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# Molecular Targeting of Melanoma in a Biomimetic Scaffold: Apoptosis and Autophagy Induced by Cold Atmospheric Plasma

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## ABSTRACT

**Introduction:** Cold atmospheric plasma (CAP) has previously been explored in two-dimensional cultures of melanoma cancer cells and shown to enhance apoptosis and autophagy. However, in vivo, cancer cells reside within a three-dimensional (3D) microenvironment surrounded by neighboring cells and the extracellular matrix, making 3D culture models more reflective of physiological conditions. In this study, we investigated the effects of CAP on the survival, apoptosis, and autophagy of B16-F10 melanoma cells compared to L929 normal cells using a 3D culture model.

**Materials and Methods:** We fabricated polycaprolactone nanofiber scaffolds to serve as 3D culture substrates. B16-F10 and L929 mouse cells were seeded into these scaffolds for 3D culture. Cell viability following CAP exposure was evaluated by the MTT assay, while nitrogen radical levels were quantified using the Griess method. To evaluate apoptosis, we performed Annexin V/PI staining and flow cytometry. The expression levels of apoptotic genes (*BAX*, *BCL-2*, and *CASPASE-3*) and autophagy-related genes (*ATG-5* and *LC-3*) were analyzed using real-time PCR. Data analysis was conducted using Excel 2016 and SPSS v23, with statistical significance set at  $p < 0.05$ .

**Results and Discussion:** CAP treatment significantly decreased the capability of B16-F10 cancer cells, enhanced nitric oxide radical generation, and induced apoptosis, without showing cytotoxic effects on normal L929 cells. Furthermore, the expression levels of *BAX* and *ATG-5* genes were markedly elevated following CAP exposure ( $p < 0.001$ ).

**Conclusion:** Our findings demonstrate that CAP selectively increases reactive oxygen species production, cytotoxicity, and apoptosis, while also increasing the expression of apoptosis- and autophagy-related genes in invasive melanoma cells within a 3D microenvironment that closely resembled in vivo tumor conditions. Elucidation of additional signaling pathways involved could support the future application of CAP as a primary or adjuvant therapeutic strategy for invasive melanoma.



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**Keywords:** Apoptosis, Autophagy cold atmospheric plasma, Invasive melanoma cancer, Three-dimensional culture

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