Investigation of Na+/K+ -ATPase role in cancer cells’ functioning

S A Perkov1, A K Emelyanov1,4,5, S V Shmakov1, A A Bogdanov2-4
1 Nanobiotec Lab, St. Petersburg Academic University, St. Petersburg 194021, Russia
2 Saint Petersburg clinical scientific and practical center for special types of medical care (oncology-oriented), Russia
3 Bionics Lab, ITMO University, St. Petersburg, Russia
4 The Petersburg Nuclear Physics Institute, Gatchina, Russia
5 Pavlov First Saint-Petersburg State Medical University, St. Petersburg, Russia

Abstract. Na+/K+ -ATPase is an essential protein for cell functioning which has a great impact on osmotic stabilization, electrochemical potential and volume of the cell. According to the research of A Bogdanov and co-authors there is an overexpression of ATP1A1 and down-regulation of ATP1A2 in breast cancer cells [1]. Therefore, we assumed an existence of correlation between cancer cells proliferation and ATPase expression, which gives a possibility to regulate cancer cells proliferation via ATPase inhibition. In this research we have found a down-regulation of ATP1A1 in K562 cells after its processing with AgNO3. We expect that our research could help in investigating Na+/K+ -ATPase role in cancer cells’ functioning.

1. Introduction

One of the most important problems for a living cell is maintaining its volume and osmolarity. There are plenty of ions that are essential for cell’s existence. The pressure created by the osmolarity differences between intracellular and extracellular space can easily reach 1 atm or more [2], which is too high for plasma membrane to withstand. Now it is clear that osmotic stabilization of a living animal cell is related to the active transport of Na+ from and K+ into the cell, which is provided by Na/K ATPase, also known as Na/K pump. Therefore, we can see a great role of Na+/K+ -ATPase in cells’ existence.

Na+/K+ -ATPase’s (NKA) main function is the creation and maintenance of electrochemical gradients for sodium and potassium ions in living cells. These gradients have a huge influence on cells’ volume, osmolarity and resting potential. The minimal functional NKA consist of two associated subunits: α- and β-. The catalytic α-subunit is responsible for ATP energy conversion to transport Na+ and K+ ions and has ATP and cardiac glycosides binding sites. In human tissues it is presented in four isoforms (α1, α2, α3, α4). The beta subunit is responsible for delivery and insertion of α1 subunits into the cell membrane and it presented in three isoforms (β1, β2, β3).

A series of experiments has shown that cardiac glycosides actually inhibit the cellular sodium-potassium pump and due to this may have an antitumor effect [3]. From the other side, silver nitrate also inhibits the Na+/K+ -ATPase, probably acting like a potassium mimic and actually blocking the K-site of NKA [4]. Furthermore silver has antitumor effect [5]. Taking into account the fact that many cell cultures have an overexpression of NKA [1], and combining all of the above together, occurs an assumption that silver’s antitumor effect is related to the NKA inhibition.
2. Materials and methods
In present study we focused on $\alpha_1$ subunit ($ATP1A1$ gene) expression in K562 cells (chronic myeloid leukemia). K562, chronic myelogenous leukemia, culture was taken from the Russian vertebrates’ cells collection of Cytology Institute, RAS. Cells were cultivated in RPMI-1640 medium (HyClone, USA) with the addition of 10% embryonic bovine serum (HyClone, USA) in presence 40 µg/ml of gentamicine (Sigma, USA) in temperature of 37°C in 5% CO$_2$ atmosphere. Then processed with AgNO$_3$ in two concentrations: $9 \times 10^{-5}$ (9-5) and $10^{-4}$ (1-4) mol/L. Control group was processed with the same amount of water. We chose this concentrations because processing cells with it causes a metabolism grow, which has shown via MTT testing. After purifying mRNA from cells (Qiagene RNeasy kit, Netherlands) and synthesis of cDNA from mRNA via reverse transcription (Thermo Scientific, RevertAid First Strand cDNA synthesis kit, USA), we used real-time PCR (Eco Real-Time PCR System, Illumina) with GAPDH as a control gene to detect an expression change of ATP1A1.

Furthermore we compared an expression of $ATP1A1$ via qRT-PCR (Eco Real-Time PCR System, Illumina) with GAPDH as a control gene in two similar cell lines: 3T3b and 3T3-SV40. 3T3b is cell line of mice fibroblasts and 3T3-SV40 is the same cell line, but transformed by SV40 virus. They have highly similar structure, but different sensibility to silver (Fig. 1).

![Figure 1](image.png)

**Figure 1:** comparison of cells’ viability after processing with silver nitrate in different concentrations.

3. Results
We have shown the downregulation of $ATP1A1$ expression in investigated samples (9-6 and 1-5) after inhibition with AgNO$_3$ (Fig. 2a). This may explain an antitumor effect of silver: majority of cancer cells have an overexpression of ATP1A1 and processing cells with silver nitrate lowers the expression levels, which could be the reason of silver’s antitumor effect.

An experiment with 3T3b and 3T3-SV40 cell lines has shown a difference in $ATP1A1$ expression: 3T3-SV40 contain more NKA then 3T3b (Fig. 2b). This may explain why 3T3b cells more sensible to silver: silver inhibits NKA, and 3T3b have less NKA, then 3T3-SV40. This mean that in presence of silver nitrate in equal concentrations, percent of blocked NKA in 3T3b cells would be higher, than in 3T3-SV40 cells.
Figure 2(a,b): (a) Comparison of ATP1A1 expression in proceeded and non-proceeded samples. Each bar represents level of relative expression ATP1A1 gene to GAPDH gene; (b) Comparison of ATP1A1 expression in 3T3b and 3T3-SV40 cell lines.

4. Discussion

Considering all of the above, we can assume that NKA has a great role in mechanism of antitumor activity of silver. Still it is not clear what happens after NKA inhibition and what processes lead to cancer cells’ death.

References
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