Non-invasive physical plasma (NIPP) activates STING pathway in triple negative breast cancer and is associated with increased host immune response

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Abstract: In this contribution, we present an analysis of STING pathway activation following both direct and indirect plasma treatments in triple-negative breast cancer (TNBC) cells. Additionally, we explore the M1 polarization of THP-1-derived M0 macrophages cultured with conditioned medium from plasma-treated TNBC cells. Findings demonstrate that STING pathway activated by non-invasive physical plasma (NIPP) is responsible for further modulating of tumor immune microenvironment.

1. Introduction

Modulating the tumor immune microenvironment (TIME) has emerged as a promising strategy in cancer therapy. The STING pathway, activated by cytosolic DNA fragments arising from viral infections or DNA damage, orchestrates the recruitment and activation of immune cells, shifting the TIME into a robust anti-tumor state[1]. This pathway is known to be activated in various cancer therapies that induce the accumulation of aberrant cytosolic DNA, such as radiotherapy and certain chemotherapies[2]. Although NIPP is well-documented for its cytotoxic effects on breast cancer cells, including DNA damage induction, the role of CAP in activating the STING pathway and reshaping the TIME remains largely unexplored. Here, we explore the potential of plasma treatment in activating STING pathway and assess its ability in mediating immune response, potentially bridging NIPP's cytotoxic and immunomodulatory effects in breast cancer therapy.

2. Methods

The electrosurgical device VIO® 3/APC 3 was used to generate plasma, to which MDA-MB-231 was exposed directly (APC) or indirectly (plasma treated medium, PTS). Western blot was used to detect expression of γ-H2AX (DNA damage marker), STING, p-STING, TBK-1 and p-TBK-1. Interferon stimulated genes (ISGs) were measured by RT-qPCR. To investigate the immune modulatory effects of NIPP, MDA-MB-231 cells treated with plasma were cultured in complete medium to create a conditioned medium. This medium was then transferred to THP-1-derived M0 macrophages. Flow cytometry was utilized to measure the proportion of M1-polarized macrophages—a phenotype associated with anti-tumor immunity. STING inhibitor H-151 was used to explore the connection between M1 polarization and STING activation[3].

3. Results and Discussion

Figure 1 shows changes in protein expression involving γ-H2AX, p-STING, and p-TBK-1 presented in (A) representative WB, (B) quantitative densitometric analysis, and (C) fold changes in mRNA expression of ISGs in MDA-MB-231 after plasma treatment.

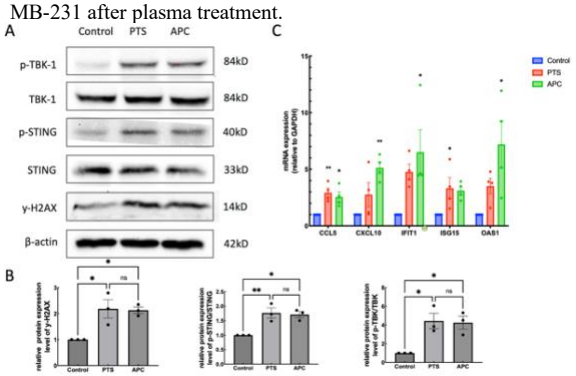
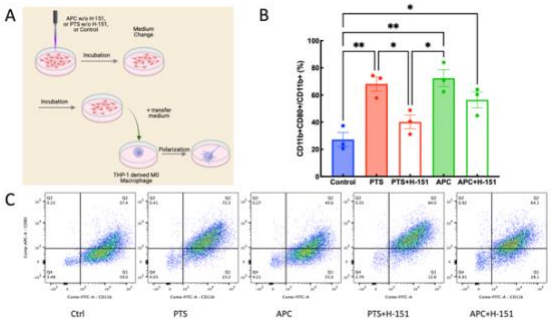


Figure 2 illustrates (A) the schematic of the induction of polarization in THP-1 derived M0 macrophages, (B) quantitative analysis of the change in the proportion of M1 cells (CD11b+CD80+), and (C) representative flow cytograms.



The STING pathway was activated in TNBC following both direct and indirect plasma treatments, as evidenced by an increased p-STING/STING and p-TBK1/TBK1 ratio. This activation led to the upregulation of downstream ISGs, resulting in the production of pro-inflammatory cytokines. These cytokines, present in the culture medium, facilitated M1 polarization of M0 macrophages. The polarization effect was partially attenuated when treated with the STING inhibitor H-151, suggesting that the M1 polarization induced by the plasma treatment was dependent on STING pathway activation.

4. Conclusion

This study highlights that CAP, administered both directly and indirectly, induces DNA damage, which subsequently activates the STING pathway. This activation transforms TIME into a pro-inflammatory state. Such a shift in the TIME shows the potential for combining plasma treatment with other therapies, which depend significantly on the immunological profile of the tumor microenvironment for their efficacy. This demonstrates NIPPS's promising role as an adjunct in immunotherapy strategies, leveraging its ability to modulate the TIME to boost anti-cancer immune responses.

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References

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