benign and borderline. While the difference in the IHC expression of EGFR between benign and malignant were significant (p = 0.027) and also between borderline and malignant were significant (p = 0.014), there was no different relation between benign and borderline cases.

Conclusions: The results of this study provide evidence that immunohistochemical CD10 and EGFR over-expression is significantly related with the degree of malignancy and pathogenesis of phyllodes tumors.

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Flow Cytometry Analysis of Circulating Endothelial Cells in Women with Breast Cancer. Preliminary Results

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Background: Despite advances in diagnosis and treatment, breast cancer (BC) remains one of the main causes of cancer death in women. Currently, all relevant prognostic information should be obtained integrating traditional clinicopathological parameters with the molecular classification of BC, and measurement of different tumor-specific markers. Angiogenesis is crucial for tumor growth, and it has been shown that mature and immature endothelial cells, such as circulating endothelial cells (CECs) or endothelial progenitor cells, are present in the blood. CECs are extremely rare in normal peripheral blood, but their number increases significantly in cancer patients. Different cell surface markers have been used to detect these cells, and flow cytometry is a method well suited for their detection and quantitation. The aim of this study was to assess the levels of the microenvironmental cell marker $CD146^+CD45^-$ phenotype, which is linked to angiogenesis, vessel damage, and disease progression, in patients with metastatic and localized BC.

Patients and Methods: Blood samples from 34 women (median age 48 years, range 33−76 years) with primary invasive ductal carcinoma were collected for analysis of CECs. Patients with distant metastases (M+) have been excluded, as well as those who underwent adjuvant chemotherapy. All samples were stained with antihuman CD146-PE and CD45-FITC, and immunophenotyping was obtained using a Beckman Coulter XL-MCL flow cytometer to assess levels of $CD146^+CD45^-$ CECs, using a 600 s acquisition time for each sample. Using a post-hoc criteria, two age-matched groups of patients were obtained: Group A (18 patients) with local disease and negative (N0) axillary lymph nodes (AN), and Group B (16 patients) with positive (N+) AN. Overall, in this group, 329 AN (median 19, range 15−26 per patient) have been removed, of which 34 (10.3%) were N+.

Results: Patients with localized BC (Group A) had a level of $CD146^+/CD45^-$ cells significantly increased (451±357 *vs.* 87±72 CECs, $p < 0.0001$) in respect to those with metastatic disease (Group B). No correlation (R = 0.11, $p = 0.16$) was found between number of the N+ and level of putative CECs.

Conclusions: Although the rare nature of CECs ($<10^{-4}$ elements per mononuclear blood cell) and their not yet completely established phenotype represent a technical challenge, our preliminary data suggest a possible increased angiogenic activity in patients with metastatic BC, which can be demonstrated using flow cytometry analysis of CECs. However, with the aim of increasing sensitivity (note the very high standard deviation), further endothelial cell markers, alone or in combination, should be studied.

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The Reference of Immunodeficiency and Molecule-Genes Characteristics of Breast Cancer (BC)

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Immunological disorders have variability and can change hormone answers of cancer treatment, it mainly concerns Luminal A (LA) BC.

The aim of the research was to compare immunological characteristics of common variable immunodeficiency (CVID) and of serious combination immunodeficiency (SCID) on different molecule-genes forms of BC. 33 patients of the USMA department of oncology have been included in the research since 2007 with complex treatment of BC ($T_{1-4}N_{0-2}M_0$), on LA, Luminal B (LB) and triple-negative (TN) forms of BC of different genes of San-Gallen consensus 2011, at the age of 50.2±10.9 years. All the patients were divided into three groups. Group I were LABC (n = 15), Group II were LBBC (n = 10) and Group III were TNBC (n = 8). The division in groups was in cases of decrease of any index of immunoglobulin (A, M, G) smaller than standard with B-cells less than $0.2*10^9$/l − it was CVID. The SCID was a mixture with decrease CD4+ less than $0.5*10^9$/l cells by D. Mail classification 2007. We compared indexes of leucocells, limfocells, CD3+, CD4+, CD8+. The Coefficient Reaction was index “Cr” (Cr = nCD3+stimulation/nCD3+wild where n = count CD3+ that can synthesize cytokines TNF-α, IL-2, IFN-γ in the tests after stimulation and in wild). The CVID and the SCID were typical compare points for groups I and III, with the reliability index X^2 >2.

Luminal A BC with CVID and SCVD has differenced by the index of leucocells that was ($6.2\pm1.5\times10^9$l and $3.5\pm0.7\times10^9$l), the count of limphocells was ($1.9\pm0.2\times10^9$l and $0.9\pm0.2\times10^9$l), CD3+ ($1.3\pm0.4\times10^9$l and $0.6\pm0.1\times10^9$l), CD4+ ($0.8\pm0.3\times10^9$l and $0.3\pm0.05\times10^9$l), CD8+ ($0.5\pm0.1\times10^9$l and $0.3\pm0.1\times10^9$). LB of BC with CVID and SCID has differenced by CD4+ ($0.7\pm0.2\times10^9$l and $0.4\pm0.06\times10^9$l). TN has defined in groups with CVID and SCVD of indexes of leucocells ($6.7\pm0.7\times10^9$l and $5.2\pm0.9\times10^9$l), CD4+ ($0.7\pm0.06\times10^9$l and $0.3\pm0.07\times10^9$l). “Cr” in groups I and III was different in CVID for TNF-α (30.9±12.0 and 123.5±24.3, X^2 >2) and in SCID for IFN-γ (35,9,1±40.3 and 138.5±82.9, X^2 >2).

As a conclusion, the difference between CVID and SCID can be the core prognostics problem for LA, LB and TN of BC. The prognosis of LA of BC can be the worst for SCID. And the prognosis of aggressive TNBC may be better if patients have only CVID. This difference can be a good way of immunology correction during hormone therapy for LA or chemotherapy of TNBC among the women of the Ural region. The “Cr” may be an interesting aim for individual correction of TNF-α and IFN-γ.

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Inhibition of Breast Cancer Angiogenesis and Metastasis by P16 Gene Therapy − Downregualting VEGF Expression Via Viral-mediated P16is Interaction with HIF-1a

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Background: One effective approach to suppress malignant breast cancer (BCa) progression is to block tumor angiogenesis. Vascular endothelial growth factor (VEGF) plays a pivotal role in tumor angiogenesis. Because the degree of tumor malignancy directly correlates with the expression of VEGF, but inversely correlates with the expression of tumor suppressor gene p16, we examined whether restoration of p16 in BCa cells would modulate VEGF expression and consequently suppress BCa angiogenesis and metastasis.

Materials and Methods: To facilitate induction of p16 expression, a recombinant adenovirus expressing p16 (AdRSVp16) was generated and used to transduce BCa cells. The p16 effects on BCa angiogenesis and metastasis were examined by a series of assays including the dorsal air sac model and spontaneous metastasis animal model. The mechanism of p16-mediated modulation of VEGF expression was further analyzed by studying p16’s effect on hypoxia inducible factor-1a (HIF-1a), the transcriptional factor of VEGF gene promoter, by both co-immunoprecipitation and colocalization assays.

Results: We found that adenoviral-mediated p16 expression down-regulated VEGF gene expression in breast cancer cells, inhibited BCa cell-induced angiogenesis and suppressed breast tumor metastasis in a spontaneous metastasis animal model. Moreover, p16 appears to bind directly to HIF-1a, and consequently translocates cellular location of HIF-1a from the nucleus to cytoplasm in BCa cells.

Conclusions: These results demonstrated that p16 modulates VEGF expression and inhibits tumor-induced angiogenesis and metastasis. The binding between p16 and HIF-1a protein appears to alter HIF-1a’s cellular localization and HIF-1a’s ability to transactivate VEGF expression. This study reveals a novel function of p16, namely, p16’s anti-angiogenesis function by its interaction with HIF-1a and downregulation of VEGF gene expression. The dual function of p16’s anti-angiogenesis and its well-known anti-proliferation should warrant p16 gene transfer as an effective therapeutic strategy for clinical treatment of BCa patients.