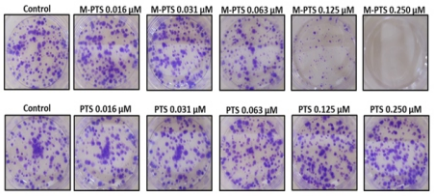
पादपरसायन की ट्यूमर-रोधी प्रभावशीलता

13

कैंसर की वृद्धि में माइटोकॉन्ड्रियल रेडॉक्स संतुलन की भूमिका

राहुल चेकर [1,2], **शिवानी आर. नंदा** [1,2], **आर. एस. पटवर्धन** [1,2], **दीपक शर्मा** [1,2] **और संतोष के संदुर**[1,2]*
[1]विकिरण जीवविज्ञान एवं स्वास्थ्य विज्ञान प्रभाग, भाभा परमाणु अनुसंधान केंद्र, ट्रांबे-400085, भारत
[2]होमी भाभा राष्ट्रीय संस्थान, अणुशक्ति नगर, मुंबई-400094, भारत



A549 कोशिकाओं की कोशिका व्यवहार्यता पर PTS और M-PTS का प्रभाव।

सारांश

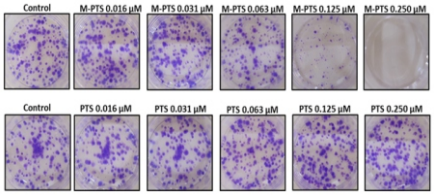
माइटोकॉन्ड्रिया ऑक्सीडेटिव फॉस्फोराइलेशन के माध्यम से ऊर्जा उत्पन्न करके, कोशिकीय उपापचय को नियमन करके और कोशिका उत्तरजीविता और कार्य के लिए महत्वपूर्ण विभिन्न सिग्नलिंग मार्गों में भाग लेकर कोशिका वृद्धि में महत्वपूर्ण भूमिका निभाते हैं। दिलचस्प बात यह है कि कैंसर के विकास और मेटास्टेसिस के सभी लक्षण जैसे कि कोशिका मृत्यु से बचना, जैव और्जिकी में बदलाव और जीनोमिक अस्थिरता भी माइटोकॉन्ड्रिया की शिथिलता से जुड़े हैं। इसके अलावा, माइटोकॉन्ड्रियल रेडॉक्स संतुलन का रखरखाव कैंसर की वृद्धि में महत्वपूर्ण है, क्योंकि यह क्रम प्रसरण और उत्तरजीविता सहित सेलुलर सिग्नलिंग मार्गों को सीधे प्रभावित करता है। कैंसर कोशिकाओं में माइटोकॉन्ड्रिया की यह महत्वपूर्ण भूमिका उन्हें चिकित्सीय हस्तक्षेप के लिए प्रमुख कारक बनाती है। वर्तमान अध्ययन में, हमने ट्राइफेनिलफॉस्फोनियम (टीपीपी+) अंश के साथ संयोजन करके फाइटोकेमिकल्स के नए माइटोकॉन्ड्रिया-लक्षित व्युत्पन्नों की ट्यूमर-रोधी प्रभावकारिता का अध्ययन किया है।

Anti-tumour Efficacy of Phytochemicals

13

Role of Mitochondrial Redox Balance in Cancer Progression

Rahul Checker[1,2], **Shivani R Nandha**[1,2], **R. S. Patwardhan**[1,2], **Deepak Sharma**[1,2] **and Santosh K. Sandur**[1,2]*
[1]Radiation Biology & Health Sciences Division, Bhabha Atomic Research Centre, Trombay-400085, INDIA
[2]Homi Bhabha National Institute, Anushakti Nagar, Mumbai-400094, INDIA



Effect of PTS and M-PTS on the cell viability of A549 cells

ABSTRACT

Mitochondria play a pivotal role in cell growth by generating energy through oxidative phosphorylation, regulating cellular metabolism, and participating in various signaling pathways crucial for cell survival and function. Interestingly, all the hallmarks of cancer growth and metastasis such as cell death evasion, altered bioenergetics, and genomic instability are also linked to mitochondria dysfunction. Further, the maintenance of mitochondrial redox balance is crucial in cancer progression, as it directly affects cellular signaling pathways involved in proliferation and survival. This important role of mitochondria in cancer cells makes them prime candidate for therapeutic intervention. In the present study, we have studied the anti-tumour efficacy of novel mitochondria-targeted derivatives of phytochemicals by conjugating with triphenylphosphonium (TPP+) moiety.

KEYWORDS: *Mitochondrial ROS, Clonogenic potential, Cell death, Triphenylphosphonium, Natural product*

*Author for Correspondence: Santosh K. Sandur
E-mail: sskumar@barc.gov.in

Introduction

Cancer, a multifaceted disease which is characterized by uncontrolled cell proliferation, remains a major challenge in healthcare worldwide. Over the last several decades, researchers have delved into different aspects of cancer biology, including the role of mitochondria (powerhouse of the cell), to understand their role in cancer progression. It is well known that mitochondria play a central role in cellular energy production through oxidative phosphorylation. In addition to their role in fulfilling the metabolic requirements of tumour cells, mitochondria also act as dynamic signaling organelles that control cancer cell survival, motility, stemness and resistance to treatment [1]. Interestingly, mitochondria are also a major source of reactive oxygen species (ROS), which are natural by-products of cellular metabolism. ROS function as signaling molecules at low to moderate levels wherein they regulate cell proliferation, differentiation, and survival. However, excessive ROS production can lead to oxidative stress, causing damage to cellular macromolecules.

Mounting evidence suggests that dysregulation of mitochondrial redox balance plays a pivotal role in cancer initiation, progression, and therapeutic resistance. Furthermore, oncogenic signaling pathways and mutations in key mitochondrial proteins can disrupt mitochondrial function and redox homeostasis in cancer cells. Targeting mitochondrial redox balance represents a promising therapeutic strategy for combating cancer [2]. However, mitochondrial structure and localization are major impediments towards efficient administration of small molecule modulators. Hence, targeting potent anti-cancer compounds to the tumour cell mitochondria by conjugating with delocalized lipophilic cations (DLCs) like triphenylphosphonium (TPP+) may perturb redox balance leading to apoptosis in tumour cells and increase the cytotoxic efficacy of these molecules. The molecule of interest is usually linked via a carbon linker to triphenylphosphonium (TPP+) cation [3]. Several compounds, such as mitochondria-targeted antioxidants and modulators of mitochondrial metabolism, have shown efficacy in preclinical studies and clinical trials. By selectively perturbing mitochondrial redox homeostasis in cancer cells, these agents have the potential to induce oxidative stress-mediated cytotoxicity while sparing normal cells.

In the present study, novel mitochondria-targeted derivatives of Ethyl ferulate (ethyl-3-hydroxy-4-methoxycinnamate; EF) and pterostilbene (3,5-dimethoxy-4-hydroxystilbene; PTS), a methoxylated form of resveratrol (Fig 1) were synthesized. Further, the efficacy of mitochondria targeted derivative of EF (M-EF) and pterostilbene (M-PTS) was compared with with their respective parent molecules in human lung adenocarcinoma cell line (A549) [4, 5].

Materials

Pterostilbene (PTS) was a kind gift from Sami Labs Limited, Bangalore. Ethyl ferulate (ethyl-3-hydroxy-4-methoxycinnamate; EF) was procured from TCI Chemicals. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA, 3-(4,5-dimethyl-2-thiazole)-2,5-diphenyltetrazolium bromide (MTT reagent), dimethyl sulfoxide (DMSO) and crystal violet were obtained from Sigma Chemical Co. (MO, USA).

Methods

Cell culture

A549 lung cancer cell line was purchased from European Health Protection Agency, cultured in DMEM supplemented with 10% FBS and 1X antibiotic antimycotic solution in a humidified 5% CO2 incubator at 37°C.

Cytotoxicity assay

Anti-proliferative effects of EF and PTS and their mitochondria-targeted counterparts were assessed in A549 cells by performing the MTT cell viability assay. Briefly, A549 cells (3000 cells/well seeded in 96 well plate) were incubated with the indicated concentrations of EF or M-EF or PTS or M-PTS for 72h. 4 hours prior to the completion of 72h, 10µl of MTT tetrazolium dye (5mg/ml) was added to every well. After 4h of MTT incubation, media was replaced with 200µl DMSO in order to dissolve the formazan crystals and the absorbance (570nm) was recorded using a microplate reader (Synergy H1 Hybrid, USA).

Clonogenic assay

A549 cells seeded at a density of 500 cells/2ml in a 6-well plate were allowed to attach overnight followed by incubation with varying concentrations of EF or M-EF or PTS or M-PTS for 14 days. The colonies so formed were rinsed with PBS, methanol-fixed and stained with crystal violet. Colonies with at least 50 cells were counted.

Results and Discussion

Mitochondria-targeted derivative of EF and PTS demonstrated superior anti-tumour effect

Incubation with the parent compounds EF or PTS caused

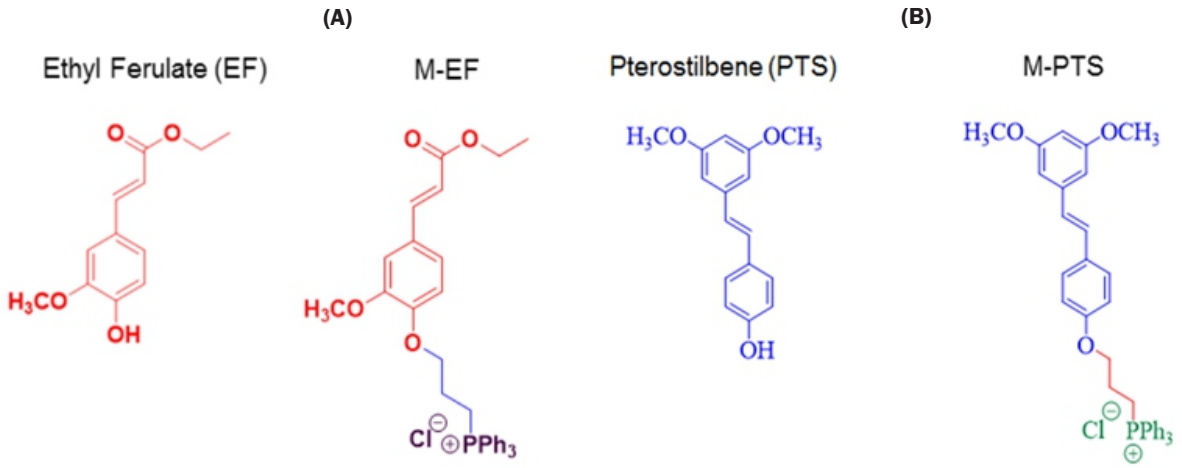


Fig.1: (A) Structure of EF and M-EF and (B) Structure of PTS and M-PTS. Adapted from Patil AS et al., Appl Biochem Biotechnol. 2023 Mar;195(3):2057-2076 and Ibrahim MK et al., Advances in Redox Research 8 (2023) 100071.

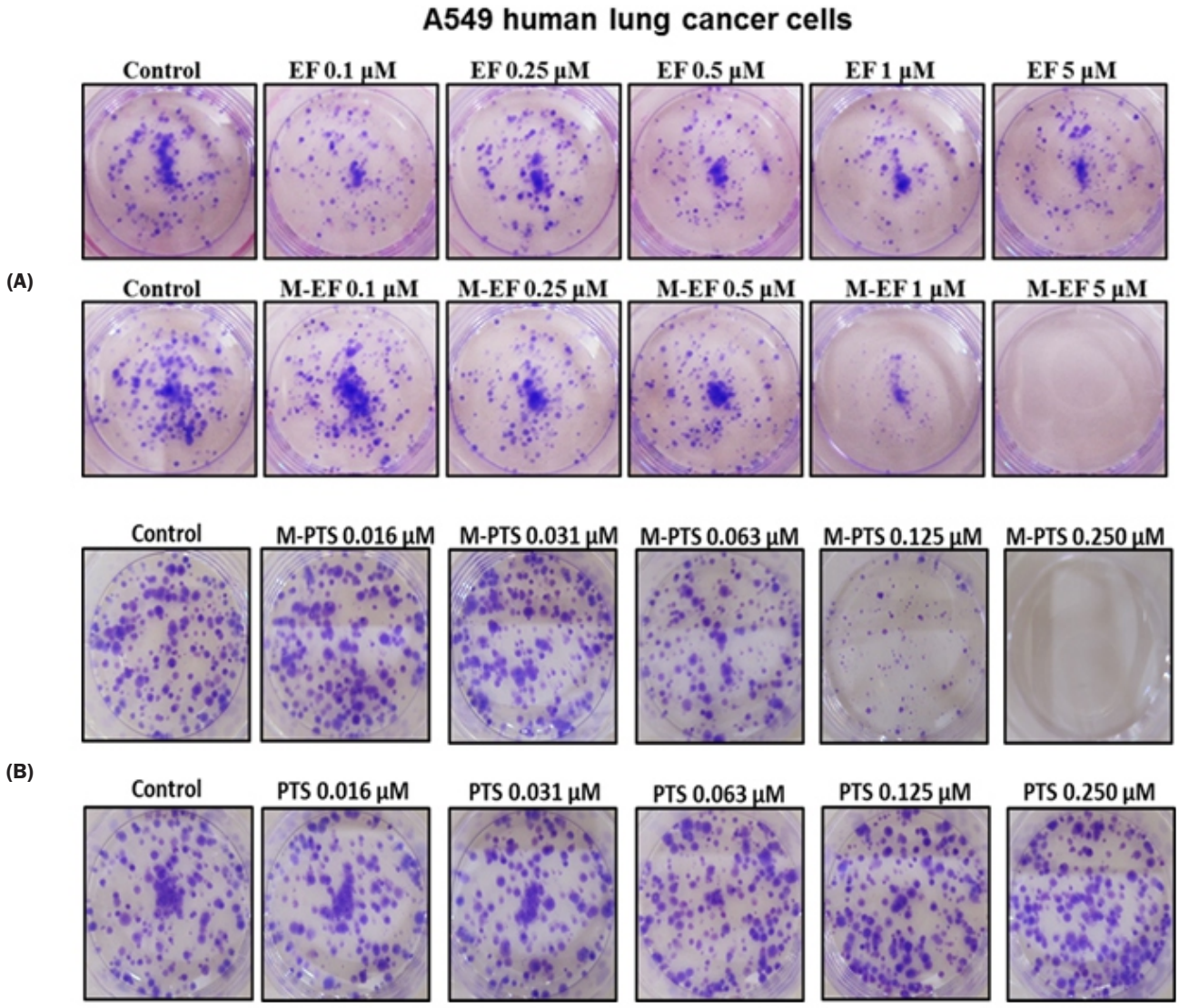


*Fig.4: Effect of (A) EF and M-EF (B) PTS and M-PTS on the mitochondrial ROS levels in A549 cells. **p<0.005; ***p<0.001; ****p<0.0001; as compared to control. Adapted from Patil AS et al., Appl Biochem Biotechnol. 2023 Mar;195(3):2057-2076 and Ibrahim MK et al., Advances in Redox Research 8 (2023) 100071.*

minimal reduction in the cell viability (around 10%) compared to M-EF or M-PTS treatment which resulted in a profound dose-dependent decrease up to 80-90% in the viability of A549 cells (Fig.2(A) & (B)). It was observed that M-EF is around 400-fold more effective than EF and M-PTS is around 200-fold effective than PTS in inducing cell death in A549 cancer cells. Taken together, these results highlight better anti-cancer potency of M-EF and M-PTS over EF and PTS, respectively.

M-EF and M-PTS displayed better efficacy in suppressing the colony-forming potential of lung cancer cells

Addition of EF led to partial reduction in the clonogenic

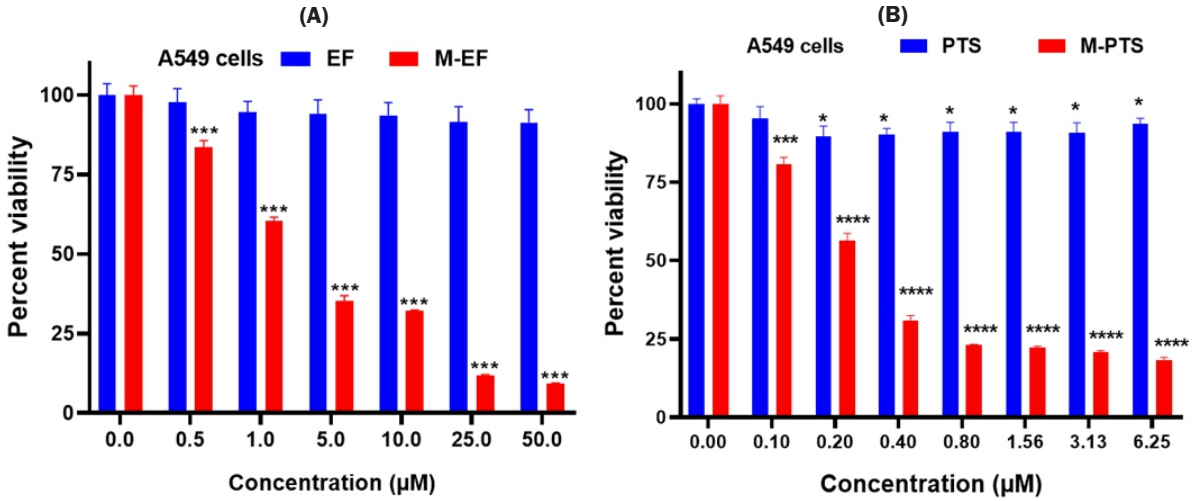


Fig.4: Representative images showing the effect of (A) EF and M-EF and (B) PTS and M-PTS on the clonogenic capacity of A549 cells. Adapted from Patil AS et al., Appl Biochem Biotechnol. 2023 Mar;195(3):2057-2076 and Ibrahim MK et al., Advances in Redox Research 8 (2023) 100071

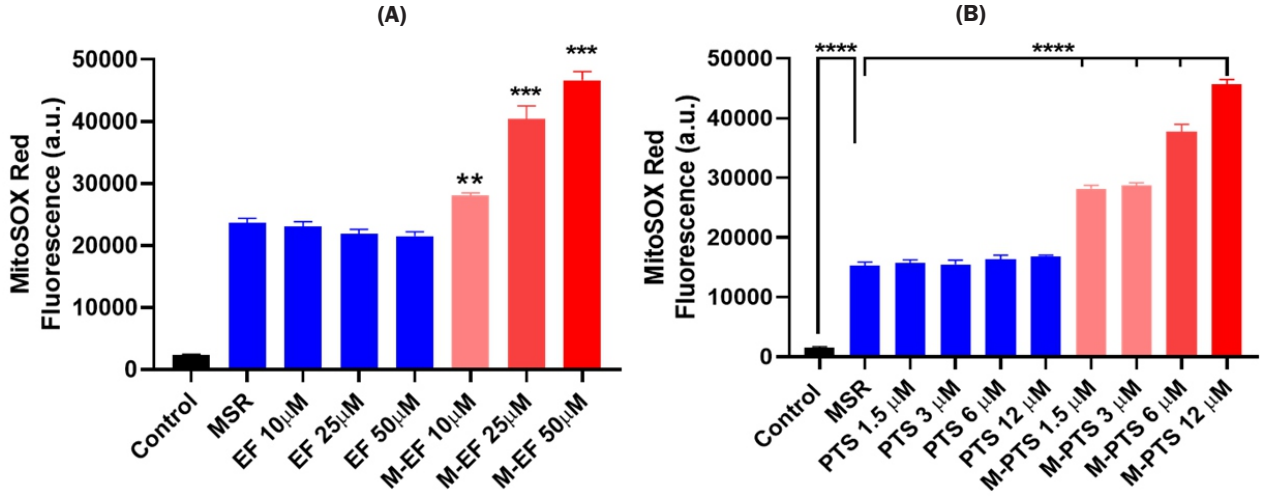


*Fig.4: Effect of (A) EF and M-EF (B) PTS and M-PTS on the mitochondrial ROS levels in A549 cells. **p<0.005; ***p<0.001; ****p<0.0001; as compared to control. Adapted from Patil AS et al., Appl Biochem Biotechnol. 2023 Mar;195(3):2057-2076 and Ibrahim MK et al., Advances in Redox Research 8 (2023) 100071.*

potential of A549 cells while PTS had no significant effect on reducing the colony-forming ability of A549 cells (Fig.3). In contrast, M-EF and M-PTS showed a concentration-dependent decrease in survival fraction of cells with complete loss of clonogenicity observed at 5μM and 0.25μM respectively (Fig.3). These results corroborated M-EF and M-PTS to be more effective than the parent molecules in inhibiting the clonogenic ability of A549 cancer cells.

M-EF and M-PTS increased mitochondrial ROS in cancer cells

Since these derivatives are targeted to the mitochondria, their effect on mitochondrial ROS levels was investigated. It was observed that treatment with both M-EF and M-PTS led to a dose-dependent increase in generation of mitochondrial superoxide in A549 cells (Fig.4(A) & (B)).

Conclusion

In conclusion, mitochondrial redox balance plays a critical role in cancer progression by influencing various cellular processes, including metabolism, signaling, and genomic stability. Combination therapies that integrate mitochondrial-targeted agents with conventional chemotherapeutic agents or targeted therapies may enhance treatment efficacy and overcome drug resistance in cancer patients. However, further research is warranted to elucidate the complex interplay between mitochondrial redox balance and cancer biology and optimize therapeutic interventions.

Acknowledgment

The authors acknowledge that these studies were carried out in collaboration with Institute of Chemical Technology, Matunga, Mumbai.

References

[1] Lopez, J. and S.W. Tait, Mitochondrial apoptosis: Killing cancer using the enemy within, Br J Cancer, 2015, 112(6), 957-62.

[2] Huang, M., et al., Mitochondria as a Novel Target for Cancer Chemoprevention: Emergence of Mitochondrial-targeting Agents, Cancer Prev Res (Phila), 2021, 14(3), 285-306.

[3] Dong, L., et al., Mitocans Revisited: Mitochondrial Targeting as Efficient Anti-Cancer Therapy, Int J Mol Sci., 2020, 21(21).

[4] Patil, A.S., et al., Mitochondriotropic Derivative of Ethyl Ferulate, a Dietary Phenylpropanoid, Exhibits Enhanced Cytotoxicity in Cancer Cells via Mitochondrial Superoxide-Mediated Activation of JNK and AKT Signalling, Appl Biochem Biotechnol., 2023, 195(3), 2057-2076.

[5] Ibrahim, M.K., et al., Mitochondria-targeted derivative of pterostilbene, a dietary phytoestrogen, exhibits superior cancer cell cytotoxicity via mitochondrial superoxide mediated induction of autophagy, Advances in Redox Research, 2023, 8.