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Morphological characteristics of mitochondria in normal and malignant human breast epithelial cells correlate with estrogen and progesterone receptors: a stereological analysis

Ljiljana Amidžić [1], Radoslav Gajanin[1], Vesna Ljubojević[2]

[1]*University Hospital Clinical Center Banja Luka, University of Banja Luka, Faculty of Medicine, Bosnia and Herzegovina*
[2]*University of Banja Luka, Faculty of Medicine, University Hospital Clinical Center Banja Luka, Bosnia and Herzegovina*

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Summary. The biological and pathological effects of estrogen and progesterone in hormone-sensitive breast tissue are due - on a molecular level - to estrogen and progesterone receptor signaling responses at the cell membrane, nucleus and mitochondria of breast epithelial cells. The present study focuses on the involvement of mitochondria in mammary gland carcinogenesis. Here we provide a quantitative comparison of mitochondria in normal vs. malignant human breast epithelial cells. Using stereological analysis, the volume (Vvm), surface (Svm), specific surface (Svm/Vvm) and numerical (Nvm) density of mitochondria were estimated for a total of 600 cells: 150 breast cancer cells positive for estrogen and progesterone receptors (group 1C); 150 breast cancer cells negative for estrogen and progesterone receptors (group 2C); and two compatible groups of 150 normal mammary epithelial cells (groups 1K and 2 K). Comparison of stereological parameters of mitochondria from complementary groups 1K and 1C shows that the surface, specific surface and numerical density of mitochondria is significantly reduced ($p < 0.000$) in cancer cells (1C). Comparison of complementary groups 2K and 2C shows that the numerical ($p < 0.015$) and specific surface ($p < 0.000$) density was significantly reduced, while the surface ($p < 0.009$) and volume density ($p < 0.000$) increased in cancerous cells (2C). Comparison of complementary groups 2K and 2C shows that the numerical ($p < 0.015$) and specific surface ($p < 0.000$) densities of the mitochondria were significantly reduced and the surface ($p < 0.009$) and volume density ($p < 0.000$) increased in cancerous cells (2C). Based on stereological analysis, we conclude that mitochondria in breast cancer cells are significantly different from mitochondria in normal cells in breast glandular epithelia. In addition, differences in mitochondria were observed which correlate with the presence/absence of estrogen and progesterone receptors.

Keywords: cancer of the breast, estrogen and progestreone receptors, stereological analysis, the mitochondria.

INTRODUCTION

The sex hormones, estrogen and progesterone play important roles in the development and function of mammary glands in mammals. The molecular mechanisms underlying the biological and pathological effects of these sex hormones are due in part to their interactions with estrogen and progesterone receptors (Gilbert 2006; Brisk and O'Malley 2010).

Our present understanding of the influence of estrogen and progesterone in normal and malignant human breast epithelium has been achieved following years of careful collection and analysis of data obtained by biochemical, immunohistochemical, and molecular methods on the function of estrogen and progesterone receptors of breast epithelial cells in bioptic material (Nadji et al. 2005; Arihiro et al. 2007; Badve et al. 2008; Li et al. 2010; Oda et al. 2010), experimental animals (Cheng et al. 2005; Gilbert 2006; Beleuta et al. 2010; Brisken and O'Malley 2010) and selected cell cultures (Jacobsen et al. 2005). Due to their roles as mediators of hormone action, assessment of the expression levels of hormone receptors in cancer cells has become an important factor in the selection of appropriate therapy and the prognosis of breast cancer. For a long time, estrogen and progesterone receptor expression was monitored almost exclusively in the cytoplasm and nuclei of breast epithelial cells (Cordera and Jordan 2006; Dowsett et al. 2006; Rhodes 2006; Varghese

*Corresponding author, e-mail: amidzicljiljana@gmail.com

2007; Farzami et al. 2009; Hammond et al. 2011; Lonard and O'Malley 2012). However, there is far less information about the expression levels of estrogen receptors in mitochondria, or their impact on normal vs. cancer cells: via the rapid, non-genomic action of estrogen hormones (Chen et al. 2004; Felty and Deodutta 2005). More recently however, special attention has been given to the direct and/or indirect effects of estrogen on the biogenesis and function of mitochondria (Klinge 2008; Mattingly et al. 2008), regulation of electron transport chains and production of reactive oxygen species (Chen et al. 2009), control of mitochondrial apoptosis times (Pedram et al. 2006; Yager and Chen 2007), induction of mutations of mitochondrial DNA (Radpour et al. 2009). In particular, the possibility of inhibiting mitochondrial estrogen steroidogenesis as a means to prevent breast carcinogenesis (Yager and Chen 2007; Elliot et al. 2012), or the application of anti-mitochondrial therapies to block energy metabolism and inhibit fast-growing tumors (Mandujano-Tinoco et al. 2013) has potential in cancer therapeutics.

Literature data on the morphological and morphometric characteristics of mitochondria in normal vs. malignant human breast epithelial cells is scarce (Stenger et al 1983; Wolf et al 1985; Stegner et al 1986; Beneduci et al 2005; Elliot and Barnett 2011). Therefore, in the present study we tested the hypothesis that mitochondria differ in normal vs. malignant human breast epithelia with respect to morphological and morphometric characteristics. In addition, we also examined whether these differences in mitochondrial morphology reflect differences in estrogen and pogesterone receptor status (positive or negative). These hypotheses are based on the fact that the function and morphology of subcellular units, such as mitochondria, are closely linked, and that study of their morphological characteristics may enable assessment of their functional status.

To enable quantitative, objective data analysis, quantitative stereological analysis of volume density (Vvm), surface density (Svm), the specific surface density (Svm/Vvm) and numerical density (Nvm) of mitochondria in the reference area of the cells of normal and malignant human breast epithelium were performed.

MATERIALS AND METHODS

In the present study, breast tissue samples were obtained from women of reproductive age, who had been biopsied for primary invasive cancer. This study includes determination of the status of estrogen and progesterone receptor (positive or negative) in cancerous mammary epithelial cells, and stereological analysis of morphological changes in mitochondria in malignant vs. normal breast epithelial cells.

In order to determine the relationship between the amount of estrogen and progesterone receptors and stereological mitochondrial parameters, tissue samples were divided into 4 different groups of cells.

First group (150 cells): normal mammary epithelial cells from the environment around cancer cells positive for estrogen and progesterone receptors (group 1K). Second group (150 cells): normal mammary epithelial cells from the environment around carcinomas negative for estrogen and progesterone receptors (group 2K). Third group (150 cells): estrogen/progesterone receptor positive malignant epithelial cells (ER+PR+) (group 1C). Fourth group (150 cells): estrogen and progesterone receptor negative malignant epithelial cells (ER-PR-) (group 2C).

Histological analysis of breast tissue was performed using a Leitz Wetzlar Orthoplan light microscope.

Determination of estrogen and progesterone receptor status

For immunohistochemical detection of estrogen and progesterone receptors, monoclonal mouse anti-human Estrogen Receptor α (ER α), Clone 1D5 and monoclonal mouse anti-human Progesterone Receptor, clone 1A6 (Dako, DakoCytomation, Ink, Carpinteria, USA) were used. Visualization was performed by standard protocols using the LSAB method and DAB chromogen (all reagents from DakoCytomation, Ink, Carpinteria, USA).

Quantification of estrogen and progesterone receptors was performed using the Allred scoring system (Qureshi and Pervez 2010). Qualitative and stereological analyses of mitochondria were performed on micrographs of malignant and normal breast epithelium cells.

Electron microscopy

For electron microscope examinations, five to ten samples of normal and malignant human breast tissue, were used. Samples, 1 mm 3 were prefixed in 4% glutaraldehyde and postfixed in 1% osmium tetroxide (Serva, Heidelberg, Germany), then dehydrated with acetone and embedded in Araldite resin (Ciba-Geigy, Basel, Swiss). Semithin sections were cut at a thickness of 500 nm and stained with toluidine blue (Merck, Darmstadt, Germany). These sections were used for the selection of regions of interest for electron microscope analysis. Ultrathin sections of 80 nm were contrasted with 2% uranyl acetate (Merck, Darmstadt, Germany) and lead citrate prepared after Reynolds.

Ultrastructural analysis and photography of cells with the most representative mitochondria was performed on a transmission electron microscope (OPTON 9S-2, Oberkochen, Germany). Electron micrographs were used for qualitative and stereological analysis of mitochondria.

Stereological analysis

Stereological analysis of mitochondria was performed

on electron micrographs of normal vs. malignant human breast epithelia using a transparent test system (mesh) with an unlimited number of test points and test lines. Using stereological point-counting methods, the volume density (Vvm), surface density (Svm), specific surface density (Svm/Vvm) and numerical density (Nvm) of mitochondria in the reference area were calculated (Weibel 1969; Rigoglio et al 2012).

Statistical analysis

The significance of differences between the mean values of all examined stereological parameters of mitochondria among the studied cells was analyzed by t-test with *p*-values set at 0.05. The contribution of each parameter to discrimination between groups was determined by the coefficient of discrimination. All tests were carried out using STATISTICA 10.0 software.

RESULTS

Malignant cells with high levels of estrogen and progesterone receptors (Allred score, ER = 8/PR = 8, 150 cells) were selected for analysis and grouped into phenotypicaly differentiated, ER+PR+ cancer cells (group 1C).

In contrast, breast cancer cells lacking estrogen and progesterone receptor (Allred score, ER = 0/PR = 0, 150 cells) were grouped as undifferentiated phenotype ER-PR- cancer cells (group 2C).

In addition to cancer cells (group 1C and group 2C), normal cells were collected from the respective surrounding environments and were also analyzed. These cells served as control groups (group 1K and group 2K).

Stereological analysis of all 600 selected cells revealed differences in volume density, surface density, specific surface density and the numerical density of the respective mitochondria. The significance of differences for each parameter between the groups of normal and cancerous cells was analyzed by the t-test (Table 1). Results presented in this Table show that there are significant differences in the stereological parameters between the examined cells groups. Stereological analysis of the volume density of mitochondria in cells from groups 1K and 2K show that volume density is significantly higher ($p < 0.0001$) in the control group 1K (normal breast epithelial cells surrounding carcinomas positive for estrogen and progesteron receptors) compared to control group 2K (normal breast epithelial cells from the environment surrounding carcinomas negative for estrogen and progesterone receptors).

In addition, as can be seen, the volume density of mitochondria in cells from group 2C (ER-PR- cancer cells) was significantly higher ($p < 0.000$) compared to the appropriate control group (2K). Stereological analysis of the surface density of mitochondria from control cells in groups 1K and 2K revealed significantly higher values for this parameter in group 1K compared to group 2K. Comparison of these parameters between control groups (1K, 2K) and cancer cells (1C, 2C) suggests that the surface density of mitochondria in group 1K are significantly higher ($p < 0.001$) than in group 1C, while between groups 2K and 2C this relationship is inverted: the surface density of mitochondria is higher in group 2C (ER-PR- cancer cells) than in the corresponding control group 2K. Comparison between groups of cancer cells (1C and 2C) shows significantly higher ($p < 0.000$) surface density of mitochondria in cells from group 1C than those from group 2C. According to these stereological analyses of the specific surface density, which indicate changes in mitochondrial shape or changes in the ratio between surface area and volume, higher values of this parameter were observed in mitochondria from cells in 1K group (normal mammary epithelial cells surrounding invasive cancer cells positive for estrogen and progesterone receptors) compared to group 2K (normal mammary epithelial cells surrounding cancer cells negative for estrogen and progesteron receptors) ($p < 0.001$), as well as in mitochondria from group 1C (ER+PR+ cancer cell) vs. mitochondria from group 2C (ER-PR- carcinoma cells). In addition, larger values for this parameter were observed in mitochondria from the 2K cell group compared to group 2C (ER-PR- carcinoma cells); and in mitochondria from group 1C (ER+PR+ cancer cell) vs. group 2 C (ER-PR- carcinoma cells). These numerical density values indicate that the number of mitochondria per reference area is higher in the control groups of cells (1K and 2K) compared to the cancer cell groups (1C and 2C). The numerical density of mitochondria was also higher in the control cells (2K) compared to those from group 2C. Among groups of cancer cells, significantly higher values of this parameter ($p < 0.000$) were observed in mitochondria from group 1C compared to group 2C ($p < 0.000$).

In order to more clearly determine the ability of each stereological parameter to discriminate between different groups, a coefficient of discrimination was calculated for each monitored stereological parameter. Analyses of the coefficient of discrimination (Table 2) reveal that the largest contributions to the significant differences between the studied groups of cells stem primarily from numerical density (kd = 0.124) and specific surface density (kd = 0.082), with less of a contribution from surface (kd = 0.028) and volume density (kd = 0.004).

Based on the above results, we observed that in normal human breast epithelial cells (groups: 1K and 2K) there are on average a higher number of mitochondria of homogeneous structure: elliptical shaped, with conserved membrane and parallel oriented christae and transparent intra-mitochondrial matrix with no signs of swelling. In contrast, breast cancer cells (group 1C and group 2C) displayed altered mitochondrial morphology characterized by swollen mitochondria with short and disoriented christae, and with a "pale"

Table 1. The statistical significance of differences in the investigated stereological mitochondrial parameters: volume (Vvm), surface (Svm), specific surface (Svm/Vvm) and numerical (Nvm) density between the two groups of normal, and two groups of breast cancer cells.

	Group 1	Group 2	mean		t	p
Vvm (μm^0)	*norm.cell/+phen.ca.*(1K)	*norm.cell/-phen.ca.* (2K)	0.148	0.116	3.541	0.000
	norm.cell/+phen.ca.(1K)	*cell ca./-phen.* (2C)	0.148	0.163	2.170	0.031
	norm.cell/-phen. ca. (2K)	*cell ca./+phen.* (1C)	0.116	0.152	3.810	0.000
	norm.cell/-phen. ca. (2K)	*cell ca./-phen.* (2C)	0.116	0.163	5.438	0.000
Svm (μm^{-1})	*norm.cell/+phen.ca.*(1K)	*norm.cel./-phen.ca.* (2K)	0.542	0.325	9.077	0.000
	norm.cell/+phen.ca. (1K)	*cell ca./+phen.* (1C)	0.542	0.450	3.373	0.001
	norm.cell/+phen.ca. (1K)	*cell.ca./-phen* (2C)	0.542	0.369	7.379	0.000
	norm.cell/-phen.ca. (2K)	*cell ca./+phen.* (1C)	0.325	0.450	5.703	0.000
	norm.cell/-phen.ca. (2K)	*cell ca./-phen.* (2C)	0.325	0.369	2.616	0.009
	cell ca./+phen.ca. (1C)	*cell ca./-phen.* (2C)	0.450	0.369	3.779	0.000
Svm/Vvm (μm^{-1})	*norm.cell/+phen.ca.*(1K)	*norm.cell./-phen.ca.* (2K)	3.807	3.119	5.620	0.000
	norm.cell/+phen.ca. (1K)	*cell ca./+phen.* (1C)	3.807	3.100	5.807	0.000
	norm.cell/+phen.ca. (1K)	*cell ca./-phen.* (2C)	3.807	2.337	13.296	0.000
	norm.cell/-phen.ca. (2K)	*cell ca./-phen.* (2C)	3.119	2.337	8.056	0.000
	cell ca./+phen.ca. (1C)	*cell ca./-phen.* (2C)	3.100	2.337	7.916	0.000
Nvm (μm^{-3})	*norm.cell/+phen.ca.* (1K)	*norm. cell/-phen.ca.* (2K)	0.939	0.316	8.920	0.000
	norm.cell/+phen.ca. (1K)	*cell ca./+phen.* (1C)	0.939	0.451	6.749	0.000
	norm.cell/+phen.ca. (1K)	*cell ca./-phen.* (2C)	0.939	0.261	9.774	0.000
	norm.cell/-phen.ca. (2K)	*cell ca./+phen.* (1C)	0.316	0.451	4.453	0.000
	norm.cell/- phen. ca. (2K)	*cell ca./-phen.* (2C)	0.316	0.261	2.439	0.015
	*cell ca./+phen.*ca. (1C)	*cell ca./-phen.* (2C)	0.451	0.261	6.507	0.000

norm.cell/+phen.ca. (1K) – normal breast epithelial cells taken from the environment of cancer tissue positive for estrogen and progesterone receptors (+ phenotype); *norm. cell/-phen.ca.* (2K) – normal breast epithelial cells taken from the environment of invasive cancer tissue negative for estrogen and progesterone receptors (- phenotype); *cell ca./+phen.ca.* (1C) – cells from breast cancer positive for estrogen and progesterone receptors (ER+PR+ cancer cells); *cell ca./-phen.* (2C) – cells from breast cancer negative for estrogen and progesterone receptors (ER-PR- cancer cells).

Table 2. The coefficients of discrimination between the four studied groups of cells compared to individual stereological mitochondrial parameters.

Stereological mitochondrial parameters	Coefficient of discrimination
Nvm (μm^{-3})	0.124
Svm/Vvm (μm^{-1})	0.082
Svm (μm^{-1})	0.028
Vvm (μm°)	0.004

Nvm – numerical density; Svm/Vvm – specific surface density; Svm – surface density; Vvm – volume density.

intra-mitochondrial matrix (group 1C). These cells are also characterized by the appearance of irregularly shaped, giant mitochondria with tubular christae and with pronounced crystallization of the inner mitochondrial membrane (2C).

DISCUSSION

Previous studies conducted over the last few decades in connection with the malignant transformation of human breast epithelial cells undoubtedly confirmed the connection of this process with the presence/absence of receptors for estrogen or progesterone, which essentially determines the biological potential of a breast tumor (Dowsett et al. 2006; Farzam et al. 2009; Hammond et al. 2010; Dobrescu et al. 2011). Previous studies of the morphological characteristics of mitochondria in various physiological and pathophysiological conditions have indicated that there is an obvious variability in their number, size and shape, depending on their functional state, but that these changes cannot be considered specific for certain tumors (Collins et al. 2002; Elliot and Barnett 2012).

The morphological changes in mitochondria observed in the present study are in agreement with the changes in these organelles observed by other authors who have examined the correlation between the structure and function of mitochondria and the status of estrogen and progesterone receptors (Stenger et al. 1983; Stenger et al. 1986; Beneduce et al. 2005).

The results of the present study reveal statistically significant differences in the analyzed stereological parameters between all groups of cells examined. In control cells (1K and 2K), numerical, specific surface, surface and volume density of mitochondria were significantly higher in normal epithelial cells surrounding cancers positive for estrogen and progesterone receptors (1K) than in normal epithelial cells surrounding cancers negative for estrogen and progesterone receptors (2K). These differences indicate that, in normal

cells (group 1K) there are a greater number of mitochondria occupying a larger area and volume of the cells than in normal cells surrounding cancer cells negative for the estrogen and progesterone receptors (group 2K).

However, comparison of the investigated stereological mitochondrial parameters between breast cancer cells with estrogen and progesterone receptors (group 1C) vs. control cells (1K) shows that the numerical, surface and specific surface density of mitochondria is significantly reduced in cancer cells. In contrast, comparison of stereological mitochondrial parameters in breast cancer cells without estrogen and progesterone receptors (group 2C) showed a significant decrease of numerical and specific surface density, and increase their surface and volume density vs. controls (group 2K); suggesting that cancer cells from group 2C have a smaller number of mitochondria occupying a larger area and volume in the cell reference area.

In particular, interesting differences were found in the values of stereological parameters of mitochondria between the two groups of cancer cells (groups 1C and 2C). In cancer cells negative for estrogen and progesterone receptors (group 2C) we observed that numerical, surface and specific surface density are significant reduced comparing to cells with a high content of these receptors.

The observed differences in volume density between the mitochondria of receptor positive/negative cancerous cells may be due to several factors. For example, because they are receptor negative, cells from group 2C are not exposed to hormonal activity, but, due to their rapid proliferation are more exposed to the effects of oxygen reduction and lack other necessary elements for reproduction. This indicates that the tumor mass in tumors negative for estrogen and progesterone receptors increase faster. In fact, previous studies have shown that receptor negative cancers are poorly differentiated, have a poorer prognosis, and are more difficult to treat (Dowsett et al. 2006).

In contrast, in cancer cells positive for estrogen and progesterone receptors (which show better responses to estrogenic stimulation) mitochondria are more numerous, more uniform in shape, and occupy a larger area and smaller volume. Hormonal influence on mitochondria is undoubtedly a factor, because clear differences between cancer cell groups in all measured stereological mitochondrial parameters were observed.

The increased mitochondrial volume in cancer cells vs. normal cells may be explained by the fact that cancers (both receptor positive and negative) increase in cellular mass relatively quickly (with receptor negative cells growing faster than receptor positive cells). However, this fast growing cellular mass is very vulnerable because of poor vascularization. The vascular neogeneza in tumors that quickly increase in mass does not adequately follow the growth of the tumor, resulting in different morphological signs of hypoxia. These earliest morphological changes expressed in mitochondria could be monitored. However, changes in the number and volume of mitochondria caused by lack of oxygen at the earliest stages of cancer are very difficult to distinguish, even at the ultrastructural level, from mitochondria which *a priori* possess these characteristics. For these reasons, it is possible that this phenomenon can be explained by a faster increase in tumor mass and compromised circulation in the cancer tissue.

Based on the coefficient of discrimination for the investigated stereological mitochondrial parameters, we conclude that observed differences between the analyzed groups of cells are mostly due to changes in numerical density (kd = 0.124) and specific surface density, which determines the shape of the mitochondria (kd = 0.082), and that surface (kd = 0.028) and volume density (kd = 0.004) are more minor contributing factors. This suggests that the number and shape of mitochondria in the reference area are the most important indicators of morphological differences in normal vs. malignant cell groups.

Taken together, our results suggest that mitochondria in breast cancer cells are significantly different from the mitochondria of normal cells in breast glandular epithelia. In addition, mitochondrial differences observed between breast cancer cell types correlate with the status of estrogen and progesterone receptors.

REFERENCES

Arihiro K, Umemura S, Kurosumi M, Moriya T, Oyama T, Yamashita H, Umekita Y, Komoike Y, Shimizu C, Fukushima H, et al. 2007. Comparision of evaluations for hormone receptors in breast carcinoma using two manual and three automated immunohistochemical assays. American Journal of Clinical Pathology. 127:356–365.

Badve SS, Baehner FL, Gray RP, Childs BH, Maddala T, Liu ML, Rowley SC, Shak S, Perez EA, Shulman LJ, et al. 2008. Estrogen- and progesterone-receptor status in ECOG 2197. Comparison of immunohistochemistry by local and central laboratories and quantitative reversetranscription polymerase chain reaction by central laboratory. Journal of Clinical Oncology. 26(15):2473–2481.

Beleuta M, Rajarama RD, Caikovskia M, Ayyanana A, Germanob D, Choic Y, Schneiderd P, Briskena C. 2010. Two distinct mechanisms underlie progesterone-induced proliferation in the mammary gland. Proceedings of the National Academy of Science of the United States of America. 107(7):2989–2994.

Beneduci A, Chidichimo G, Tripepi S, Perrotta E. 2005. Transmission electron microscopy study of the effects produced by wide-band low-power millimeter waves on MCF-7 human breast cancer cells in culture. Anticancer Research. 25:1009–1014.

Brisken C, O'Malley B. 2010. Hormone action in the mammary gland. Cold Spring Harbor Perspective in Biology. 2(12):a003178, doi:10.1101/cshperspect.a003178, epub 2010 Aug 25.

Chen JQ, Cammarata PR, Baines CP, Yager JD. 2009. Regulation of mitochondrial respiratory chain biogenesis by estrogens/estrogen receptors and physiological, pathological and pharmacological implications. Biochimica et Biophysica Acta. 1793(10):1540–1570.

Chen JQ, Delannoy M, Cooke C, Yager JD. 2004. Mitochondrial localization of ER α and ERβ in human MCF7 cells. The American Journal of Physiology –Endocrinology and Metabolism. 286:E1011–1022.

Cheng G, Li Y, Omoto Y, Wang Y, Berg T,Nord M, Vihko P, Warner M, Pio YS, Gustafsson JA. 2005. Differential regulation of estrogen receptor

(ER)α and (ER)β in primate mammary gland. The Journal of Clinicl Endocrinology and Metabolism. 90(1):435–444.

Collins TJ, Berridge MJ, Lipp P, Bootman MD. 2002. Mitochondria are morphologically heterogenous within cells. The EMBO Journal. 21(7):1616–1627.

Cordera F, Jordan VC. 2006. Steroid receptors and their role in the biology and control of breast cancer growth. Seminars in Oncology. 33(6):631–641.

Dowsett M, Houghton J, Iden C, Salter J, Farndon J, A'Hern R, Sainsbury R, Baum M. 2006. Benefit from adjuvant tamoxifen therapy in primary breast cancer patients according oestrogen receptor, progesterone receptor, EGF receptor and HER2 status. Annals of Oncology. 17(5):818–826.

Elliot R, Barnett B. 2011. Ultrastructural Observation of Mitochondria in Human Breast Carcinoma Cells. Microscopy and Microanalysis. 17(S2):194–195.

Elliot RI, Jiang XP, Head JF. 2012. Mitochondria organelle transplatation: introduction of normal epithelial mitochondria into human cancer cells inhibits proliferation and increases drug sensitivity. Breast Cancer Research and Treatment. 36(2):347–354.

Farzami MR, Anjarani S, Safadel N, Amini R, Moghaddam MG, Roosta B, Nazari S, Saneet S. 2009. Association between the expression of hormone receptors, HER-2/neu overexpression and tumor characteristic in women with primary breast cancer. The Internet Journal of Pathology. 8(2):1–7.

Felty Q, Deodutta R. 2005. Estrogen, mitochondria, and growth of cancer and non-cancer cells. Journal of Carcinogenesis. 4(1):1–35.

Gilbert S. 2006. Multiple hormonal and paracrine interactions in the formation of the mammary glands. In: Gilbert S, editor. Developemental biology. 8th ed, Sunderland MA USA: Sinauer Associate Inc. p. 211-253.

Hammond MEH, Hayes DF, Dowsett M, Allred DC, Hagerty KL, Badve S, Fitzgibbons PL, Francis G, Goldstein NS, Hayes M, et al. 2010. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. Archives of Pathology and Laboratory Medicine. 134(7):e48-72. doi: 10.1043/1543-2165-134.7.e48.

Jacobsen BM, Schittone SA, Richer JK, Horwitz KB. 2005. Progesterone-independent effects of human progesterone receptors (PRs) in estrogen receptor-positive breast cancer: PR isoform-specific gene regulation and tumor biology. Molecular Endocrinology. 19(3):574–587.

Klinge CM. 2008. Estrogenic control of mitochondrial function and biogenesis. Journal of Cellular Biochemistry. 105(6):1342–1351.

Li S, Han B, Liu G, Li S, Ouellet J, Labrie F, Pelletier G. 2010. Immunocytochemical localization of sex steroid hormone receptors in normal human mammary gland. Journal of Histochemistry and Cytochemistry. 58(6):509–515.

Lonard DM, O'Malley BW. 2012. Nuclear receptor coregulators: modulators of pathology and therapeutic targets. Nature Reviews Endocrinology. 8:598–604.

Mandujano-Tinoco EA, Gallardo-Pérez JC, Marín-Hernández A, Moreno-Sánchez R, Rodríguez-Enríquez S. 2013. Anti-mitochondrial therapy in human breast cancer multi-cellular spheroids. Biochimica et Biophysica Acta - Molecular Cell Research.1843(7):1237–1426.

Mattingly KA, Ivanova MM, Riggs KA, Wickramasinghe NS, Barch MJ, Klinge CM. 2008. Estradiol stimulates transcription of nuclear respiratory factor-1 and increases mitochondrial biogenesis. Molecular Endocrinology. 22:609–622.

Nadji M, Gomez-Fernandez C, Ganjei-Azar P, Morales AR. 2005. Immunohistochemistry of estrogen and progesterone receptors reconsired. Experience with 5993 breast cancer. American Journal of Clinical Pathology. 123(1):21–27.

Oda M, Arihiro K, Kataoka T, Osaki A, Asahara T, Ohdan H. 2010. Comparison of immunohistochemistry assays and real-time reverse transcription-polymerase chain reaction for analyzing hormone receptor status in human breast carcinoma. Pathology International. (4):305–315.

Pedram A, Razandi M, Wallace DC, Levin ER. 2006. Funcional estrogen receptors in the mitochondria of breast cancer cells. Molecular Biology of the Cell. 17(5):2125–2137.

Qureshi A, Pervez S. 2010. Allred scoring for ER reporting and it's impact in clearly distinguishing ER negative from ER positive breast cancers. Journal of the Pakistan Medical Association. 60(5):350–353.

Radpour R, Fan AX, Kohler C, Holzgrave W, Zhong XY. 2009. Current understanding of mitochondrial DNA in breast cancer. Breast Journal. 15(5):505–509.

Rhodes A. 2006. Oestrogen receptors and breast cancer. BMS. 6:1055–60.

Rigoglio NN, Mendes Silva MV, Pavanelo Junior V, Ferreira Lima SA, Luiz Nogueira J, Avancini Fernandes R, Miglino MA. 2012. Ultrastructural morphometric analysis by transmission electronmicroscopy associated with stereology methods. In: Méndez-Vilas A, editor. Current Microscopy Contributions to Advances. p. 309–314.

Russo J, Bradle RH, McGrath C, Russo IH.1977. Scanning and transmission electron microscopy study of a human breast carcinoma cell line (MCF-7) cultured in collagen-coated cellulose sponge. Cancer Research. 37:2004–2014.

Stenger HE, Bahnsen J, Trams G. 1983. 9. Steroid receptors. Correlation of estrogen and progesterone receptor status with ultrastructural differentiation in breast tumors. Journal of Steroid Biochemistry. 19(1A):297–303.

Stegner HE, Brörken W, Bahnsen J. 1986. Relation between receptor status and ultrastructure in breast cancer: E+P+ versus E-P-. Pathology, Research and Practice. 181(4):377–381.

Varghese C. 2007. The significance of oestrogen and progesterone receptors in breast cancer. Journal of Clinical and Diagnostic Research. 1(3):198–203.

Weibel ER. 1969. Stereological principes for morphometry in electron microscopic cytology. International Review of Cytology. 26:235–302.

Wolf B, Thompson J, Schwinde A. 1985. An ultrastructural morphometric analysis of normal human mammary tissue and human breast cancer. Anticancer Research. 5:211–230.

Yager JD, Chen JQ. 2007. Mitochondrial estrgen receptors – new insights into specfic functions.Trends in Endocrinology and Metabolism. 18(3):89–91.