

DOI: 10.29256/01.03.2017.escbm11

B-CELL LYMPHOMA-2 RECEPTOR EXPRESSION IN HUMAN BREAST CANCER

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Introduction: BCL2 is a member of regulator proteins that regulate cell death. It is considered as an important anti-apoptotic protein, which apart from its well-known function, apoptosis inhibition, can also block progression of the cell cycle by delaying entry into the S phase and maintaining cells in the G0 phase. This marker can improve individualization of patient therapy management, by predicting response to chemotherapy, hormone therapy and radiotherapy. Due to its anti-apoptotic function BCL2 is considered an important factor in the modulation of hormonal/anti-hormonal responsiveness exhibited by tumors. Patients with elevated BCL2 expression have the greatest benefit from endocrine therapy.

The **aim** of this study was to highlight BCL2 expression according to ER, PR, HER2, basal cytokeratin CK5 status and molecular subtype of breast cancer. As result was determined an affinity of BCL2 expression to Luminal tumors.

Materials and methods. Patients. There were analyzed breast carcinomas of no special type (NST) from 84 patients of 33-86 years old (57.7 ± 1.3 , with a median of 58.5). In all cases patients underwent radical mastectomy and lymph nodes dissection, without prior chemo- and radiotherapy.

Specimen processing and immunohistochemistry. The specimens were fixed in 10% phosphate buffered formalin for 24-48h and paraffin embedded (Paraplast High Melt, Leica Biosystems). The immunohistochemical assessment were processed automatically with Leica Bond-Max autostainer and included 6 surrogate markers: ER (clone Er/6F11, Leica Biosystems), PR (clone Pr16, Leica Biosystems), human epidermal growth factor receptor 2 (HER2/polyclonal, DakoCytomation), marker of proliferation Ki67 (clone K2, Leica Biosystems), basal cytokeratin CK5 (clone XM26, Leica Biosystems) and BCL2 (clone BCL2/100/D5, Leica Biosystems). All cases were evaluated also by FISH as international rules recommended (PathVysion HER-2 DNA

Probe Kit II, Abbot). The Harris modified (HHS32, SigmaAldrich) hematoxylin solution was used for counterstaining.

Microscopic evaluation. The guidelines of ER and PR assessments purposed by Allred et al. were used ^[1]. The threshold of positivity was 10%. The HER2 assessments were done with LeicaBond Oracle HER2 IHC System (LeicaBiosystem). The HER2 status was interpreted in accordance with American Society of Clinical Oncology recommendations ^[2]. For Ki67 we used a 14% threshold. The basal cytokeratin CK5 was interpreted in accordance with Azoulay et al. recommendations ^[3]. Cases evaluated as +1 to +3 were considered positive. The BCL2 evaluation was based on Callagy et al. recommendations ^[4]. Cases scored as +2 and +3 were considered positive.

Results. The most frequent histological grade was G2, determined in 45 cases (53.6%), followed by G3 with 40.5%/34 cases and G1 (6%/5 cases).

In relation to BCL2 expression, the majority of positive scores were noticed in cases with G2 (36 cases/42.9%) and G3 grades (23 cases/27.4%).

BCL2 was positive in 62 cases/73.8%. The positive BCL2 highest score was determined in cases with high expression of ER (56 cases/66.7%) and PR (44 cases/54.8%) receptors, and *vice versa* increasing of the HER2 (10 cases/11.9%) and CK5 (4 cases/4.8%) expressions led to BCL2 score diminishing.

The proliferation marker Ki67 was considered positive (≥ 14) in 50 cases/59.52% of tumors. A positive BCL2 was followed by an elevated Ki67 (≥ 14) in 32 cases/38.10% and in 30 cases/35.71% with a Ki67 < 14. In 18 cases/21.43% of high Ki67 level, BCL2 was considered negative.

The most often determined subtypes were related to Luminal B group (45 cases/53.5%), structured as: 8 cases/9.5% of Luminal B/HER2 and 37 cases/44% of Luminal B/Ki67. Tumors described as Luminal A constituted 26 cases/31% followed by HER2 (8 cases/9.5%) and triple-negative subtypes (5 cases/6%).

By comparing the molecular profile with BCL2 score the highest rate of positivity was determined in Luminal B (39.29%) and Luminal A subtype (28.57%). The ratio of positive/negative BCL2 expression vs HER2⁺ subtype was 50/50 - (4.76% positive)/(4.76% negative). Appropriate ratio was determined also for Luminal B/HER2 – 4.76% positive/3.57% negative. A single triple-negative case of 5 expressed BCL2.

Discussion. Breast carcinoma is the most common cause of death among women. Failure to undergo apoptosis is one of mechanisms of carcinogenesis and chemoresistance.

BCL2 contributes to oncogenesis because in cooperation with c-myc, ras or viral genes it can transform and immortalize cells. Its expression was associated with a better differentiation of the cancers and particularly, G1 – 100% of BCL2-positive tumors, G2 – 81%, G3 – 60%. Contradictory, Binder et al. (1995) presented a significant inverse correlation between histological grading and immunoreactivity for BCL2, recently confirmed by Ermiah et al. too [5]. In the present study a statistical significant correlation between histological grade and BCL2 was not found.

BCL2 is considered as modulator of hormonal/anti-hormonal responsiveness exhibited by tumors. Binder et al. (1995) supposed that loss of BCL2 expression induce the loss of hormonal regulation, increased dedifferentiation and deregulated proliferation. Later, Linjawi et al. (2004) determined that expressions of hormone receptors were strongly associated with BCL2 score, results which are in line with present study too.

BCL2 expression was inversely related to c-erbB-2 oncoprotein. Petry et al. (2010) purposed the concept that ERBB2 influences the expression of BCL2 family members to induce an anti-apoptotic phenotype. Authors indicated that ERBB2 alters the expression of BCL2 in a way that leads to adverse prognosis. In our assays BCL2 correlated negatively with HER2 expression.

The anti-apoptotic function of BCL2 should predispose tumor to high proliferation. No associations were observed with Ki67 proliferative status by some researches. More, a high proliferative activity assessed by Ki67 correlated inversely with BCL2 expression in primary tumor in Binder et al. (1995) experiments. The results of the present study are complementary to above mentioned.

CK5/6-positive breast carcinomas have a low BCL2 expression and highly proliferation rate. Same data arise from this study too.

Korsching et al. (2002) considered that different cellular subgroups in the female breast give rise to subgroups of breast carcinomas with different protein expression and cytogenetic alteration patterns that may be

related to clinical behavior. Approximately 80% of patients develop hormone positive tumors. Dawson et al. (2010) established that prognostic value of BCL2 was present across molecular subtypes (ER⁺/Luminal, HER2⁺, HER2⁻ and triple negative), and was independent of tumor size, grade and stage. Cases with ER⁺/BCL2⁻ pattern had a worse prognosis than those with ER⁻/BCL2⁺. The present study revealed the affinity of BCL2 expression to the ER, PR positive tumors.

Conclusion: The BCL2 expression is not dependent on carcinomas proliferative activity. The highest scores were determined in cases of BCL2 co-expression with ER and PR receptors. The HER2 and CK5 exhibition led to decrease BCL2 score. Luminal B and Luminal A carcinomas are leaders in BCL2 positivity.

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Accepted for printing on 23 Oct 2017