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## Biological Effects of Cold Atmospheric Plasma on Breast Cancer Tissue

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### OPEN ACCESS

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### ABSTRACT

**Introduction:** Cold atmospheric plasma (CAP) is a non-thermal ionized gas that generates reactive oxygen and nitrogen species (RONS) at room temperature. Due to its low temperature, strong chemical reactivity, and ability to operate without vacuum systems, CAP has become a promising tool in biomedical applications. In cancer therapy, CAP selectively induces oxidative stress, DNA damage, and apoptosis in tumor cells while causing minimal harm to surrounding healthy tissue.

**Materials and Methods:** This study conducted at the Cancer Research Center of Shahid Beheshti University of Medical Sciences, Tehran, Iran, where we evaluated the biological alterations induced by CAP in breast cancer patients. Following tumor excision, a margin measuring 0.5 × 0.5 cm was collected before and after plasma exposure to assess changes at the cellular, molecular, and protein levels. CAP treatment significantly increased RONS production, leading to elevated nitric oxide levels and enhanced apoptosis ( $p < 0.0001$ ). Inflammatory cytokines IL-6 and IL-1 $\beta$  also increased significantly, indicating acute immune activation. Additionally, STAT-1, an important mediator of antitumor immunity, was markedly elevated after CAP treatment.

**Results and Discussion:** CAP treatment reduced the expression of several tumor-promoting components of the extracellular matrix. The level of  $\alpha$ -SMA, a marker of activated myofibroblasts involved in tumor progression, significantly decreased. The expression of type III collagen, which is associated with invasion and metastasis, reduced from 40% to 20%. Cancer stem cell markers CD133 and CD24 also showed decreased expression after plasma therapy. Furthermore, fibroblast activation protein, a key driver of recurrence and metastasis, dropped from 40% to 20%.

**Conclusion:** The results demonstrated a shift towards reduced invasiveness in epithelial-mesenchymal transition-related markers, with decreases in N-cadherin and vimentin and alterations in cadherin structure. Apoptotic markers, including cleaved-caspase-3 and cleaved-PARP1, significantly increased, confirming CAP-induced apoptosis. Overall, CAP effectively modulates the tumor microenvironment, enhances cell death, and reduces metastatic potential in breast cancer tissue.



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