doi 10.18699/bgrs2024-11.3-07

Molecular mechanisms of cell death under cold plasma jet exposure: potential enhancement with gold nanoparticles

Koval O.[1, 2]*, Biryukov M.[1, 2, 3], Polyakova A.[1, 2, 3], Krychkova N.[1, 2], Semenov D.[1], Gorbunova E.[1, 2], Pyshnaya I.[1], Zakrevsky Dm.[2, 4], Milakhina E.[2, 4], Schweigert I.[2]

[1] *Institute of Chemical Biology and Fundamental Medicine, SB RAS, Novosibirsk, Russia*
[2] *Khristianovich Institute of Theoretical and Applied Mechanics, SB RAS, Novosibirsk, Russia*
[3] *Novosibirsk State University, Novosibirsk, Russia*
[4] *A.V. Rzhanov Institute of Semiconductor Physics, SB RAS, Novosibirsk, Russia*
* *o.koval@niboch.nsc.ru*

Key words: cold plasma jet; cytotoxic activity; apoptosis; cell cycle

Motivation and Aim: Cold physical plasma (CP) is a new modality in cancer research that can help enhance the results of chemotherapy and immunotherapy. The result of plasma interaction with biological object is the generation of ions, oxygen- and nitrogen-related species (RONS) in the gas and water environment that actively react with the cells of biological tissue, causing cell death under certain irradiation regimes. The sensitivity of various healthy and tumor cells to plasma irradiation has been shown to be different. Therefore, the study of the mechanisms of resistance of grown human cells to the cold plasma jet of atmospheric pressure and the search for overcoming such resistance is an urgent task of plasma medicine.

Methods and Algorithms: The helium plasma jet was initiated with a generator of sinusoidal voltage with the amplitude of (3.3–3.5) kV at a fixed frequency f_U = 50 kHz and with a generator of unipolar positive pulses with adjustable repetition rate f = 1–40 kHz and amplitude of 3.8 kV and 4.2 kV. Under these modes of plasma jet generation, the near-selective conditions of tumor cell death were determined in *in vitro* experiments. In irradiated cells, dynamic changes in the transcriptome were assessed using the RNA-Seq (Illumina 1500 NextSeq platform) and subsequent gene sets enrichment analysis. Data verification for the particular transcripts was done by RT-PCR and western blot for corresponding proteins. Gold nanoparticles (GNP) were synthesized and covered with polyethylene glycol (PEG) for the co-treatment cells with cold plasma exposure.

Results: The adding of GNP to the CAP-treated cells increased cell death rate compared to cells treated with plasma alone. Based on the transcriptome-analysis data, the genes KLF4, FOS, ATF3, and GADD45B were selected to confirm changes in their mRNA levels in response to CAP exposure and combinatorial treatment of CAP with GNP. The results of the RT-PCR assay showed that the expressions of the *KLF4*, *FOS*, and *ATF3* mRNAs were up-regulated 3 h after the treatment, and GNP did not alter the level and type of transcriptional response. Such a transcriptional response is implicated in the p53 pathway, KRAS signaling, UV response, TNF-alpha signaling, and apoptosis-related processes. The amplitude of the response to CAP treatment was more variable in the A549 cells.

Conclusion: The combination of gold nanoparticles with CAP exposure was a quite successful in the *in vitro* experiments against various cancer cells. We suppose that this way is a promising strategy for treatment of cancerous *in vivo*, especially using modified GNP.

Funding: The study is supported by the RSF grant No. 22-49-08003.