 ml O2/kg/min. If 1% would turn into superoxyd radical, would result in 0,147 mol/day. In terms of effort, increasing the oxygen 10 times has an increase in parallel and the amount of O2-(assuming that is the percentage of 1% of the square superoxyd) [8] appearance of ROS (in small quantities) and lipoperoxydations are the major triggers and mediators of apoptosis, which can remove precancerous or cancerous cells viral infection, or other cells that are likely to affect the health of the body [9]

The idea that lipoperoxydation would be a destructive process has slowly changed in the last decade, participation in this process by demonstrating the signaling cascade controlling cellular, cellular proliferation, induce differentiation, maturation, and apoptosis (via fat hidroperoxizis of oxygenated products of degradation and very lipoperoxydation-ROS).Thus, under normal circumstances, lipoperoxydation is a metabolic process. [10, 11, 12]. The stress exerted on the body, increases the appearance of ROS, interventions are required of many antioxidant and non-enzymatic enzyme systems.

Bcl2 gene in the regulation of cell death take action planned in a number of physiological and pathophysiological situations. It was shown that the expression of bcl-2 in clonal expansion by increasing the survival of cells in carcinomas neoplastic and leads to a slow development of neoplasia over other oncogenes.

Bcl-2 is used to regulate the apoptosis in the development and progression of malignancy. Moreover, recent studies indicate the importance of the bcl2 gene to prognostic in breast tumors. The comparison of expression of bcl2 in mammary epithelium in breast invasive intraductal birth marks provides important data on the role of apoptosis in the progression and development of this type of neoplasia.

Material and Methods

Tissues and histopathological processing

Twenty-one formalin-fixed, paraffin-embedded breast tissue blocks from the archive of the department of pathology (No. 1 Emergency County Hospital, Craiova) were included in the current study. All these samples originated from complete resection material.

Sections from these paraffin-embedded blocks were stained with Hematoxylin and Eosin (HE). Two experienced pathologists (S.C. and F.C.) without knowledge of the clinical data performed re-evaluation of the HE stained sections. Diagnosis and tumoral grading were performed according to *WHO* criteria.

Histopathological data

The histopathological re-evaluation of the section was summarized in Table 1. The most numerous studied lesions was malignant (14) with ductal invasive carcinoma best represented (eight). The most encountered benign lesion was breast adenosis (three cases), especially of sclerosing type (two cases)

Table 1. Histopathological breast lesion type

Histopathological breast lesion type	No. of cases
Benign breast lesion	7
Sclerosing adenosis	*2*
Microglandular adenosis	*1*
Ductal hyperplasia	*1*
Fibroadenomas	*3*
Malignant breast lesion	14
G3 ductal invasive carcinoma	*5*
G1 ductal invasive carcinoma	*3*
Lobular invasive carcinoma	*3*
Mucinous invasive carcinoma	*3*

To view the expression I used the streptavid-biotin immunoassay technique of bcl2 detection system using polyvalent ready UltraVision work Plus DAB/HRP from company Lab Vision Corporation, USA.

There were cut sections of 5 microns thickness of paraffin. Then they were successively in xylene deparfinate, rehydrated by passage through successive baths of alcohol concentration increasing.

It was used as a type of antigenic exposure - unmasking by boiling in hot 10mmol/L Tris buffer, 1mmol/L EDTA, pH 9.0. for 20 minutes on cooling thermostat, followed by 20 minutes at room temperature.

Incubation.with primary antibody - monoclonal mouse anti- human BCL2, 124 Clone man from Dako overnight at 4 ° C in 1: 50 dilution on PBS shawl in 1% album in serum gifted BOV in A and 0.005% Tween.

The next day the sections were abundant splalat (3 x 5 minutes) and incubated and then

using the enzyme system of polymer/HRP DAKO EnVision marked kits, code Nos. K4004, incubation is made for 30 minutes at room temperature.

Contracolouring was made with HE, after which it passed to the dehydration and clarification. Mounting section has been made in the anhydrous Entelan.

In the end, the reaction was viewed with DAB.

Image acquisition was made using the microscope Nikon Eclipse 90i equipped with a 5 megapixel camera.

Pictures were taken in tiff format at the magnification of X 20 and X40 using NIS-Elements of Nikon software.

Results

Bcl-2 overexpression than mitochondrial membranes inhibits the growth of ROS in cells exposed to a number of apoptotic triggers.

It was shown on experimental mice that the deficiency of bcl2 causes severe phenotypic alterations associated with chronic oxidative stress, suggesting that one of the functions of the bcl-2 is to adjust the antioxidant pathways. [13, 14].

Initiating apoptosis studies under conditions of hypoxia, the ROS production is expected to be suppressed, they have shown that bcl-2 can protect these cells by apoptotic stimulus regardless of the generation of reactive oxygen species, bcl2 is modifying cells by apoptotic threshold of multiple mechanisms, only some of these function-dependent antioxidant [15]. I recorded a citoplamsatic benign lesions mark greater than the malignant ones.

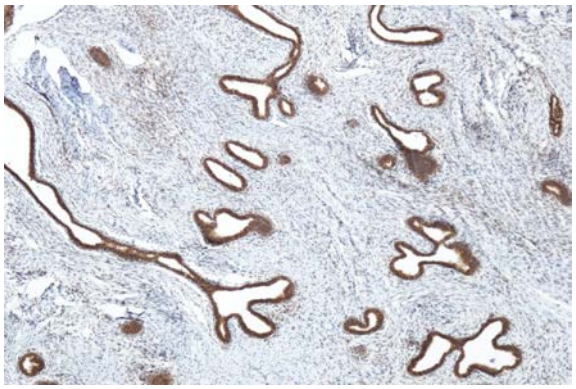

Figure 1. The expression of bcl-2 in breast fibroadenoma X 20

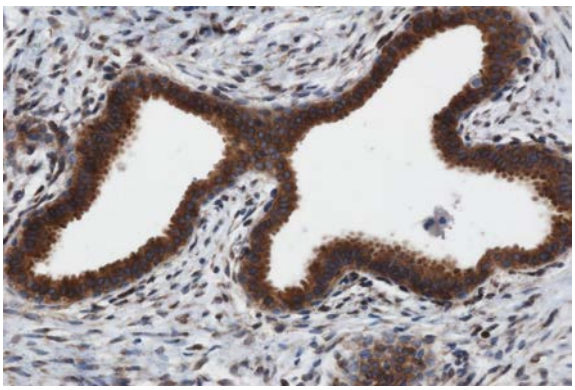

Figure 2. The expression of bcl-2 in breast X40 fibroadenoma

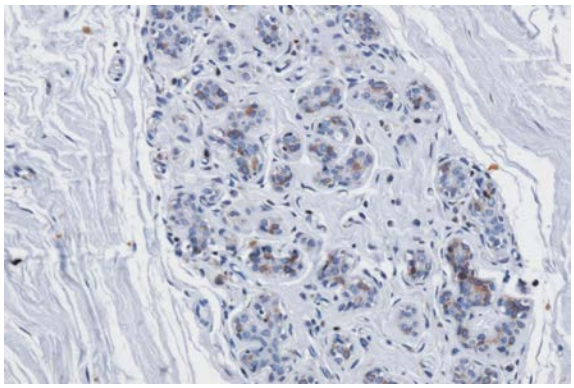

Fig. 3. The expression of bcl-2 in sclerosing adenosis X20

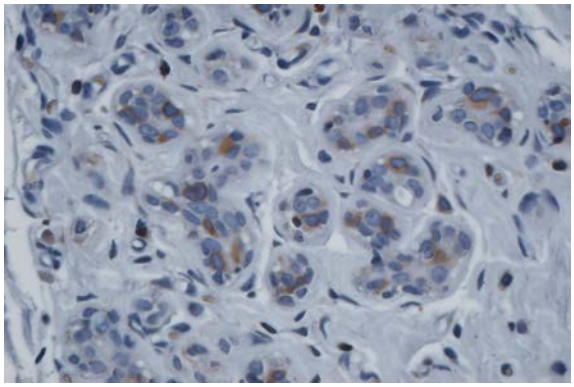

Fig. 4. The expression of bcl-2 in sclerosing adenosis X40

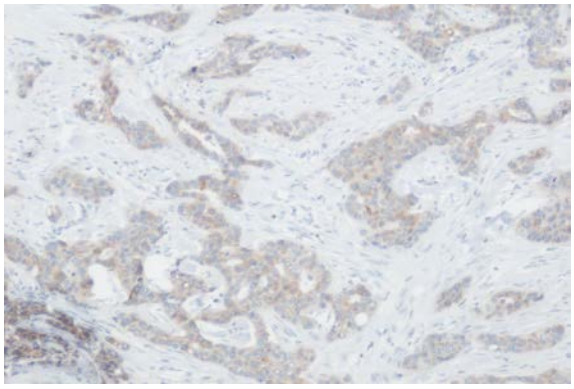

Fig. 5. Bcl-2 expression in ductal adenocarcinoma G1X20

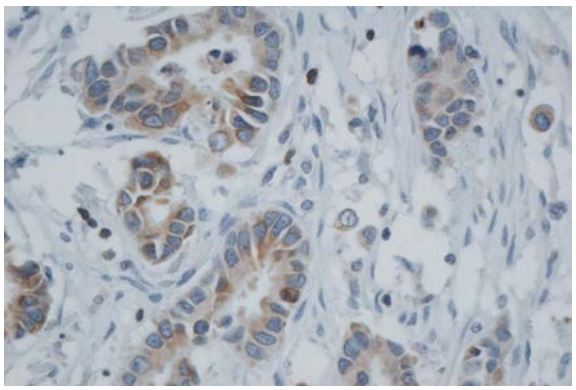

Fig. 6. Bcl-2 expression in ductal adenocarcinoma X40

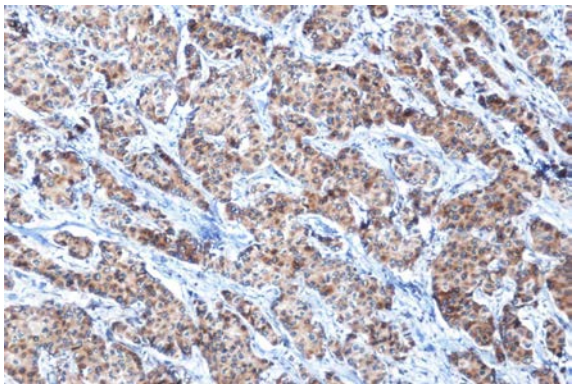

Fig. 7. Bcl-2 expression in ductal adenocarcinoma G3X20

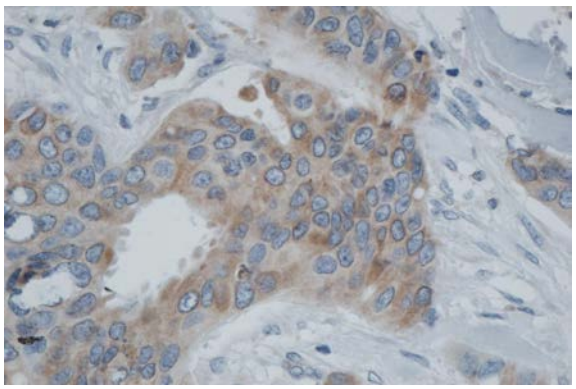

Fig. 8. The Bcl-2 expression in ductal adenocarcinoma X40 G3

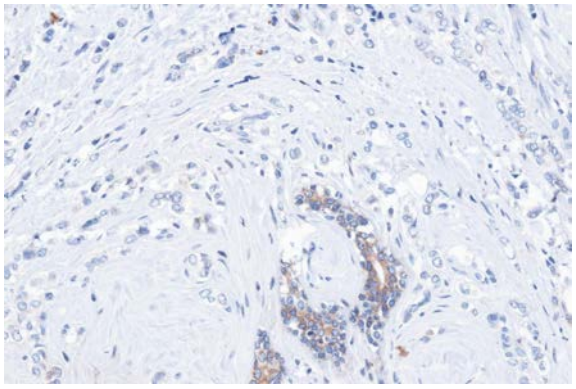

Fig. 9. The expression of bcl-2 n X lobular adenocarcinoma

In the case of invasive ductal adenocarcinomas the intensity of the mark is lower in the same time with the increase of tumoral grading.

A weak citoplasmatic mark on bcl-2 was observed in breast lobular-type birth marks, while it was absent in the mucinous type.

Discussion

Bcl-2 gene is a regulator of the cell cycle of death scheduled in a variety of physiological and pathological situations. It was shown that the expression of bcl-2 can contribute to the expansion of clonal neoplastic cells by extending survival in carcinomas [16] and it may lead to a proliferation of smaller neoplastic than other induced oncogene [17].

All these data suggest that bcl-2 is used to regulate apoptosis in epithelial development and progression of neoplasmas [18].

Furthermore, previous studies have shown the important role of expression of bcl-2 in breast cancers as important prognosis factor [19,20,21].

It was observed that bcl-2 is expressed in normal mammary epithelium [19], but at the rate of 60-80% in invasive breast cancer [22,23]

Conclusions:

1.These records suggest that bcl-2 contributes to the development and progression of breast cancer. The comparison of expression of bcl-2 in normal mammary epithelium with the expression of intraductal and invasive lesions, can provide information on the role of apotosis in the development and progression of breast cancer.

2.The expression of bcl-2 in clonal expansion by increasing the survival of cells in carcinomas neoplasia and leads to a slow development over other oncogenes neoplasia. Supraexpression of bcl-2 inhibits mitochondrial membrane in addition to increased ROS in cells exposed to a number of triggers I recorded a apoptotic citoplamatic benign mark lesions greater than in the case of the malignant invasive ductal adenocarcinom of the low intensity of the bookmark along with raising.

3.A weak mark of citoplasmatic tumoral grading from bcl-2 was observed in breast lobular-type birth marks while it was absent in mucinous.

References

1. M. Fevzi Polat, Seyithan Taysi, Mustafa Gul AT. all, Oxidant/antioxidant status in blood of patients with malignant breast tumour and benign breast disease Cell Biochem Funct 2002; 20: 327–331.
2. Elena M.V. de Cavanagh, Alba E. Honegger, Erica Hofer. at all Higher Oxidation and Lower Antioxidant Levels in Peripheral Blood Plasma and Bone Marrow Plasma from Advanced Cancer Patients, CANCER June 15, 2002/Volume 94/Number 12; 3247-3248.
3. Duck-Hee Kang. Oxidative Stress, DNA Damage and Breast Cancer, AACN Clinical Issues Volume13, Number 4, pg. 540-549© 2002, AACN.
4. Nakashima I., Kato M., Akhand A., Suzuki H., Takeda K., Hossain K., Kawamoto Y. Redox-linked signal transduction pathways for protein tyrosine kinase activation. Antioxid redox signal. 2002 jun; 4(3): 517-531.
5. Hicks Kk., Shin Jt., Opalenik Sr., Thompson Ja. Molecular mechanisms of angiogenesis: experimental models define cellular trafficking of fgf-1. Pr Health Sci J 1996 SEP; 15(3): 179-86
6. Iscan M., Coban T., Cok I., Bulbul D., Eke BC., Burgaz S. The organochlorine pesticide residues and antioxidant enzyme activities in human breast tumors: is there any association? Breast Cancer Research and Treatment, vol 72, nr.2, 2002, 173-182.
7. Ebubekir Bakan and Fazli Erdogan. Oxidant/antioxidant status in blood of patients with malignant breast tumour and benign breast disease, cell biochemistry and function. Cell Biochem Funct 2002; 20; pg. 327.
8. Zouzoui Boudjeltia K., Moguilevsky N. et al. Oxidation of low density lipoproteins by myeloperoxidase at the surface of endothelial cells: an additional mechanism to subendothelium oxidation. Biochem Biophys Res Commun 2004; 325(2): 434–8
9. Das U. A radical approach to cancer. Med Sci Monit. 2002; 8:RA79–92.
10. Barrera G., Pizzimenti S., Dianzani MU. 4-hydroxynonenal and regulation of cell cycle: effects on the pRb/E2F pathway. Free Radic Biol Med. 2004; 37: 597–606;
11. Blokhina O., Virolainen E., Fagerstedt KV.. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot (Lond). 2003, 179–194 ;
12. Cejas P., Casado E., Belda-Iniesta C. et al. Implications of oxidative stress and cell membrane lipid peroxidation in human cancer (Spain). Cancer Causes Control. 2004; 15: 707–719.
13. Veis D.J., Sorenson C.M., Shutter J.R. and Korsmeyer S.J. Bcl-2-deficient mice demonstrate fulminant lymphoid apoptosis, polycystic kidneys, and hypopigmented hair. Cell, 75: 229–240, 1993.
14. Gillardon F., Moll I., Meyer M., and Michaelidis, T.M. Alterations in cell death and cell cycle progression in the UV-irradiated epidermis of bcl-2-deficient mice. Cell Death Differ., 6: 55–60, 1999.
15. Jacobson M.D. and Raff M.C. Programmed cell death and Bcl-2 protection in very low oxygen. Nature (Lond.), 374: 814–816, 1995
16. Lu Q.L., Elia G., Lucas S. and Thomas J.A. Bcl-2 protooncogene expression in Epstein-Barr virus associated nasopharyngeal carcinoma. Int. J. Cancer, 53: 29-35, 1993
17. Hockenbery D.M., Zutter M., Hickey W., Nahin M., and Korsmeyer S.J. bcl-2 protein is topographically restricted in tissues characterized by apoptotic cell death. Proc. Nati. Acad. Sci. USA, 88: 6961-6965, 1991
18. Watson A.J.M., Merritt A.J., Jones L.S., Askew J.N., Anderson E., Becciolini A., Balzi M., Potten C.S. and Hickman J.A. Evidence for reciprocity of bcl-2 and p53 expression in human colorectal adenomas and carcinomas. Br. J. Cancer, 73: 889-895, 1996
19. Leek R.D., Kaklamanis L., Pezzella F., Gatter K.C. and Harris A.L. bcl-2 in normal human breast and carcinoma, association with oestrogen receptor-positive, epidermal growth factor receptor-negative tumors and in situ cancer. Br. J. Cancer, 69: 135-139, 1994
20. Silvestrini R., Veneroni S., Daidone M.G., Benini E., Boracchi P., Mezzetti M., Fronzo G.D., Rilke F. and Veronesi U. The bcl-2 protein: a prognostic indicator strongly related to p53 protein in lymph node-negative breast cancer patients. J. Natl. Cancer Inst., 86: 499-504, 1995
21. Zhang G., Tsuda H., Adachi I., Fukutomi l., Yamamoto H. and Hirohashi S. Value of cancer-related oncoprotein expression as prognostic factors in breast cancers with one to three axillary lymph nodes positive. Jpn. J. Cancer Chemother., 23 (Suppl. I): 66-74, 1996
22. Gee J.M.W., Robertson J.F.R., Ellis I.0., Willsher P., McClelland R.A., Hoyle H.B., Kyme S.R., Finlay P., Blarney R.W., and Nicholson R.I. Immunocytochemical localization of bcl-2 protein in human breast cancers and its relationship to a series of prognostic markers and response to endocrine therapy. Int. J. Cancer, 59: 619-628, 1994;
23. Gasparmni G., Barbareschi M., Doglioni C., Palma PD., Mauri FA., Boracchi P., Bevilacqua P., Caffo 0., Morelli L., Verderio P., Pezzella F., and Harris AL. Expression of bcl-2 protein predicts efficacy of adjuvant treatments in operable node-positive breast cancer. Clin. Cancer Res., 1: 189-198, 1995

Corresponding Author: Teaching Assistant Simona Neamtu, M.D., Hematology Department, UMF Craiova, Clinical Laboratory Municipal Hospital Filantropia, st. Constantin Brancusi no.3, Craiova ; e-mail: simona_0712@yahoo.com