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

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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.

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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 digital 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)\(^9,10\). 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 available by 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).

**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\(^22\) and 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.

**Grant information**

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I think the analysis is appropriate to examine the relative expression of the \textit{ATP1A1} and \textit{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.

\textbf{Is the work clearly and accurately presented and does it cite the current literature?}
Yes

\textbf{Is the study design appropriate and is the work technically sound?}
Yes

\textbf{Are sufficient details of methods and analysis provided to allow replication by others?}
Yes

\textbf{If applicable, is the statistical analysis and its interpretation appropriate?}
Yes

\textbf{Are all the source data underlying the results available to ensure full reproducibility?}
Yes

\textbf{Are the conclusions drawn adequately supported by the results?}
Yes

\textit{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.

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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 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.