EXPRESSION OF BMI-1 PROTEIN IN CERVICAL, BREAST AND OVARIAN CANCER

Mihaela-Madalina Gavrilescu¹, Ana-Maria Todosi², Maria-Gabriela Aniței², B. Filip², V. Scripcariu²
University of Medicine and Pharmacy “Grigore T. Popa” - Iasi
1. Ph.D. student
2. Discipline of Surgery

EXPRESSION OF BMI-1 PROTEIN IN CERVICAL, BREAST AND OVARIAN CANCER

(Abstract): B-cell-specific Moloney murine leukemia virus integration site 1 (Bmi-1) is a member of the polycomb group, which participates in axial patterning, hematopoiesis, cell cycle regulation, and senescence. Overexpression of Bmi-1 has been reported in various human cancers and proved to be associated with poor survival. Discussion: Bmi-1 is expressed by various tumors and therefore may contribute to malignant transformation. Bmi-1 not only can lead mammary epithelial cells to senescence and immortalization, but also plays a key role in breast cancer. A significant correlation was observed between Bmi-1 expression and axillary lymph node metastases in lymph-ductal breast cancer. Bmi-1 is expressed in cervical cancer and correlated with a poorer prognosis, suggesting that this protein participates in the development and progress of cervical cancer. Regarding ovarian cancer, the results of several immunohistochemical studies revealed overexpression of Bmi-1, especially in poorly differentiated ovarian carcinoma. There is a strong correlation between histological grade, clinical stage and its expression. Conclusions: Human genes of polycomb group correlated with various hematologic and epithelial cancers identify new mechanisms of malignant transformation and pave the way for developing new cancer treatments and identifying new diagnostic markers. Bmi-1 and its expression in tissues taken from patients with cervical, breast and ovarian cancer could be a marker for diagnosis and prognosis, and not least a potential target of antitumor therapy. Keywords: BMI-1 PROTEIN, IMMUNOHISTOCHEMISTRY, ANTITUMORAL THERAPY

B-cell-specific Moloney murine leukemia virus integration site 1 (Bmi-1) is a member of the polycomb group, involved in hematopoiesis, cell cycle and cell aging. Recently, Bmi-1 overexpression has been reported in many cancers and was associated with a low survival rate (1). Human polycomb group genes related to various hematologic and epithelial cancers, identify new mechanisms of malignant transformation and open a path to developing new cancer treatments and identifying new diagnostic markers.

Polycomb group proteins are silent genes with key roles associated with body development by at least two multimeric complex: polycomb repressive complex 1 (PRC1) and repressive complex 2 (PRC2). Bmi-1 protein, a core member of PRC1 complex, was identified as oncogene and
Expression of Bmi-1 protein in cervical, breast and ovarian cancer

together with c-myc initiates the occurrence of lymphoma. Bmi-1 gene is located on human chromosome 10p11.23 and extends over 4.9 kb, comprising 10 exons and 9 introns. It contains a RING finger domain type in its N-terminal end, and a central helix-turn-helix motif, which is required for inducing telomerase activity and immortalization of human epithelial cells (2).

It has been shown that Bmi-1 would be involved in several biological processes, such as embryonic development, organ formation, tumorigenesis, stabilization and differentiation of stem cells (3). Bmi-1 is expressed ubiquitously in almost all tissues and its expression was found to be slightly higher in the brain, spinal cord, kidneys, lungs, gonads, and placenta. However, several studies have shown that Bmi-1 expression is frequently increased in various human cancers, including lung cancer, ovarian cancer, acute myeloid leukemia, nasopharyngeal carcinoma, breast cancer, and neuroblastoma, indicating that Bmi-1 could play important roles in the initiation and progression of cancer. The oncogenic feature of Bmi-1 was also reported to be associated with cell protection from apoptosis (4).

DISCUSSION

Ovarian cancer. Prognosis of ovarian cancer has not improved substantially by standard classical treatment, in spite of new surgical techniques, chemoresponsivness of this malignancy to classical cytostatic agents, and efforts to detect it in early stages, accounting for the current global interest in ovarian cancer research. Recent molecular biology and genetic researches focus on the detection of molecular-genetic defects associated with this type of cancer, and their relation with morphopathological and clinical-imaging aspects and distinct clinical developments, which ultimately have direct therapeutic implications: molecular treatment targeting the molecular defect, customized treatment, with impact on survival (5).

Ovarian cancer has the highest mortality rate of all gynecologic malignances (6). Despite an initial response to surgery and first-line platinum- or taxane-based chemotherapy, most tumors eventually develop drug-resistant relapse. Evidence suggests that resistance to cisplatin may be the result of a defective apoptotic program. In this case, increased levels of DNA damage would be required to induce apoptosis initiation signal (7).

To investigate whether the abnormal presence of Bmi-1 is involved in the pathogenesis of ovarian carcinoma in a study conducted at the Oncology Laboratory of Sun Yat-Sen University, China in 2010, protein expression was first examined immunohistochemically in normal, benign, and borderline ovarian tissue and tissues from epithelial ovarian cancers. The results demonstrated that Bmi-1 expression in all normal ovary specimens was absent or low. In ovarian tumor specimens, a significantly increased Bmi-1 expression was observed in benign cystadenoma to borderline tumor, and carcinoma. Moreover, it was found that the frequency of Bmi-1 expression in undifferentiated ovarian carcinomas was significantly higher than in other types of carcinoma. Higher Bmi-1 expression in ovarian cancers was strongly correlated with tumor histological grade and clinical stage (pT / pN / pM). These findings suggest that Bmi-1 overexpression in ovarian carcinoma may be an acquired feature of the malignant phenotype (8).

Breast cancer is the most common malignancy in women, its mortality and morbidity continuing to grow despite the re-
markable progress in diagnosis and therapeutic approach in recent years. In the past decades, there was a continuous concern for the early detection of breast cancer and development of effective treatment modalities which to lead to a decline in breast cancer deaths and improved quality of life for women struggling with this disease. Along with conventional histopathological diagnosis of malignant breast tumors, which grades and classifies them into microscopic subtypes, significant progress in assessing the prognosis of these patients was made with the investigation of some immunohistochemical markers involved in tumor carcinogenesis.

Invasion and metastasis processes that cause great mortality are extraordinary distinctive features of breast cancer progression. Although lymph node metastases, tumor size, and histological grade of differentiation are usually considered to be prognostic markers for metastases, these occur at distant sites in 20-30% of the patients without lymph node involvement (9). So far, Human Epidermal Growth Factor Receptor (HER-2/neu), c-myc, and HOXB9 emerged as metastasis risk predictors in breast cancer. Their aberrant expression can induce the expression of growth and angiogenic factors, resulting in higher local concentrations of these factors in the tumor microenvironment, thus favoring tumor progression (10).

Bmi-1 overexpression increases motility and invasive properties of mammary immortalized epithelial cells, which is concomitant with increased expression of mesenchymal markers and decreased expression of epithelial markers. Consistent with these observations, Bmi-1 repression in metastatic breast cancer cells remarkably reduces cell motility, invasion, and transformation, as well as metastasis and tumor-igenesis in mice. It has been demonstrated that Bmi-1 promotes invasion and metastasis of breast cancer and predicts poor survival (11).

Bmi-1 mRNA is significantly higher in adjacent normal breast tissue in breast cancer patients compared with normal breast tissue from patients with non-cancerous lesions. By contrast, Mel-18 mRNA level is lower in normal tissues of patients operated for breast cancer compared with breast tissue from mammoplasty. When protein expression in these two genes was evaluated, it was noticed that most epithelial cells were positive for Bmi-1 in both groups of tissue samples, although expression intensity was stronger in the normal tissue of cancer patients. Bmi-1/Mel-18 ratio can be used as a tool to stratify women at risk for developing malignancies (12).

Early detection of breast cancer is critical for improving patients’ chances of survival. Techniques designed to detect recurrent or metastatic disease in the preclinical stages can contribute to this goal. The presence of Bmi-1 oncogene in plasma was assessed in a wide range of primary breast carcinomas with the aim at investigating its correlation with tumor clinicopathological parameters and survival rate. Study results suggest that Bmi-1 expression may be a marker of poor prognosis and clinically it may become a noninvasive diagnostic marker (13).

Cervical cancer. Despite significant progress in the etiology, diagnosis and treatment of cervical cancer, its incidence and epidemiology factors were not correspondingly influenced its resurgence in parallel with increased mortality both in as our country and worldwide being recognized. The reality is hard to justify and, especially, to accept, since it is the only
Expression of Bmi-1 protein in cervical, breast and ovarian cancer

cancer with known etiology, viral, sexually transmitted, and cured 100%, of course in early forms, by complex, multimodal treatment. Without minimizing the undeniable progress in its etiopathogenesis, diagnostic and therapeutic means, and methods of prevention and early detection, we believe that in this field modern medicine owes, and stronger action must be taken for decreasing the incidence and improving the treatment of the disease at curable stage. It is noteworthy that this is a cancer benefiting from early detection and prevention and coded therapy, likely to cure the patients. Cervical cancer is the second most common cancer in women worldwide, with approximately 500,000 new cases diagnosed and 250,000 deaths each year. Almost 80% of cases are from developing countries (14).

Virtually all cervical cancers (99%) are related to HPV (Human Papilloma Virus) infection. In our country, every year approx. 2,800 new cases are detected, situation similar to that in developing countries from Africa, Asia and South America. According to a study by the World Health Organization, the mortality rate in Romania is 2 to 2.7 times higher than in most Central and Eastern European countries, and 6.3 times higher than EU average, the main cause responsible for this high mortality being the same factor: late diagnosis, and absence of mass screening programs, respectively. And this, in the conditions of a well organized and equipped network for the current practice of cervicovaginal exfoliative smear cytology. The natural corollary of the high incidence of cervical cancer mortality in our country is its proportional mortality, which, constantly in the last two decades, is the highest in Europe: 11.01 to 100,000 women (14).

The role of neoadjuvant chemotherapy in locally advanced cervical cancer has been studied in the past 25 years; this therapy reduces tumor size and lymph node positivity and allows minimizing the postoperative radiation therapy (15). Neoadjuvant chemotherapy, which can reduce the size and thereby increase tumor resectability, has been proposed as treatment of locally advanced cervical cancer. A study on a series of 110 patients, of which 68 received neoadjuvant chemotherapy and 42 were subjected to surgical procedure as the first therapeutic choice was conducted at the Peking University "People" Hospital. The results showed that 70.6% of patients achieved complete or partial response to neoadjuvant chemotherapy. Estimating the blood loss, duration of intervention, number of lymph nodes removed, the rate of complications during and after surgery, no significantly differences between the 2 groups were recorded. However, the rate of lymph-vascular space involvement was lower in the group who received neoadjuvant chemotherapy (16).

Neoadjuvant chemotherapy together with radical surgery increase the disease-free interval (DFS) and overall survival (OS) of patients with locally advanced cervical cancer compared with surgery as the first therapeutic act (17).

Bmi-1 is expressed in cervical cancer and correlates with a poorer prognosis, suggesting that this protein could participate in the development and progression of cervical cancer. It can be an independent prognostic of overall survival rate (18).

In a study by the Department of Gynecology and Obstetrics, University of Wurzburg, Bmi-1 expression in ovarian, breast, cervical and endometrial cancer tissues was determined by Western blot and
immunohistochemistry techniques. The results showed that Bmi-1 protein was significantly increased (19).

CONCLUSIONS
Bmi-1, polycomb group gene, regulates the proliferative activity of normal stem and progenitor cells. It is also essential for self-renewal of neural cells and hematopoietic stem cells. Bmi-1 is frequently increased in ovarian cancer and correlates with clinical stage, presence of lymph node metastasis, and poor prognosis.

Bmi-1 protein expression is more pronounced in primary breast cancer tissues compared to adjacent non-cancerous tissues. Increased Bmi-1 expression is correlated with advanced clinicopathological classifications (T, N, M) and clinical stage.

In addition, high Bmi-1 levels indicate a lower overall survival and serves as a marker of increased risk for breast cancer.

Polycomb group genes related to various hematological and epithelial cancers identify new mechanisms of malignant transformation and open the door to developing new cancer treatments and identify new diagnostic markers.

Bmi-1 expression in tissues collected from patients with cervical, breast and ovarian cancer could be a diagnostic and prognostic marker, and last but not least a potential target of antitumor therapy.

ACKNOWLEDGEMENTS
This work was supported by European Social Founds, project POSDRU/107/1.5/S/78702.

REFERENCES
Expression of Bmi-1 protein in cervical, breast and ovarian cancer


**NEWS**

**UNIVERSAL DNA VACCINE VECTOR**

Kong *et al.* describe the development of recombinant attenuated *Salmonella Typhimurium* strains that can be used as universal platform for delivering DNA vaccines to stimulate mucosal, systemic and cellular protective immunities. These *S. Typhimurium* strains have features that enable them to colonize host tissues and allow the release of bacterial contents after lysis. The purpose of the study was to transform a recombinant attenuated *Salmonella* vaccine (RASV) strain into a universal DNA vaccine-vector. The strains were genetically modified to display a hyperinvasive phenotype, in order to maximize host entry and cell internalization, to enable endosomal escape and release of the DNA vaccine into the cytosol and to decrease *Salmonella*-induced apoptosis. This will allow efficient nuclear trafficking of the DNA vaccine for synthesis of encoded protective antigens. According to this study, a DNA vaccine encoding influenza virus HA antigen, delivered by RASV strain, induced complete protection to mice against influenza virus (Kong W, Brovold M, Koeneman BA et al. Turning self-destructing Salmonella into a universal DNA vaccine delivery platform. *Proceedings of the National Academy of Sciences*, 2012; DOI: 10.1073/pnas.1217554109).

*Teodora Vremeră*