CORRELATIONS BETWEEN SEVERAL GENE POLYMORPHISMS AND BREAST CANCER

Alina BELENGEANU[*], Andrei ANGHEL[**], Elena LAZAR[***], Dorina STOICANESCU[****]

[*] *University of Medicine and Pharmacy "Victor Babes", Department of Cell and Molecular Biology, Timisoara, Romania*
[**] *University of Medicine and Pharmacy "Victor Babes", Department of Biochemistry, Timisoara, Romania*
[***] *University of Medicine and Pharmacy "Victor Babes", Department of Anatomopathology, Timisoara, Romania*
[****] *University of Medicine and Pharmacy "Victor Babes", Department of Medical Genetics, Timisoara, Romania*
Corresponding author: Alina Belengeanu, Department of Cell and Molecular Biology, University of Medicine and Pharmacy "Victor Babes", 2 E. Murgu Square, 300041 Timisoara, Romania, tel.: 0040256204476, e-mail: alinabele@yahoo.com

Abstract. Breast cancer is among the most common forms of cancer. The molecular mechanisms involved in hormone dependence of breast cancer have been largely investigated. The role of the estrogen and progesterone receptors has been documented, but the role of androgen receptor is less well known. A great part of the researches is focused on identifying the gene profile for disease predisposition, as well as the gene profiles characteristic to different stages of the disorder. Gene instability is the result of a minor alteration at the level of the microsatellites from the genomic DNA sequence. Changes of the stability of microsatellites are considered markers in the colon cancer and in different types of solid tumors. Breast cancer samples were analyzed to determine the existence of several polymorphisms in the structure of the genes that code for hormone receptors such as estrogen, progesterone and androgen receptors. The following tandem repeats were sized by gel electrophoresis: CA for ER-α, TA for ER-β and CAG for androgen receptor, together with PROGINS polymorphism. Correlated with the stage of malignancy it was noticed that majority of cases with a higher number of CAG repeats as well as those who had the allele of 481 bp in PROGINS segment were in stage III or IV, with invasive ductal carcinoma and a severe prognosis. It was also noticed that polymorphisms of estrogen receptors genes may be correlated with a severe prognosis, but due to the low number of samples, no ferm conclusions could be drawn.

Keywords: gene polymorphism, estrogen receptor, androgen receptor, PROGINS fragment, breast cancer

INTRODUCTION

Breast cancer is among the most common forms of cancer in women. Its incidence and mortality show significant disparities among different racial and ethnic groups for which clinical data are available. Thus, the incidence of breast cancer in African women is lower than that of non-Hispanic white women. Breast cancer shows heterogeneity at the clinical, microscopic, and molecular level [17]. The spreading and gravity of breast cancer have made it become a testing polygon for different therapies, besides the classical ones, such as gene therapy, auto-vaccine, and molecular targets in medication. Even if there is a large number of newly appeared drugs, their efficiency is reduced and hard to detect when associated as adjuvants. In these conditions, a great part of the researches is focused on identifying the gene profile for disease predisposition, as well as the gene profiles characteristic to different stages of the disorder. When the gene profile is identified, research is then focused on identifying those gene alterations that are correlated with the appearance and development of the cancerous process.

Breast cancers are staged clinically based on tumor size, presence of nodal metastasis and distant metastasis (the TNM classification). Another classification is based on the presence or absence of some hormones receptors. Hormones send signals through the receptors, thus cells with hormone receptors grow and multiply when certain hormones attach to the receptors. Hormones may also cause malignant cell grow and multiply. After surgical removal of the malignancy, the cancer cells are tested to see if they have hormone receptors. If either estrogen or progesterone receptors are present, a response to hormonal therapy is very likely. The more estrogen or progesterone receptors present on those cells, the higher the probability that hormonal therapy will be effective [1].

It is known that estrogens play a role in the development of the breasts and it was suggested that increased sensitivity or longer exposures to estrogens could cause higher risk for tumorigenesis [7]. Estrogen receptors (ER) represent a group of receptors that are activated by the hormone 17β-estradiol (estrogen) [5]. There are two different forms of estrogen receptors, usually referred to as α and β, each encoded by separate genes, ESR1 and ESR2, located on chromosome 6q24-q27 and 14q21-q22, respectively. It is estimated that estrogen receptors are over-expressed in around 70% of breast cancers, which are called ER-positive, the others are ER-negative. The progesterone receptor (PR), which is also called NR3C3 (nuclear receptor, subfamily 3, group C, member 3), is a steroid receptor that specifically binds progesterone. The progesterone receptor is encoded by a single gene called PGR located on chromosome 11q22-q23 and it has two main forms, A and B, that differ in their molecular weight [9].

It is estimated that about 65% of ER-positive breast cancers are also progesterone-receptor-positive. About 25% of breast cancers are ER-negative and PR-negative or of "unknown" status. Also, about 10% of breast cancers are ER-positive and PR-negative, and around 5% of breast cancers are ER-negative and PR-positive. If there are no hormone receptors present, if they cannot be measured, or if the status is "unknown," the cancer is said to be hormone-receptor-negative. The presence of the estrogen receptor and of the progesterone receptor allows oncologists to choose targeted therapy. The presence of the positive ER-α status is essential in making clinical decisions about introducing anti-estrogens for the endocrine therapy, as they will inhibit the mitogenic activity of estrogens in breast cancer. Currently three classes of anti-estrogens

are used: selective estrogen receptor modulators (e. g. tamoxifen); aromatase inhibitors; and 'pure' estrogen antagonists (e. g fulvestrant.), which –like tamoxifen– binds competitively to ERs [14]. Patients with ER-positive breast cancer also have a better prognosis, including a lower rate of cell proliferation and histologic evidence of tumor differentiation. ER-α status is also prognostic for the site of gross metastatic spread. ER-α-positive tumors seem more likely to initially manifest metastases in bones, soft tissues, or reproductive and genital tracts, whereas ER-α-negative tumors tend more commonly to metastasize to brain and liver [13]. If ER-α-positive cells were implanted in mice, tumors appeared only in the presence of estrogens and were poorly metastatic as compared with those developed from ER-α-negative breast cancer cell lines [8]. Expression patterns present in primary breast cancers are also seen in their metastases [18].

The molecular mechanisms involved in hormone dependence of breast cancer have been largely investigated and the role of the estrogen and progesterone receptors in promoting this type of malignancy has been well documented. However, the role of androgens receptors in breast cancer etiology and progression has been less profoundly studied [6]. The androgen receptor (AR), also known as NR3C4 (nuclear receptor subfamily 3, group C, member 4), is a type of nuclear receptor, which is activated by binding of androgenic hormones: testosterone or dihydrotestosterone. Its main function is as a DNA binding transcription factor, which regulates gene expression [12]. The gene for the androgen receptor is located on the X chromosome at Xq11-q12. There is evidence showing that androgens can directly stimulate growth of human breast cancer cell lines. It is estimated that AR is expressed in approximately 70% - 90% of invasive breast cancers, frequency comparable with the one reported for ER (70–80%) and PR (50–70%) [11]. Certain types of breast carcinoma, may be ER- and PR-negative, but AR-positive and a typical example is the apocrine breast carcinoma [2].

Gene instability of malignant cells generates different mutations: insertions, deletions, duplications, inversions, located in different regions of the investigated genes, appearance of these mutations not only being able to modify the level of gene expression, but also the structure and biological activity of the final product, the coded protein. The existence of the tendency of gene instability itself makes the object of investigations. Gene instability is the result of a minor alteration at the level of the microsatellites from the genomic DNA sequence. Changes of the stability of microsatellites are considered markers in the colon cancer and in different types of solid tumors.

MATERIALS AND METHODS

This study is part of a larger one, whose goal was to promote the systematic study of the biology of human breast cancers together with characterization of some of their molecular features. In this part we aimed to identify polymorphisms in the structure of the genes that code for estrogen receptors, progesterone receptors and androgen receptors, variations that could be correlated with the prognosis of the case and with the response to different hormonal therapies. The obtained data are important for developing early detection and treatment strategies.

We analyzed 60 tumor samples from breast cancer cases to determine the existence of several polymorphisms in the structure of the genes that code for hormone receptors such as estrogen, progesterone and androgen and compared them to results obtained from the blood analysis of healthy subjects, without any medical history or positive family history. Thus, genomic DNA was isolated from around 35 mg of paraffin-embedded mamary tissue samples and the following tandem repeats were sized by gel electrophoresis: CA for ER-α, TA for ER-β and CAG for androgen receptor, together with PROGINS polymorphism. We examined how these variations in the number of nucleotide repeats, specific for each gene, are associated with each other and their relationship with the size of the primary breast tumor, lymph node invasion, and development of metastasis.

The DNA of affected subjects was extracted from each tissue and a set of primers was used for PCR to amplify the DNA. In order to analyze the dimension of the resulted fragments through their comparison with markers, which have a standard molecular weight, gel electrophoresis was used. Compared to the normal tissue, the malignant one often presents 2 bands with different lengths of the repetitive dinucleotidic tandems, typical for the microsatellites instability (MIN). All subjects gave their permission to be taken into the study, after being informed.

RESULTS

The study lot was formed by affected subjects with a mean age of 53.66, ranging between 37 and 81 years and age-matched control cases. The affected ones had different histopathologic subtypes of breast cancer and were in different stages of the malignancy. The majority of the cases (73.33%) had ductal carcinoma, which is known to be the most frequent type of breast cancer and were in stage II (54.83%), as revealed by the preliminary results that are presented in Table 1.

Several gene polymorphisms were tested in these subjects: number of repeats for the three nucleotides CAG in the gene that codes the androgen receptor, number of CA tandem repeats for the gene coding estrogen receptor α, number of TA repeats for estrogen receptor β gene, together with PROGINS fragment from the progesterone receptor gene.

Regarding the number of CAG repeats in the gene that has the information for the androgen receptor synthesis, it ranged from 8 repeats to 27 repeats. The majority of cases had a number of repeats that ranged between 17 and 24, the size of the PCR products being around 230bp (Fig. 1). 15 alleles (12.5%) had less than 16 CAG repeats and 9 alleles (7.5%) had 25 or 27

CAG repeats. Considering the lower number of CAG tandem repeats, all cases with 11/8 repeats were in stage III and from the 2 cases with 11/9 repeats, 1 was in stage II and the other one in stage IV. PROGINS polymorphism in the progesterone receptor gene was examined by polymerase chain reaction, followed by electrophoresis. The size of the PCR products was 175 bp for the majority of the cases, only 6 (5%) longer alleles with 481 bp were detected (Fig. 2). One of the detected cases was in stage IV of malignancy, the others were in stage III and all had ductal carcinoma.

As for CA tandem repeats in the gene coding estrogen receptor α the majority of cases had a number varying between 20 and 25 and were in stage II of malignancy. 10 alleles (8.33%) were detected with less than 21 repeats and 6 alleles (5%) were detected with 26 or 27 repeats. We present PCR products having almost similar size and similar pattern of migration for 7 of the collected samples revealing the amplification for CA tandem repeats (Fig. 3).

Table 1. Partial results of gene analysis from paraffin-embedded mamary tissue samples from 31 cases.

Nr.	Age (yrs.)	TNM Classif.	Stage	Histopathological form	G	άEstrogene Receptor	βEstrogene Receptor	Progesterone Receptor	Androgen Receptor
						Nr.CA repeats	Nr.TA repeats	Progins	Nr. CAG repeats
1	73	$T_2N_1M_0$	II B	Ductal carcinoma, invasive, with solid cancer zones	3	23/25	13/13	175 bp	22/22
2	54	$T_2N_{Ibii}Mo$	II B	Ductal carcinoma, invasive, trabecular and glanduliform	2	23/21	19/16	175 bp	15/15
3	69	$T_2N_{Ibii}M_0$	II B	Lobular carcinoma, invasive	2	25/25	19/21	175 bp	22/25
4	47	$T_2N_0M_0$	II A	Atypical medullary carcinoma	N/A	26/24	16/11	175 bp	24/23
5	53	$T_2N_0M_0$	II A	Lobular carcinoma, invasive	2	20/20	23/16	175 bp	24/24
6	37	$T_2N_2M_0$	III A	Fibrocystic mastopathy	N/A	22/14	16/16	175/481 bp	18/22
7	50	$T_2N_{Ibiii}M_0$	II B	Lobular carcinoma, invasive	2	22/24	13/13	175 bp	21/25
8	54	T_2N_0Mo	II A	Medullary carcinoma	3	23/20	14/14	175 bp	15/14
9	49	$T_3N_{Ibiii}M_0$	III A	Ductal carcinoma, invasive, trabecular and glanduliform	2	22/23	13/22 |	175 bp	18/18
1 0	72	$T_{4b}N_1M_0$	III B	Ductal carcinoma, invasive, glanduliform	N/A	24/22	14/14	175 bp	11/8
1 1	69	$T_{4b}N_0M_0$	III B	Ductal carcinoma, invasive, trabecular and glanduliform	X	21/27	14/16	175/481 bp	16/14
1 2	58	$T_3N_0M_0$	II B	Ductal carcinoma, invasive, and lobular invasive	2	23/21	19/16 J	175 bp	15/15
1 3	63	$T_3N_2M_0$	III A	Ductal carcinoma, invasive, desmoplastic stroma, intravascular embolism	2	25/25	19/21	175 bp	22/25
14	55	$T_2N_{Ibii}M_0$	II B	Atypical medullary carcinoma	3	22/20	14/14	175 bp	15/14
15	49	$T_2N_0M_0$	II A	Ductal carcinoma, invasive	2	23/21	14/25	175 bp	15/12
16	66	T_2N_0IVE§	II A	Papillary carcinom, invasive	2	23/25	23/23	175 bp	15/18
18	67	T2N,biHM0	II B	Ductal carcinoma, invasive	2	25/22	17/16	175 bp	24/23
19	3S	$T_3N_1M_0$	III A	Ductal carcinoma, invasive	N/A	23/21	24/16	175/481 bp	16/12
20	58	$T_3N_1M_0$	III A	Trabecular carcinoma	2	20/22	12/12	175 bp	23/24
21	52	T_2N_1Mo	II B	Ductal carcinoma, invasive	N/A	25/25	19/21	175 bp	22/25
22	35	$T_3N_1M_0$	III A	Ductal carcinoma, invasive, glanduliform	x	23/24	17/21	175 bp	14/13
23	54	$T_2N_0M_0$	II A	Mucinous carcinoma	2	19/21	16/27	175 bp	18/20
24	55	T_2NoMo	II A	Ductal carcinoma, invasive	3	22/24	13/13	175 bp	21/25
25	65	$T_2N_{Ibii}Mo$	II B	Ductal carcinoma, invasive, solid, trabecular and glanduliform, lobular invasive	N/A	23/25	14/26	175 bp	20/25
26	81	$T_3N_2M_0$	III A	Ductal carcinoma, invasive	2	24/24	14/21	175 bp	27/27
27	39	T_2N_1Mo	II B	Ductal carcinoma, invasive	2	22/22	23/23	175 bp	22/22
28	43	T_1NoMo	I	Ductal carcinoma, invasive	2	20/21	24/15	175 bp	21/21
29	50	TxN_1M_1	IV	Ductal carcinoma, invasive	3	23/24	26/24	175 bp	17/16
30	52	T_3N_2Mo	III A	Ductal carcinoma, invasive	N/A	23/21	15/15	481 bp	11/9
31	70	$T_2N_2M_0$	II A	Ductal carcinoma, invasive with mucinous carcinoma zones	1	20/20	17/16	175/481 bp	24/23

Regarding the number of TA repeats (Fig. 4) in the gene that has the information for estrogen receptor β it was noticed that in 16 cases (26.66%) the number of repeats did not exceed 15 and were in stages II or III, respectively. 9 alleles (7.5%) were found to have more than 25 TA tandem repeats.

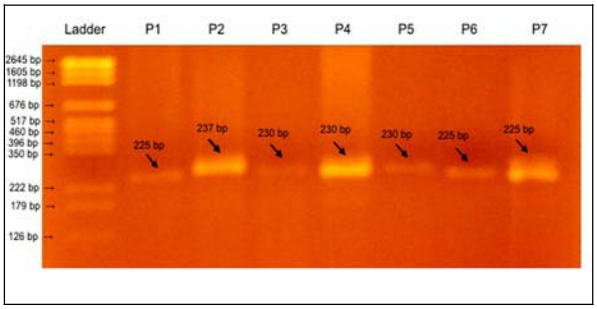

Figure 1: Electrophoregrame of PCR amplified products. Agarose gel was used to verify the amplification of CAG repeats (androgenic receptor) for probes P1 –P7 representing samples of malignant mamary tissue. Ladder is Pgem (Promega).

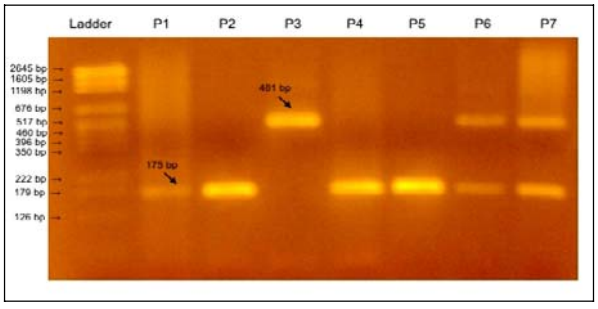

Figure 2. Electrophoregrame of PCR amplified products. Agarose gel was used to verify the amplification of PROGINS for probes P1 –P7 representing samples of malignant mamary tissue. Ladder is Pgem (Promega)

Figure 3. Electrophoregrame of PCR amplified products. Agarose gel 2% was used to verify the amplification of CA repeats (estrogen receptor α) for probes P1 –P7 representing samples of malignant mamary tissue. Ladder is Pgem (Promega)

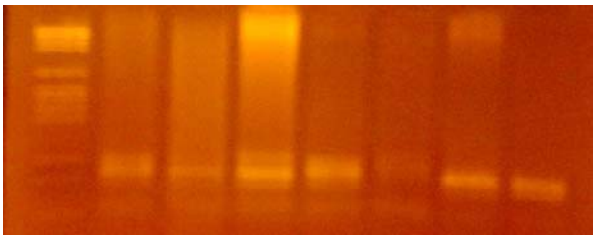

Figure 4. Electrophoregrame of PCR amplified products. Agarose gel 2% was used to verify the amplification of TA repeats (estrogen receptor β) for probes P1 –P7 representing samples of malignant mamary tissue. Ladder is Pgem (Promega).

DISCUSSIONS

The prevalence of breast cancer has been increasing in almost all populations in the last decades. It is possible that higher detected incidences may also be due to the introduction of early detection measures. Breast cancer is among the most common cancers, especially in western countries, but the mortality rate in many of these countries has been reduced, especially due to introduction of new diagnostic techniques and therapeutic methods [10].

Prognosis has been improving, but for cases with advanced breast cancer the prognosis remains still poor. These forms are essentially incurable and the optimal management remains a therapeutic challenge. The main objectives of the therapy are represented by restricting tumour growth, providing effective palliative treatment and maintaining quality of life. In some cases such as hormone-sensitive cases or those with a long disease-free interval, with soft tissue or bone metastases and with associated disorders, hormonal therapy may be appropriate. This therapy is well tolerated and it is thought to be an option to be explored [15].

In breast cancer, type of cancer that is part of the hormone-dependent category, gene changes with high influence are the ones that interfere modulation of the influence of sex hormones on the mammary tissue.

The most important element in the hormonal action is the hormone-receptor interaction and for this reason changes that affect the structure and the expression of the genes that code sex hormones receptors will have a major contribution in the reduction or stimulation of the interaction between the mammary tissue and sex hormones [3].

Breast cancer was associated with the steroid hormone estrogen more than a century ago. Estrogens are essential for normal growth and proliferation of target cells. In order to achieve their physiological effects they bind to specific nuclear proteins, called estrogen receptors, which are located especially in the breast, liver and uterus in females. It is estimated that estrogens also play an important role in the etiology and progression of breast cancer. The discovery of estrogen receptors provided not only a powerful predictive and prognostic marker for this malignancy, but also an efficient target for the treatment of hormone-dependent breast cancer using antiestrogens.

The role of estrogen receptors is complicated by the receptor's interaction with co-regulatory proteins, its cross-talk with other signal transduction pathways, and its involvement in the development of antiestrogen resistance.

At the transcriptome level, gene expression analysis has revealed that different molecular subtypes exist within ER-α-positive and ER-α-negative breast cancers, and these are associated with different clinical outcomes. Several genetic alterations identified in the estrogen receptor α and β genes are thought to influence the expression or function of this protein, and many have been evaluated for their role in breast cancer predisposition.

The associations between genetic polymorphisms in the estrogen receptor gene and the risk for several types of cancers, including breast cancer, have been a subject of increasing interest. Thus, several DNA sequence variations in the estrogen receptor gene have been reported. Recent studies suggested that genetic polymorphisms of the estrogen receptor-alpha gene may be associated with breast cancer risk. Evaluation of the role of this gene in the risk of breast cancer, was made by genotyping a newly identified GT dinucleotide repeat polymorphism located in the promoter region in breast cancer cases. Sixteen alleles were identified, the most common one having 16 GT repeats. Compared with homozygote cases, those with 17 or 18 repeats had a decreased risk of breast cancer. Among breast cancer patients, the presence of one of these alleles was associated with a reduced expression of progesterone receptor [4].

The human genome contains many sequences that are repeated many times within the genome. One group of such repeated sequences consists of 2-5 bp that are reiterated from a few times up to 50 times. Studies aiming to reveal whether nucleotide repeats have any biological importance have gained much attention.

Changes affecting tandem sequences containing CA repeats, respectively TA repeats, from the genes that code for α and β estrogen receptors as well as those of the CAG trinucleotidic sequence of the gene coding androgen receptors, exert the marking effects in the occurrence and progress of the cancerous disease through: change gene transcription; occurrence of the microsatellites instability associated or not with the occurrence of some alleles (loss of heterozygosity) and increase of susceptibility to the action of some carcinogenetic factors.

The human progesterone receptor is a ligand-dependent transcription factor and has two isoforms called PRA and PRB. PROGINS, which is a PR polymorphic variant, affects PRA and PRB and it was

demonstrated that it acts as a risk-modulating factor in several gynecological disorders. PROGINS is characterized by a PV/HS-1 Alu insertion in intron G and two point mutations, V660L in exon 4 and H770H (silent substitution) in exon 5 [16].

Regarding the gene coding for the progesterone receptor, the discovery of an insertion of 306 bp in intron 7 of the gene (PROGINS segment), which is in a non-equilibrium relationship with a mutation in exon 4 and 5, has revealed the association between the existence of this insertion with a profound change of the progesterone receptor structure, with direct repercussions over its biological function.

The study revealed that segment A from the insertion zone presents 2 alleles:

- A_1, 175bp, without insertion of 306 bp
- A_2, 481bp, with the insertion of 306 bp

It is considered that the polymorphism of segment A from the progesterone receptor gene increases transcription and stability of the receptor and in the same time represses the activity of the androgen receptor.

Correlated with the stage of malignancy it was noticed that majority of cases with a higher number of CAG repeats as well as those who had the allele of 481 bp in PROGINS segment were in stage III or IV, with invasive ductal carcinoma and a severe prognosis. It was also noticed that polymorphisms of estrogen receptors genes may be correlated with a severe prognosis, but due to the low number of samples, no ferm conclusions could be drawn.

Gene expression profiling has also allowed researchers to gain new insights into the cell biology of breast cancers, providing clinically useful classification of tumors that can be used to stratify patients and decide the best treatment options.

REFERENCES

[1] Ascenzi, P., Bocedi, A., Marino, M., (2006): Structure-function relationship of estrogen receptor alpha and beta: impact on human health. Molecular Aspects of Medicine, 27(4): 299–402.

[2] Bratthauer, G.L., Lininger, R.A., Man, Y.G., Tavassoli, F.A., (2002): Androgen and estrogen receptor mRNA status in apocrine carcinomas. Diagnostic Molecular Pathology, 11(2): 113-118.

[3] Cahill, M.A., (2007): Progesterone receptor membrane component 1: an integrative review. Journal of Steroid Biochemistry and Molecular Biology, 105: 16-36.

[4] Cai, Q., Gao, Y.T., Wen, W., Shu, X.O., Jin, F., Smith, J.R., Zheng, W., (2003): Association of breast cancer risk with a GT dinucleotide repeat polymorphism upstream of the estrogen receptor-alpha gene. Cancer Research, 63(18): 5727-5730.

[5] Dahlman-Wright, K., Cavailles, V., Fuqua, S.A., Jordan, V.C., Katzenellenbogen, J.A., Korach, K.S., Maggi, A., Muramatsu, M., Parker, M.G., Gustafsson, J.A. (2006): International Union of Pharmacology. LXIV. Estrogen receptors. Pharmacological Reviews. 58(4): 773–781.

[6] Diaz-Chico, N.B., German-Rodriguez, F., Gonzalez, A., Ramirez, R., Bilbao, C., Cabrera de Leon, A., Aguirre-Jaime, A., Chirino, R., Navarro, D., Diaz-Chico, J.C., (2007): Androgens and androgen receptors in breast cancer. Journal of Steroid Biochemistry and Molecular Biology, 105(1-5): 1-15.

[7] Fisher. B., Jeong, J.H., Dignam, J., Anderson, S., Mamounas, E., Wickerham, D.L., Wolmark, N., (2001): Findings from recent National Surgical Adjuvant Breast and Bowel Project adjuvant studies in stage I breast cancer. Journal of the National Cancer Institute. Monographs, 30: 62-66.

[8] Fuqua, S.A., Cui, Y., (2004): Estrogen and progesterone receptor isoforms: clinical significance in breast cancer. Breast Cancer Research and Treatment, 87(suppl 1): S3-10.

[9] Gadkar-Sable, S., Shah, C., Rosario, G., Sachdeva, G., Puri, C. (2005): Progesterone receptors: various forms and functions in reproductive tissues. Frontiers in Bioscience. 10: 2118–2130.

[10] Kaplan, R.M., Wingard, D.L., (2000): Trends in breast cancer incidence, survival, and mortality. Lancet, 356: 592–593.

[11] Lillie, E.O., Bernstein, L., Ursin, G., (2003): The role of androgens and polymorphisms in the androgen receptor in the epidemiology of breast cancer. Breast Cancer Research. 5(3): 164-173.

[12] Lu, N.Z., Wardell, S.E., Burnstein, K.L., Defranco, D., Fuller, P.J., Giguere, V., Hochberg. R.B., McKay, L., Renoir, J.M., Weigel, N.L., Wilson, E.M., McDonnell, D.P., Cidlowski, J.A., (2006): International Union of Pharmacology. LXV. The pharmacology and classification of the nuclear receptor superfamily: glucocorticoid, mineralocorticoid, progesterone, and androgen receptors. Pharmacological Reviews, 58(4): 782–797.

[13] Neubauer, H., Clare, S.E., Kurek, R., Fehm, T., Wallwiener, D., Sotlar, K., Nordheim, A., Wozny, W., Schwall, G.P., Poznanoviæ, S., Sastri, C., Hunzinger, C., Stegmann, W., Schrattenholz, A., Cahill, M.A., (2006): Breast cancer proteomics by laser capture microdissection, sample pooling, 54-cm IPG IEF, and differential iodine radioisotope detection. Electrophoresis, 27: 1840-1852.

[14] Neubauer, H., Clare, S.E., Wozny, W., Schwall, G.P., Poznanovic, S., Stegmann, W., Vogel, U., Sotlar, K., Wallwiener, D., Kurek, R., Fehm, T., Cahill, M.A., (2008): Breast cancer proteomics reveals correlation between estrogen receptor status and differential phosphorylation of PGRMC1. Breast Cancer Research 10:R85.

[15] Piccart, M., Parker, L.M., Pritchard, K.I., (2003): Oestrogen receptor downregulation: an opportunity for extending the window of endocrine therapy in advanced breast cancer. Annals of Oncology, 14: 1017-1025.

[16] Romano, A., Delvoux, B., Fischer, D.C., Groothuis, P., (2007): The PROGINS polymorphism of the human progesterone receptor diminishes the response to progesterone. Journal of Molecular Endocrinology, 38: 331-350.

[17] Sant, M., Capocaccia, R., Coleman, M.P., Berrino, F., Gatta, G., Micheli, A., Verdecchia, A., Faivre, J., Hakulinen, T., Coebergh, J.W.W., Martinez-Garcia, C., Forman, D., Zappone, A., the EUROCARE Working Group. (2001): Cancer survival increases in Europe, but international differences remain wide. European Journal of Cancer, 37: 1659–1667.

[18] Weigelt, B., Glas, A.M., Wessels, L.F., Witteveen, A.T., Peterse, J.L., van't Veer, L.J., (2003): Gene expression profiles of primary breast tumors maintained in distant metastases. Proceedings of the National Academy of Sciences. USA, 100: 15901-15905.