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## ER signaling molecular classical and nonclassical pathways

**KEYWORDS:** Receptors, Estrogen; Signal Transduction; Gene Expression; Gene Expression Regulation; Drug Therapy; Transcription, Genetic

The ER activates gene expression in two ways. In the best understood mode of action, or classical pathway, upon binding to E2, ER dimerizes and interacts with permutations of a palindromic DNA sequence separated by three non specific nucleotides: 5'-GGTCAnnnTGACC-3', the consensus estrogen response element (ERE). The E2-ER-ERE complex subsequently recruits coactivators/regulators to promote local chromatin remodeling and the bridge with general transcriptional factors for the initiation of transcription. This pathway is called ERE-dependent ER signaling. Upon binding to E2, ER also regulates gene expression through functional contact with other DNA bound transcriptional factors, such as AP-1, SP-1 or NF-κB. Once tethered to DNA, the receptor can either positively or negatively regulate target gene transcription without directly interacting with DNA, nonclassical action. This is the DNA-dependent and ERE-independent signaling pathway. In addition, functional synergism has been documented between ER and AP-1 or SP-1, while mutual antagonism has been suggested between ER and NF-κB. Furthermore, E2 elicits effects through the membrane and cytoplasmic ERs. Membrane E2-ER complexes activate protein-kinase cascades, leading to altered functions of proteins in the cytoplasm, e.g. the functions of ER themselves, or to regulation of gene expression through phosphorylation and activation of a transcription factor. The possible convergence of genomic and nongenomic action on target genes is an attractive mechanism by which ER can finely regulate gene expression. The relative contributions of genomic and nongenomic actions to certain gene responses in vivo, however, remain to be elucidated. The aim of this review is to outline what is known about the ER signaling molecular pathways and discuss how this information can be used to identify new targets where pharmaceutical exploitation could yield novel therapeutics.

## The role of estrogen receptor isoforms in breast cancer

**KEY WORDS:** Breast Neoplasms; Receptors, Estrogen; Receptors, Progesterone; Antineoplastic Agents, Hormonal; Reverse Transcriptase Polymerase Chain Reaction

**Background:** Estrogen and progesterone receptor (ER/PR) status is an accepted predictive marker in breast cancer. It is well known that breast tumors which are ER(+) are more likely to respond to endocrine therapy. However, certain percentage of ER(+)/PR(+) tumors do not respond to endocrine therapy. Until the mid of the 1990s, it was assumed that only one form of receptor for estrogen exists (now known as estrogen receptor alpha – ERα). Identification of the second estrogen receptor, named estrogen receptor beta (ERβ), as well as the existence of numerous isoforms/splice variants of both ERα and ERβ, suggests that complex regulation of estrogen action exists. The existence of various isoforms and splice variants of both ERs additionally complicates elucidation of their involvement in the process of carcinogenesis, as well as in resistance to endocrine therapy. In this study, we analyze does the expression of two ERβ isoforms correlates with ERα/PR status.

**Methods:** Sixty samples of primary operable breast carcinomas were analyzed for ERα and PR protein levels and for mRNA expression of two ERβ isoforms (ERβ1 and ERβΔ5). ERα and PR proteins were measured by classical biochemical techniques (LBA), and ERβ mRNAs were measured by real-time RT-PCR.

**Results:** Tumors are divided in “low expressed” and “high expressed” according to relative level of mRNA for ERβ1 and ERβΔ5. We found that there is no correlation of ERβ1 mRNA expression with ERα and PR protein levels. We confirmed the existence of inverse correlation of ERβΔ5 with PR and of ERβΔ5 with ERα in the group of postmenopausal patients. In the subsets of tumors defined by ERα/PR status, we found that percentage of tumors which concomitantly expressed high levels of both transcripts, are parallel with those that do not response to tamoxifen treatment.

**Conclusion:** Inverse correlation of ERα with ERβΔ5 and PR with ERβΔ5 isoform suggests that ERβΔ5 may have inhibitory effect on ERα activity in postmenopausal patients. Until now, this activity was showed only in vitro. In addition, we wish to point out that determination of expression profiles of ERα and ERβ isoforms in the defined groups of patient are necessary for elucidating its involvement in endocrine resistance.



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## Cross-talk between ER and HER2 in breast carcinoma

## Estrogen receptor as the predictive factor for response to chemotherapy in breast cancer

**KEYWORDS:** Breast Neoplasms; Receptors, Estrogen; Receptor, Epidermal Growth Factor; Receptor, erbB-2; Receptor Cross-Talk

**KEY WORDS:** Breast Neoplasms; Receptors, Estrogen; Antineoplastic Agents; Treatment Outcome

In tumors in which estrogen receptor (ER) and growth factor signaling pathways are simultaneously active, there is a bidirectional cross-talk which results in a positive feedback cycle of cell survival and proliferation stimuli. Beside the postulated inverse correlation between ER and HER2 (human epidermal growth factor receptor 2) as a consequence of repressive feedback signaling loop, there are also other mechanisms regarding ER-HER2 interactions, involved in endocrine resistance. Numerous studies indicate that MAPK pathway that is crucial not only for cell survival and proliferation, has a central role in synergistic action between ER and HER2 in normal mammary gland development, as well in the breast cancer. MAPK pathway is hyperstimulated in cells that overexpress HER2 as a consequence of HER2 gene amplification, that is one of the most frequent gene alterations in breast cancer. MAPK are also important for ER activity in ER+ tumors because they phosphorylate and activate either ER itself or ER coregulators, enhancing the transcriptional activation potential of ER its effects on cell proliferation and survival. ER and HER2 signaling could interact on multiple levels (genomic or non-genomic) and therefore might induce reduced ER expression or, on the other hand, might increase ER function, but in all this cases the net result could be altered responsiveness to endocrine manipulation. The importance of understanding ER-HER2 cross-talk is not only because of its significance in breast cancer progression, rather because it seems to be fundamental factor in endocrine resistance, leading to improvement in treatment strategies, especially targeting MAPK pathway.

Estrogen receptor (ER) is a strong predictor to endocrine therapy in breast cancer (BC): the higher the ER content within tumor cells, the better the response to endocrine therapy. In ER-negative BC chemotherapy (CHT) is a therapy of choice. However, it was generally accepted that BC cells are equally responsive to chemotherapy irrespective of ER status. This was emphasized by the conflicting results of sensitivity of ER-positive and ER-negative metastatic BC to CHT. However, subset analyses of disease outcome in these BC cohorts in recently reported trials on neoadjuvant and adjuvant CHT brought new information about the issue. NSABP B27 was designed to evaluate if adding of docetaxel (D) to conventional neoadjuvant doxorubicin-cyclophosphamide (AC) CHT improves the clinical response rate (cRR) and pathological RR (pRR) in BC patients treated with 4 AC cycles only. Although the adding of D to AC CHT significantly improved RR in both ER-negative and ER-positive BC patients, the pCR was significantly higher in ER-negative than in ER-positive group (16.7% vs. 8.3%) irrespective to the regimen used. ECTO trial and several neoadjuvant studies confirmed the significantly inferior RR to neoadjuvant CHT in ER-positive compared to ER-negative BC patients. Three large randomized Cancer and Leukemia Group B (CALGB) studies (CALGB 8541, CALGB 9344, CALGB 9741) compared the efficacy of different adjuvant anthracycline-containing or anthracycline/taxane-containing regimens in BC patients. The absolute benefit in 5-year disease-free survival in ER-negative and ER-positive BC patients treated with adjuvant CHT were 22.8% and 7.0%, while corresponding absolute benefits in overall survival were 16.7% and 4.0%. The concept of equal sensitivity of ER-negative and ER-positive BC to CHT has been changing, which necessitates the defining in future trials a relative efficacy of CHT vs. endocrine therapy in ER-positive BC. The future task is to find more effective CHT for BC with absent steroid receptors (SR) to increase survival of these patients and to introduce endocrine therapy as primary systemic treatment in SR-expressive BC patients where appropriate.



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## Estrogen-regulated proteins cathepsin D and pS2 in breast carcinoma

**KEYWORDS:** Breast Neoplasms; Carcinoma; Cathepsin D; Receptors, Estrogen; Neoplasm Proteins; Tumor Markers, Biological

In addition to classical prognostic/predictive factors, significant biological markers have been identified to provide potentially relevant information regarding natural or clinical course of breast cancer. Steroid receptor status of the primary breast cancer has been proven to be a predictor of response to endocrine therapy since up to 80% of patients with steroid receptor-positive tumors responds to endocrine treatment. In order to improve the predictive value of steroid receptor status, attention has been paid to estrogen-regulated proteins. It was supposed that estrogen-regulated proteins, including pS2 and cathepsin D among others, may be indicators of a functional signal transduction pathway through which tumor cells respond to estrogen stimulation. pS2 is a 6.4 kDa polypeptide of 60 amino-acids secreted by MCF-7 breast cancer cells and many human breast cancer. It has been shown that pS2 protein may be constitutive product as well as estrogen-regulated product in breast carcinoma. The physiological role of this cysteine-rich protein in breast tissue remains unclear to date. pS2 appears to be positively correlated with ER, associated with a good prognosis and a predictor of response to endocrine treatment of primary and metastatic breast cancer. Cathepsin D is a lysosomal aspartic endoprotease that is ubiquitously distributed in all cells at low concentration. Cathepsin D is synthesized as an inactive 52 kDa pro-enzyme that, after proteolytical processing, yields the mature active form composed of heavy (34 kDa) and light (14 kDa) chains. Its expression may be both constitutive and overexpressed as a result of estrogen-induced transcription. It was believed that the main role of cathepsin D was to degrade protein, but many other biological functions of cathepsin D were recognized. Cathepsin D level in primary breast cancer has been demonstrated as an independent marker of poor prognosis associated with increased risk for metastasis and shorter survival times.

## *In vivo* model for research of breast cancer biomarkers

**KEY WORDS:** Breast Neoplasms; Tumor Markers, Biological; Apoptosis; Tumor Suppressor Protein p53; Proto-Oncogene Proteins c-bcl2; Ki-67 Antigen

The scenario of pre-operative (neoadjuvant) chemotherapy of breast cancer is attractive as an *in vivo* model by which to allow the characterization of tumor biological features and the significance of their expression pattern, as well as possible variations in the cell phenotype elicited by therapy, with the tumor remaining *in situ* throughout treatment as an *in vivo* measure of response. Elucidating surrogate molecular or cellular markers of tumor response to therapy may provide biological insight into both the mechanism of tumor growth dynamics and drug sensitivity/resistance. Owing to the knowledge that many drugs are effective on actively proliferating cells and more intriguingly, that many anticancer agents with differing modes of action achieve cytotoxic effects by inducing apoptosis, has led to a reappraisal of traditional views of tumor response/resistance to cytotoxic drugs *in vivo*. Accordingly, this review will focus on discussing apoptosis phenomena and the p53 and bcl-2 protein as its regulators of principal importance; a cell proliferation determined by the Ki-67 expression, as the major counterbalancing process to apoptosis is also considered. This work summarizes the rationale for the use of these proteins as indices of tumor response to therapy, as well as the published literature regarding their clinical relevance. So far, no firm conclusions can be made concerning their predictive utility.



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## Angiogenesis: bFGF and VEGF in breast carcinoma

## Prognostic role of epidermal growth factor receptor in localized breast cancer: 15-years of follow-up

**KEYWORDS:** Neovascularisation, Pathologic; Breast Neoplasms; Fibroblast Growth Factor 2; Vascular Endothelial Growth Factors; Angiogenesis Factor

**KEYWORDS:** Breast Neoplasms; Prognosis; Receptors; Steroid; Receptor, Epidermal Growth Factor

Angiogenesis, or neovascularization, is a complex process leading to formation of new blood vessels from the pre-existing vascular network of the tissue. Angiogenesis plays a central role in various physiological and pathological conditions, including embryonic development, reproduction, inflammation and wound healing, infertility, heart diseases, ulcers, rheumatoid arthritis, diabetic blindness and cancer. It is a multistep process involving endothelial cell (EC) activation, basement membrane and extracellular matrix (ECM) degradation, EC proliferation, migration and differentiation, synthesis of new basement membrane and maturation of new blood vessels. Tumor vasculature is considered to be of an "immature" nature with series of structural abnormalities. There are reciprocal paracrine interactions between endothelial cells, tumor cells, stroma and ECM. Angiogenesis plays a key role in transformation of normal to malignant cell, tumor progression and metastasis. It is similar to the metastatic process in that it requires endothelial cell attachment, proteolysis, and locomotion to proceed. A close relationship exists between the tumor and endothelial cells invasiveness of the tissue. The switch to the angiogenic phenotype involves a change in the local equilibrium between positive and negative regulators of the growth of microvessels. Basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are positive regulators of angiogenesis. Intimate cross-talk exists among bFGF and the different members of the VEGF family during angiogenesis, lymphangiogenesis, and vasculogenesis. A substantial body of experimental evidence supports the hypothesis that angiogenesis and angiogenic factors may be strong prognostic and predictive factors in breast carcinoma. This article reviews the current knowledge on angiogenesis and its positive regulators: bFGF and VEGF.

**Background:** The expression of epidermal growth factor receptor (EGF-R) in breast cancer (BC) tissue is generally thought to represent the unfavorable event during tumor progression. Its predictive role has been fairly well defined: EGF-R expression predicts tamoxifen un-responsiveness. EGF-R role in autocrine growth regulation was confirmed, and therefore it was expected that EGF-R could serve as a clinical marker of tumor aggressiveness. However, reported results on its prognostic role in BC pts were conflicting. The prognostic role of EGF-R after 15 years of follow-up is analyzed in a group of 70 BC patients, presented at diagnosis either in operable stages (I-II), or as the locally advanced BC, becoming operable after the course of preoperative radiotherapy.

**Patients and methods:** BC pts newly diagnosed from December 1990 till March 1991 were selected for EGF-R analysis. The preoperative and/or adjuvant treatment was applied in accordance to the National Protocol for early BC treatment. Steroid receptors were determined at diagnosis in cytosol, and EGF-R content in a membrane fraction of the same frozen tissue samples, using biochemical methods. Except 6 pts who were lost of follow-up at the interval less than 60 months, remaining pts were followed-up from 60-188 months (median 111.5 for relapse and 128 for survival, respectively). The total number of events was 32 relapses (46%), and 27 deaths (38.5%), respectively.

**Results:** Measurable EGF-R expression was found in 28/70 pts (40%), and the EGF-R content higher than 26 fmol – in 15/70 pts (21%). Neither the expression, nor the high content of EGF-R showed any prognostic influence. Levels of EGF-R in relapsing patients and those who remained relapse-free were similar. Among other prognostic parameters, clinical stage at diagnosis and nodal status had the strongest influence on prognosis.

**Conclusion:** Our results suggest that the controversial findings, regarding the EGF-R prognostic role in early BC, might be the consequence of a genuine weak influence of EGF-R expression on the disease outcome.



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## Identifying and testing for hereditary susceptibility to breast/ovarian cancer in Serbia: Where are we now?

**KEYWORDS:** Breast Neoplasms; Ovarian Neoplasms; Heredity; Genes, BRCA1; Genes, BRCA2; Mutation; Non MeSH Serbia

**Background:** About 90% of all breast cancers can be considered as sporadic, without inherited gene alteration. The rest of breast cancers (about 5% to 10%) are considered to be hereditary, most commonly caused by alterations of tumor suppressor genes Breast Cancer gene 1 and 2 (BRCA1 and BRCA2). BRCA1 and BRCA2 genes have been implicated in about 50% of all hereditary breast cancers. Offspring of BRCA mutation carriers have 50% chance to inherit mutated gene allele from one of the parents. Life-time risk for breast cancer among BRCA1/2 mutation carriers ranges between 40%-85%, while life-time risk in female population is about 10%. Life-time risk for ovarian cancer in female population is about 2% and in BRCA1/2 mutation carriers it is up to 40%. Due to the small proportion of hereditary form of disease, as well as to the high cost of BRCA testing, it is not screening test for general population. It is addressed to selected part of population that fit to recommended criteria. Full coding region sequencing of both genes is "gold standard" for detection of BRCA mutation.

**Patients and methods:** Concerning BRCA testing in Serbia, complete or partial sequencing of BRCA1 coding region was performed in 60 samples.

**Results:** The presence of 4 known mutations, previously detected elsewhere, has been shown: 185delAG (exon 2), C61G (exon 5) 3447del4 (exon 11) and 5382insC (exon 20) which was detected twice. Three mutations were detected in site-specific breast cancer, one in breast/ovarian cancer family and another one in family with ovarian cancer only. Besides deleterious mutations, in BRCA1 gene, exon 16, we detected an unclassified variant M1652I. Polymorphic variants in BRCA1 (8 polymorphisms) and BRCA2 (5 polymorphisms) genes were also detected. The majority of found BRCA1 and BRCA2 polymorphic variants are the missense ones and their influence on breast/ovarian cancer risk in our population has to be proved.

**Conclusion:** Identification of BRCA mutations carriers, establishment of spectra and frequency of BRCA mutations, together with long-term follow-up of mutation carriers, should allow the research on the factors affecting BRCA genes penetrability. Systemic BRCA analysis will enable introduction of clinical management for mutation carriers into the clinical practice of Serbia, resulting with the real benefit for this highly vulnerable part of population.

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## Serum biomarker Ca 15-3 and bone scintigraphy pathological findings

**KEY WORDS:** Breast Neoplasms; Neoplasm Metastasis; Ca-15-3 Antigen; Bone and Bones; Radionuclide Imaging; Diagnostic Imaging

**Background:** The aim of this study was to evaluate the correlation of CA 15-3, bone scan and complementary imaging methods (X-ray, CT and MRI) in follow up of breast cancer patients for positive bone scan findings.

**Patients and methods:** Thirty-three patients with histologically proven breast cancer were included (mean age 59, range 32-81) and followed for having positive bone scan findings. Information was confirmed with other imaging methods: X-ray, CT and MRI. CA 15-3 values were measured in the same time with the bone scan, using the same commercial test over the follow-up period. Bone scan was classified as solitary hot spot lesion (group 1), two lesions (group 2), three lesions (group 3), multiple ( $\geq 3$ ) metastatic involvement (group 4).

**Results:** Number of patients in groups 1 to 4 were: 15, 1, 1, 16, respectively, and median CA 15-3 value U/ml: 32, 10, 15, 57. Nine patients had normal CA 15-3 values in spite of skeletal metastases. Metastatic involvement of lung, liver, skin was 27.8%, 27.8%, 5.6%, respectively, (in group 1), and 11.8%, 35.3%, 5.9% and in brain 5.9% (in group 4). The statistical difference was evident in groups 4 vs. 1+2+3 ( $p < 0.01$ ) (Mann-Whitney test). Skeletal metastases were confirmed by X-ray in 16 patients (48%): multiple metastatic bone scan was found in 50%; solitary lesion in 46% patients.

**Conclusion:** Normal Ca 15-3 value does not exclude bone metastasis, and cannot be helpful in confirming solitary lesions. It has excellent specificity, and is good predictor of a progressive disease, during follow up period. Bone scan pathological findings require careful radiographic evaluation for early diagnosis.



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## Role of transforming growth factor- $\beta_1$ in breast carcinogenesis

## Correlation of HER2 positive breast carcinoma with most important clinicopathological parameters

**KEYWORDS:** Receptors, Transforming Growth Factor beta; Breast Neoplasms; Tumor Markers, Biological; Prognosis

**KEYWORDS:** Breast Neoplasms; Immunohistochemistry; Receptor, erbB-2; Receptors, Estrogen; Receptors, Progesterone; Prognosis

The main objective of this presentation is to review current knowledge regarding molecular mechanisms of Transforming Growth Factor- $\beta_1$  (TGF- $\beta_1$ ) action in breast carcinogenesis. In addition, our recent results will be presented on TGF- $\beta_1$  gene polymorphism and its relationship to TGF- $\beta_1$  secretion in breast cancer (BC) patients. Special focus will be made on potential clinical applicability of TGF- $\beta_1$  as a putative diagnostic, prognostic or predictive tool in BC detection and treatment. TGF- $\beta_1$  has a complex multifunctional profile, with tumor suppressive effects in early stages of breast carcinogenesis, but progressive dominance of tumor promoting effects with transition to more advanced malignant states. Clarification of molecular mechanisms that control parallel processing of these opposing TGF- $\beta_1$  activities might suggest new approaches for shifting the balance in favor of net tumor suppression. At the present time, a major challenge remains in more precisely defining TGF- $\beta_1$  signaling pathways and their cancer-related alterations. Current dogma views human tumorigenesis as a molecular disruption of normal physiology through genetic, epigenetic, or somatic alterations. The genetic model offers biological plausibility to epidemiological studies that link the TGF- $\beta_1$  gene polymorphism, at codon 10 due to Leu<sup>10</sup>Pro substitution in the signal peptide, with the risk of developing BC. The somatic mutations approach, provides an explanation for the TGF- $\beta_1$  overexpression in advanced BC through mutations acquired in the components of Smad-mediated TGF- $\beta_1$  signaling pathway. The available results indicate decreased T $\beta$ RII (TGF- $\beta_1$  receptor-type II) expression, rare T $\beta$ RII gene mutations, but no mutations in Smad2 and Smad4 genes, in advanced BC patients. In conclusion, the ability to further define alterations in the TGF- $\beta_1$  signaling pathways at a molecular level in an individual's tumor will allow the matching of targeted therapies developed for these alterations to make individualized cancer treatment a less toxic and more effective reality.

**Background:** Treatment of breast carcinoma as a category of hormone-dependent tumors requires the evaluation of receptor status (ER, PR, and HER2), which is fundamental for planning and application of adequate therapy. Information on HER2 status is particularly important because it selects the patients suitable for the application of Herceptin. The aim was to analyze receptor status in case of 200 breast carcinoma patients and to select all HER2 3+ findings. These findings were compared to main clinicopathological parameters: age of patients, histological type and grade of the carcinoma, TNM status, and receptor status.

**Material and methods:** Samples for IH analysis of receptor status were examined by means of ER, PR, and HER2 antibodies N and M series. LSAB2 kit was used for visualization. Estrogen and progesterone were ranged with 0, 1+, 2+ and 3+, and HER2 was ranged according to reference ranges (DAKO) 1+, 2+ and 3+.

**Results:** HER2 3+ results were found in 16.58% of patients. Those were mainly patients younger than 54 years (40.55%) with ductal (53.57%) or lobular invasive carcinoma (17.86%), stage pT1c (18.18%) or pT2 (45.45%) with positive nodal status. The results showed that HER2 3+ were most frequently associated with ER-0 (57.57%) and PR-0 (65.62%).

**Conclusion:** Our results showed that HER2 3+ status correlated most often to locoregional advanced ductal invasive carcinoma of the breast with negative ER and PR, which suggests that HER 2 status may predict the aggressiveness of the tumor.



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## MMP in the process of invasion and metastasis of breast cancer

**KEYWORDS:** Matrix Metalloproteinases; Breast Neoplasms; Neoplasm Metastasis; Neoplasm Invasiveness; Tissue Inhibitor of Metalloproteinases

As the appearance of metastasis is the leading cause of death in cancers, including breast cancer, this important biological characteristic of malignant tumors is extensively investigated. The first stage of the metastatic cascade involves the localized invasion of the host tissue requiring that tumor cells separate from each other and overcome homotypic and heterotypic cell adhesion and cell-cell contact inhibition. Metastasis requires cancer cells to switch toward expression of proteolytic enzymes such as urokinase type plasminogen activator (uPA) and matrix metalloproteinases (MMPs) which degrade basal membrane (BM) and extracellular matrix (ECM). Motility is exacerbated by "epithelial-to-mesenchymal transition" (EMT) and is a crucial event in late stage tumorigenesis. Increased expression and activity of matrix metalloproteinases (MMPs) are among the earliest and most sustained events in tumor progression, playing a role in angiogenesis, invasion and metastasis. They are a family of 23 structurally related zinc metalloproteinases, secreted as latent pro-enzymes which are activated by proteolytic cleavage and are inhibited by the tissue inhibitors of metalloproteinases (TIMPs). The most commonly connected MMPs with the processes of metastasis are MMP-2 (gelatinase A) and MMP-9 (gelatinase B), due to their ability to degrade collagen type IV, major component of vascular basement membrane. Also, MMP-2 and MMP-9, are required for the switch to the "angiogenic phenotype" during tumor progression and activation of dormant tumor cells. The reported association of the increase in serum MMP2 and MMP9 activity and clinical stage suggests the usefulness of these parameters as markers, both, in the follow up and prognosis of breast cancer patients. Active estrogen receptors have resulted in tumor progression by stimulating cell growth and invasiveness via acceleration of the expression of MMPs, including MMP-9. In early phase of breast cancer HER-2 induces stromal MMP expression and activation, which further increases disease progression and metastasis. As breast cancer may now be, based on the new molecular classification, subdivided into distinctly different, luminal, basal, and HER2 subtypes, it has been shown that basal-like subtype with the worst prognosis has constitutively active genes involved in matrix remodeling and angiogenesis, typical for the pathological "wound response" gene signature. Based on this the concept of "stromal-directed therapy" of cancer due to observed stromal MMP upregulation confers them as targets of MMP inhibitors. As MMPs have important physiological roles in tissue homeostasis and cell-mediated antitumor immune response, it follows that further refining the targets in the stromal components, more specific MMP inhibitors, and preferable testing in the bone metastasis setting, rather than in early disease, represent viable approaches to breast cancer therapy with MMP inhibition.

## BRCA2 analysis in Serbia

**KEY WORDS:** Breast Neoplasms; Genes, BRCA2; Mutation; Tumor Markers, Biological; Non MeSH Serbia

**Background:** Approximately 5%-10% of all breast cancers are hereditary. Mutations in highly penetrant BRCA1/2 tumor suppressor genes, involved in maintenance of genome stability, are associated with hereditary form of breast and ovarian cancer. BRCA mutations are scattered throughout these large genes without clustering. Therefore, automatic sequencing of whole coding region of both genes is the gold standard in mutation detection. BRCA2 mutation carriers have an increased risk for breast cancer in both men and women, as well as for prostate and pancreatic, stomach, gallbladder bile ducts carcinomas and cutaneous melanoma. Male breast cancer is very rare disease and it represents <1% of all cancers in men. BRCA2 mutations are considered to predispose to large number of male breast cancers (up to 30%), while only 4% of male breast cancer cases are BRCA1 mutation carriers.

**Materials and methods:** Genomic DNA bank has been formed in Laboratory of Molecular Genetics of the Institute for Oncology and Radiology of Serbia. So far, it is consisted of about 220 blood samples from the individuals who are, according to established criteria, potential carriers of BRCA mutations. BRCA2 analysis comprised 9 probands – 5 female and 4 male. DNA for BRCA2 gene analysis is isolated by phenol/chloroform extraction. Coding region of BRCA2 gene is further amplified by Polymerase Chain Reaction (PCR) in the presence of specific primers. PCR products were purified, labeled with fluorescent 3'-dye labeled dideoxynucleotide triphosphates and precipitated by EDTA/ethanol precipitation method. These samples were then bidirectionally sequenced by automatic ABI PRISM 310 genetic analyzer.

**Results:** Partial analysis of BRCA2 exon 10 and 11 has been performed. So far, we have not identified any deleterious mutation. Five variants were identified. Three of them are missense (1342C>A, 2024T>C, 3941T>C), while the other two are synonymous mutations (4035T>C and 3624A>G). Variants 2024T>C and 3941T>C were not reported in BIC database, while other three (1342C>A, 4035T>C and 3624A>G) were qualified as polymorphisms. 1342C>A (N372H) was found as homozygote in two patients.

**Conclusion:** Our preliminary results did not show any deleterious BRCA2 mutations. So far, we have not identified any novel mutation that would be characteristic for Serbian population, although missense variants that have not been reported yet may be considered as unclassified variants. The contribution of unclassified variants must be further clarified. It has been reported HH homozygotes of N372H in exon 10 of BRCA2 gene may influence breast cancer risk – BRCA2 N372H nonconservative amino acid change polymorphism is associated with a modest recessively inherited breast cancer risk. Investigations concerning BRCA2 mutations are in progress, with the aim to establish the frequency and types of BRCA mutations in our population.