RESEARCH NOTE

Abnormal expression of *ATP1A1* and *ATP1A2* in breast cancer [version 1; referees: 2 approved]

Alexey Bogdanov [1-4], Fedor Moiseenko[1,2], Michael Dubina[1-3]

[1]St Petersburg Academic University, St. Petersburg, Russian Federation
[2]Practical Center for Specialized Types of Medical Care (Oncologic), St-Petersburg, Russian Federation
[3]Peter the Great St. Petersburg Polytechnic University, St. Petersburg, Russian Federation
[4]The Petersburg Nuclear Physics Institute, Gatchina, Russian Federation

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Abstract

Breast cancer is the first in incidence and the second in death among all solid tumors occurring in women. The identification of molecular genetic abnormalities in breast cancer is important to improve the results of treatment. In the present study, we analyzed microarray data of breast cancer expression profiling (NCBI GEO database, accession GSE65194), focusing on Na^+/K^+ -ATPase coding genes. We found overexpression of the *ATP1A1* and down-regulation of the *ATP1A2*. We expect that our research could help to improve the understanding of predictive and prognostic features of breast cancer.

Open Peer Review

Referee Status: ✓ ✓

	Invited Referees 1	2
version 1 published 05 Jan 2017	✓ report	✓ report

1 **Mikhail Fedyanin**, N.N. Blokhin Russian Cancer Research Center Russian Federation

2 **Jen-Tsan Ashley Chi**, Duke University Medical Center USA

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Corresponding authors: Alexey Bogdanov (aleks_aa@mail.ru), Fedor Moiseenko (moiseenkofv@gmail.com)

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Introduction

Breast cancer is one of the most common and deadly female solid tumors[1]. According to reports from Perou *et al.*[2], further confirmed by other investigators[3,4], breast cancer is a highly molecularly heterogeneous disease. The identification of molecular genetic abnormalities in breast cancer is important to improve the results of treatment and, for instance, to reveal new targets for specific therapies. Recent studies based on original retrospective analysis of digitalis use in breast cancer patients have demonstrated the anticancer effect of cardiac glycosides[5] that directly inhibit Na^+/K^+-ATPase (NKA) activity. NKA signaling functions after interaction with cardiac glycosides were also shown[6]. It seems rational that expression of NKA might influence breast cancer prognosis.

NKA is a significant integral membrane protein. NKA's main function is the creation and maintenance of electrochemical gradients for sodium and potassium ions in the living cell. These gradients have critical importance for control of cell volume, osmolarity and resting potential[7,8]. The minimal functional NKA consists of two associated alpha- and beta- subunits. The catalytic alpha-subunit is responsible for conversion of ATP energy to transport of Na^+ and K^+ across cell membranes and has ATP and cardiac glycosides binding sites. It may be present in human tissues in four different isoforms (α1, α2, α3, α4 – found only in testicles). The beta-subunit is responsible for delivery and insertion of alpha one in cell membranes and has three distinct isoforms in humans (β1, β2, β3)[8–10]. NKA subunits are variably expressed in different human tissues[11]. Changes in the relative expression between different isoforms are associated with a number of pathological processes including malignant transformation[12,13]. Both down- and up-regulation of alpha- and beta- subunits were shown in solid tumors of different origin[14–19].

In the present study, we analyzed public breast cancer expression profiles made using Affymetrix Human Genome U133 Plus 2.0 Array (NCBI GEO database[20], accession GSE65194) for the expression of alpha subunits of NKA. We found abnormalities in *ATP1A1* (coding α1-subunit) and *ATP1A2* (coding α2-subunit) expression (Table 1) in breast cancer samples relative to their expression in normal breast tissue. *ATP1A1* was overexpressed approximately 1.5 times in all groups of breast cancer samples ($p<0.05$). Coincidently, *ATP1A2* expression decreased by more than 2 times ($p<0.05$). There were no differences observed in the expression of *ATP1A3* (coding α3-subunit).

Table 1. NKA genes expression in breast cancer samples relative to normal breast tissue.

Breast cancer group	Lum A	Lum B	Her2	TNBC
Gene	Relative expression/(ANOVA P-value)			
ATP1A1	1.53 (0.009016)	1.38 (0.04454)	1.66 (0.005926)	1.44 (0.015725)
ATP1A2	-2.49 ($1.85 \cdot 10^{-07}$)	-2.52 ($8.50 \cdot 10^{-09}$)	-2.78 ($5.48 \cdot 10^{-08}$)	-2.87 (2.08·10-11)
ATP1A3	-1.05 (0.429089)	1.03 (0.308298)	-1.04 (0.768041)	-1.04 (0.527878)

Methods

Preanalytical procedures consisted of a robust multichip analysis (RMA) algorithm[21], including background correction, probe set signal integration, and quantile normalization. For this purpose, we used Expression Console 1.4 software (Affymetrix, Inc. USA). We utilized Transcriptome Analysis Console 3.0 software (Affymetrix, Inc. USA) to analyze the obtained CHP files and to detect differentially expressed genes using one-way between subjects ANOVA. Array data for 41 triple negative samples (TNBC group), 30 Her2-positive (Her2 group), 30 Luminal B (Lum B group), 29 Luminal A (Lum A group) breast cancer samples and 11 normal breast tissue samples were investigated.

Conclusions

Using a public microarray dataset we found abnormalities in the expression of *ATP1A1* and *ATP1A2* in breast cancer samples. This may correlate with digitalis anticancer activity, but requires additional research. We expect that our research could help to improve the understanding of predictive and prognostic features of breast cancer.

Data and software availability

Raw data for Table 1 are available at:

https://www.ncbi.nlm.nih.gov/geo/download/?acc=GSE65194&format=file[22].

Expression Console 1.4 software and Transcriptome Analysis Console 3.0 software (Affymetrix, Inc. USA) are available after free customer registration at:

http://www.affymetrix.com/support/technical/software_downloads.affx.

Author contributions

AB, FM and MD conceptualized the study, collected data and performed data analysis. All authors were involved in the writing and revision of the draft manuscript and have agreed to the final content.

Competing interests

No competing interests were disclosed.

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Version 1

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✔ **Jen-Tsan Ashley Chi**
Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, 27708, USA

I think the analysis is appropriate to examine the relative expression of the *ATP1A1* and *ATP1A2* among different breast cancer cells. The data analysis is standard and appropriate. One helpful thing is to validate the findings in other breast cancer expression datasets beyond this discovery dataset. Another relevant thing is whether the abnormal expression of these genes are associated with varying clinical outcomes.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 21 February 2017

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Mikhail Fedyanin
Department of Clinical Pharmacology and Chemotherapy, N.N. Blokhin Russian Cancer Research Center, Moscow, Russian Federation

Over the past years several papers were published concerning prognostic role of *ATP1A1* expression in hepatocellular carcinoma, lung cancer, and esophageal cancer. The authors of the present study show that increased expression of *ATP1A1* observed at all breast cancer phenotypes compared to normal tissue.

I would like to note that the authors studied gene expression only, but did not appreciate the immunohistochemical (IHC) changes in the content of gene products. In the absence of data of the IHC expression of *ATP1A1*, it is desirable to represent the differences in gene expression of *ATP1A1* compared to referent genes for membrane transporters (http://bmcmolbiol.biomedcentral.com/articles/10.1186/1471-2199-7-29). Given a sufficiently large number of patients included in the study, it is interesting to evaluate the prognostic and predictive value of these findings. But I can conclude that this article is interesting for medical oncologists and molecular biologists.

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.