

## To Compare the Mitochondrial Complex between Metastasis Breast Cancer and Patients with Breast Cancer and Hepatitis C Virus

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### Abstract

**Background:** Breast cancer (BC) is one of the most common malignancies and the leading cause of mortality in women in Ukraine. The incidence of BC in conjunction with hepatitis C virus (HCV) is increasing at a surprisingly rapid pace. The aim of the current study was to compare the mitochondrial complex between metastasis breast cancer and patients with breast cancer and hepatitis C virus.

**Materials and Methods:** The 147 patients with metastatic breast cancer (MBC) patients we examined. Among them 102 patients were with metastatic breast cancer (MBC), 45 patients were with metastatic breast cancer and hepatitis C virus (MBCV).

**Results:** The total levels of ATP and ADP in patient's breast cancer were  $1.25 \pm 0.02$  nmoles and  $2.27 \pm 0.03$  nmoles in patients with breast cancer, respectively. The ratio of ATP to ADP in presented as 1.0: 1.13. In contrast, the amount of ATP and ADP secreted the patients with breast cancer and hepatitis C virus was  $2.16 \pm 0.4$  nmoles and  $3.42 \pm 0.55$  nmoles, respectively. The ratio of ATP to ADP, secreted from platelets upon activation, was 1: 1.59.

**Conclusion:** The affinity of NADH<sub>2</sub> and NAD<sup>+</sup> can be increased, lactate is converted to pyruvate at higher rate and lactate dependent tumor growth and proliferation can be abolished. Further investigation is needed to confirm this hypothesis. We have also identified that there is a marked difference in the amount of ADP released the patients in breast cancer. Therefore, understanding the intrinsic levels of ATP and ADP may lead to the identification of novel diagnostic method, which may assist in the early identification of patients at risk for cancer diseases. Future studies will compare ATP/ADP levels and ratios, between healthy patients and patients with cancer diseases, in order to identify any differences between their total and released amounts of ATP/ADP and the ratio between them.

**Keywords:** ATP; ADP; Breast cancer; Metastatic breast cancer; Hepatitis C virus

### Introduction

Breast cancer (BC) is one of the most common malignancies and the leading cause of mortality in women in Ukraine. The incidence of BC in conjunction with hepatitis C virus (HCV) is increasing at a surprisingly rapid pace [1,2]. The most commonly compromised sites are bones, lungs, liver, and backbone [3-5]. Traditionally, there is a need for prognostic factors to predict postoperative prognosis after curative resection of the tumor [3]. BC prognostic factors include tumor size, nodal status, histology grade, histology type, and hormone receptor status [5-12]. However, a readily measurable predictive marker predicting BC prognosis would be helpful for early prognostication and risk stratification [6].

Biomarkers are attracting increasing attention as potential predictors of BC patient's survival [7,8]. Hormonal, genetic, and environmental factors explain the disease, but its relation with HCV remains controversial [11-13]. However, HCV could be related to the genesis of tumors and its aggressive behavior. The role of HCV in the genesis of BC remains controversial, and further studies are necessary to define its influence.

Excessive growth is an important characteristic of cancer cells [6]. Glycolysis and oxidative phosphorylation are two major metabolism pathways for producing ATP in mammalian cells [5]. Although oxidative phosphorylation produces higher ATP from one mole of glucose when compared to glycolysis, many questions remain about the efficiency of these pathways for support of excessive growth in cancer cells. Cancer cells mainly generate ATP through glycolysis even in the presence of normal oxygen pressure [4]. Conversion of glucose to lactic acid in the presence of oxygen is known as aerobic glycolysis or the Warburg effect. Increased glycolysis is mostly observed in cancer cells [11]. This bioenergetics and metabolic feature not only permits cancer cells to survive under adverse conditions such as hypoxia, but also enables their proliferation, invasion and subsequent distant metastasis. This condition alters cellular microenvironment and makes it toxic for other cells, but has no harmful effect on cancer cells [5]. The aim of the current study was to compare the mitochondrial complex between metastasis breast cancer and patients with breast cancer and hepatitis C virus.

### Materials and Methods

#### Patient selection

147 patients with metastatic breast cancer (MBC) patients we examined. Among them 102 patients were with metastatic breast

cancer (MBC), 45 patients were with metastatic breast cancer and hepatitis C virus (MBCV). Control group included 25 healthy people who had no history of breast cancer and hepatitis C virus. We analyzed cohorts of consecutive primary breast cancer patients treated the Grigoriev Institute for Medical Radiology Hospital, Kharkiv in 2012–2014.

All patients were treated with curative intent, consisting of mastectomy or breast conservation, followed by external beam radiotherapy and/or systemic adjuvant therapy. Eligible patients were then classified according to geography and histology.

### Enzyme assay and mitochondrial complex parameters

To study the bioenergetic changes in serum lactate/ pyruvate and NAD/NADH<sup>+</sup> correlation were investigated. Mentioned indexes detection was performed using photometric method with a set of chemicals manufactured by firm “Filisit-Diagnosticum”, Ukraine. For determination of lactate and pyruvate test kits produced by “Olvaks”, Ukraine, were used according to manufacturer’s recommendations.

Method for determining lactate and pyruvate is based on enzymatic oxidation of lactic acid into pyruvic acid by lactate dehydrogenase while restoring NAD<sup>+</sup> in NADH<sub>2</sub>. Equilibrium reaction is shifted toward the formation of lactic acid, but when you add hydrazine formed pyruvic acid can be bind completely. Lactic acid quantity is determined by the concentration of formed NADH after measuring optical density at 340 nm. Pyruvic acid quantity is expected according to decreasing in NADH concentration.

NADH concentration (in micromole) in the sample is calculated according to formula:  $X = (\Delta A_{340} \times V) / 6.22$ , were (in which)  $\Delta A_{340}$  changing in reaction mixture optical density, V-volume of sample in ml. NADH molar absorption coefficient at 340 nm is  $6.22 \times 10^3 \text{ M}^{-1} \text{cm}^{-1}$  [5].

Washed platelets ( $5 \times 10^8 / \text{mL}$ ) were assessed with a commercial Enzyte ADP/ATP Ratio Assay Kit to evaluate the total amounts of ATP and ADP. Briefly, platelets were stimulated with TRAP, or left untreated, in a 96-well plate containing a mixture of luciferase, substrate, co-substrate and/or ADP enzyme. In parallel wells, detergent was added to platelet samples to allow determination of the total ATP and ADP load in the platelets.

### Statistical Analysis

Correlation analysis of the results between primary and metastatic breast cancer was performed using the McNemar test. Comparative statistics were performed using chi-square analysis. Statistical significance was assumed for  $P < 0.05$ . Kaplan-Meier survival curves and log-rank statistics were used to evaluate the time until tumor metastasis and the time of survival.

### Results and Discussion

This study included 147 BC women and 25 healthy women individuals. There was not statistically significant intergroup difference in age.

The 86 patients (59.0%) had an age of  $\geq 45$  year and 61 patients (41.0%) had an age of  $\leq 44$  year and 93 patients (64, 3%) were premenopausal women and 54 patients (35, 7%) were postmenopausal women. The 49 patients had tumor-mode-metastasis stage I or II and 98 patients (66, 6%) had tumor-mode-metastasis stage III. 39 patients

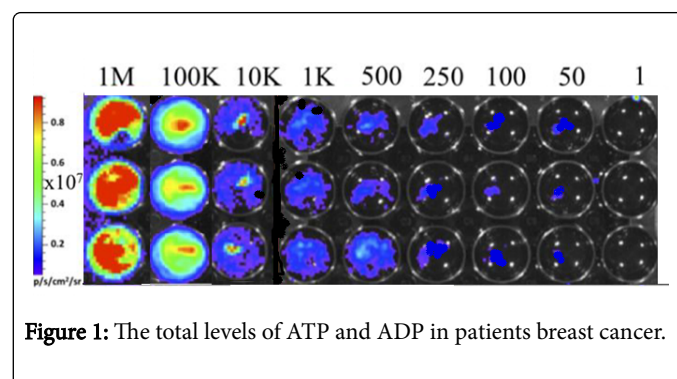
(26, 5%) had negative lymph node status and 108 patients (73, 4%) had positive lymph node status. 42 patients (29, 0%) had histological grade I or II and 105 patients (71, 4%) had histological grade III. The 73 patients (49, 8%) had positive estrogen receptor status and 74 patients (51, 2%) had negative estrogen receptor status and 85 patients (57.8%) had positive progesterone receptor status and 62 patients (42.2%) had negative progesterone receptor status.

To detect bioenergetics changes we examined the ratio of pyruvic acid and lactate as markers of carbohydrate metabolism oxidative stage (the ratio of aerobic and anaerobic phases), and NAD<sup>+</sup> and NADH<sub>2</sub> levels as mandatory participants of oxidation-reduction reactions and regulators of cell metabolism. Decreased NADH<sub>2</sub> index ( $0.002 \pm 0.0001 \text{ mmol/l}$ ) was determined in comparison with control group ( $0.01 + 0.0005 \text{ mmol/l}$ ). The NAD<sup>+</sup> concentration ( $0.494 \pm 0.03 \text{ mmol/l}$ ) was significantly ( $P < 0.05$ ) increased in patients breast cancer in comparison with normal content.

Lactate and pyruvate parameters study has found the following. In patients of all groups lactate indexes exceeded the parameters of control group and amounted  $2.12 \pm 0.23$  and  $1.89 \pm 0.45 \text{ mmol/l}$  for metastatic breast cancer and non metactatic breast cancer accordingly in comparison with control value ( $1.56 \pm 0.235 \text{ mmol/l}$ ). Pyruvate serum indexes were significantly lower than in the control group ( $0.056 \pm 0.011 \text{ mmol/l}$ ) and composed accordingly for MBC and NMBC  $0.031 \pm 0.012$  and  $0.174 \pm 0.01 \text{ mmol/l}$ .

Growth inhibition of human breast cancer cells by exogenous ATP was first shown in 1993, and it was claimed that the growth arrest was mainly due to elongation of the S-phase of the cell cycle. Chemotherapeutic release of ATP from murine breast tumour cells enhanced tumour regression via apoptosis.

The total levels of ATP and ADP in patients breast cancer were  $1.25 \pm 0.02 \text{ nmoles}$  and  $2.27 \pm 0.03 \text{ nmoles}$  in patients with breast cancer, respectively. The ratio of ATP to ADP in presented as 1.0: 1.13 (Figure 1).



**Figure 1:** The total levels of ATP and ADP in patients breast cancer.

In contrast, the amount of ATP and ADP secreted the patients with breast cancer and hepatitis C virus was  $2.16 \pm 0.4 \text{ nmoles}$  and  $3.42 \pm 0.55 \text{ nmoles}$ , respectively. The ratio of ATP to ADP, secreted from platelets upon activation, was 1: 1.59.

### Conclusions

The affinity of NADH<sub>2</sub> and NAD<sup>+</sup> can be increased, lactate is converted to pyruvate at higher rate and lactate dependent tumor growth and proliferation can be abolished. Further investigation is needed to confirm this hypothesis.

We have also identified that there is a marked difference in the amount of ADP released the patients in breast cancer. Therefore, understanding the intrinsic levels of ATP and ADP may lead to the identification of novel diagnostic method, which may assist in the early identification of patients at risk for cancer diseases. Future studies will compare ATP/ADP levels and ratios, between healthy patients and patients with cancer diseases, in order to identify any differences between their total and released amounts of ATP/ADP and the ratio between them.

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