Dual MCT1/MCT4 inhibition in Non-Small Cell Lung Cancer (NSCLC): a combined cell line and liquid biopsy approach

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Abstract

Background: Metabolic reprogramming, including the Warburg effect, is an important hallmark of cancer. Monocarboxylate transporters (MCTs) facilitate lactate transport, thereby regulating cancer progression and metastasis. MCT1 and MCT4 are overexpressed in non-small cell lung cancer (NSCLC) tissues and circulating tumor cells (CTCs), while their expression has been correlated with poor clinical outcome. Furthermore, lactate accumulation has been linked to the regulation of mitochondrial reactive oxygen species (mtROS) levels. This study investigated the effects of MCT1/MCT4 inhibitor (Syrosingopine) on NSCLC cell line (H1975), colon CTC-derived cell line (CTC-MCC-41), and NSCLC patient-derived circulating cells.

Methods: Cells were treated with Syrosingopine, a dual MCT1/MCT4 inhibitor. IC_{50} and cell viability were determined via MTT assay. Apoptotic cells were assessed by M30 immunofluorescence staining, targeting a caspase-cleaved neoepitope of Cytokeratin-18 (CK18). mtROS levels were evaluated using MitoSOX™ Red. Peripheral Blood Mononuclear Cells (PBMCs) from two NSCLC patients were also cultured after Ficoll density gradient centrifugation. Cells were treated with Syrosingopine, and stained for CK, CD45, M30, MCT1, and MCT4 antibodies. Samples were analysed using VyCAP platform, followed by statistical analyses.

Results: An IC_{50} of 13μM Syrosingopine was established after 48 hours of treatment and was used for all subsequent experiments. MCT1/MCT4 inhibition significantly reduced cell viability by 46% (p=0.0029) in H1975 and 21% (p=0.0052) in CTC-MCC-41 cells. Syrosingopine treatment increased the percentage of M30-positive cells by 370% (p=0.0004) in H1975, and 45% (p<0.0001) in CTC-MCC-41, compared to the control group. MitoSOX fluorescence analysis demonstrated a significant increase of 140% (p=0.0001) and 90% (p=0.0022) of mtROS levels in H1975 and CTC-MCC-41 cells, respectively, after treatment. In patients' samples, tumor cells were defined as [(CK+/CD45-) or CTCs], and/or (CK-/CD45-) with nuclei size ≥10μm. The total (CK-/CD45-) cell number and MCT1-positive cells were both decreased in these samples (p=0.3185 and p=0.0572, respectively), after treatment.

Conclusions: Dual MCT1/MCT4 inhibition reduced the viability of NSCLC cells and CTCs, along with increased apoptosis and mtROS levels. These findings indicate that targeting MCTs may influence cancer cell survival and metastasis through metabolic and oxidative stress-related mechanisms. Further experiments in NSCLC patients' CTCs are warranted to validate the clinical relevance of these findings.

Introduction

Monocarboxylate transporters (MCTs) hold an important role in lactate transport and have been implicated in cancer cell survival, angiogenesis and metastasis [1]. MCT1 and MCT4 overexpression has been reported in non-small cell lung cancer (NSCLC) cell lines, tissue samples and liquid biopsy components [2,3]. Beyond their role in lactate transport, MCT activity contributes to mitochondrial function, as lactate accumulation has been linked to modulation of mitochondrial reactive oxygen species (mtROS) production [4].

Given their critical role in cancer metabolism, MCT1 and MCT4 may represent attractive therapeutic targets [5]. Importantly, their overexpression in circulating tumor cells (CTCs) suggests that dual MCT1/MCT4 inhibition could affect primary tumors growth and interfere with metastatic dissemination. In this study, we examined the effects of Syrosingopine, a dual MCT1/MCT4 inhibitor, on the NSCLC cell line H1975, the colon CTC-derived cell line CTC-MCC-41, and NSCLC patient-derived circulating cells, to elucidate its impact on cell viability, apoptosis, and mitochondrial ROS regulation.

Results

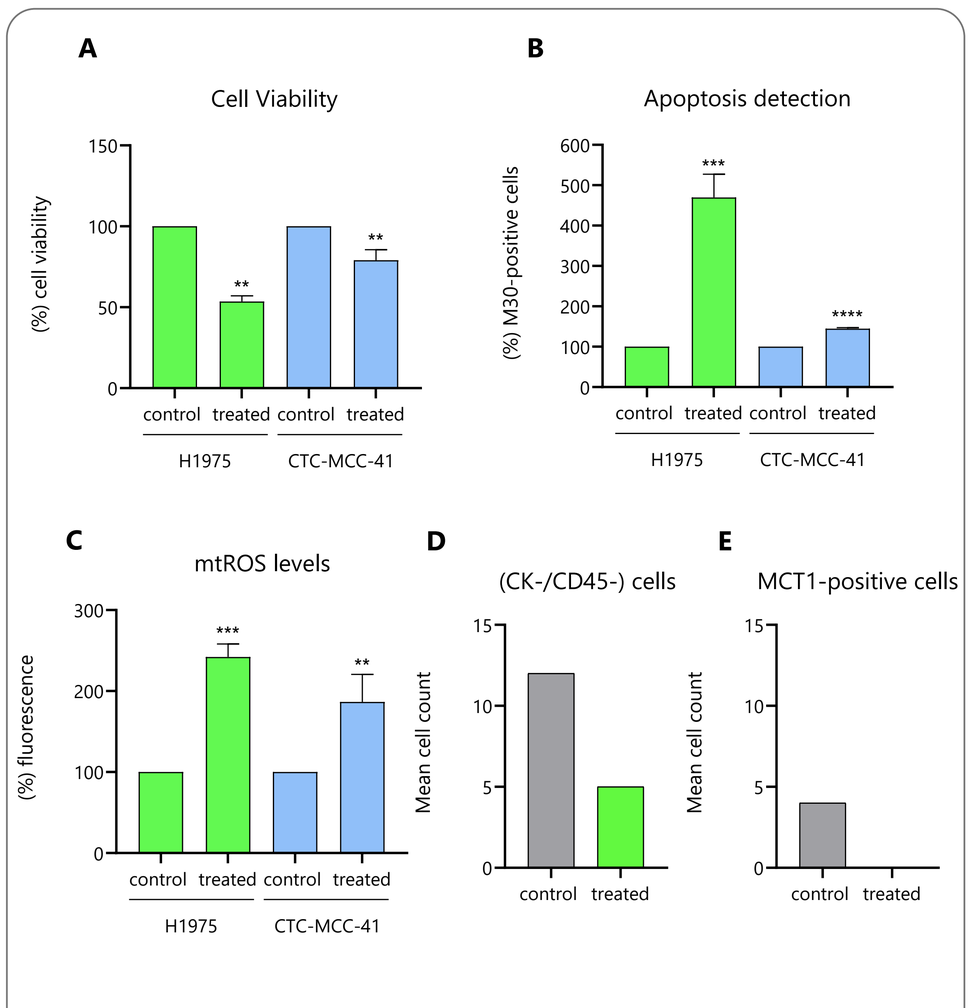

***Figure 1*. Effect of Syrosingopine treatment on cell viability, apoptosis, mitochondrial ROS levels, and MCT1 expression in cancer cell lines and NSCLC patient–derived CTCs. A)** Cell viability of H1975 and CTC-MCC-41 cells following 48h Syrosingopine treatment. Viability was reduced to 54% (p=0.0029) in H1975 cells and 79% (p=0.0052) in CTC-MCC-41 cells. Data are presented as mean ± standard deviation. **B)** Increased percentage of M30-positive cells in H1975 (p=0.0004) and CTC-MCC-41 (p<0.0001) after Syrosingopine treatment compared to control. Data are presented as mean ± standard deviation. **C)** Increased percentage of mtROS levels in H1975 (p=0.0001) and CTC-MCC-41 (p=0.0022) cells after Syrosingopine treatment compared to control. Data are presented as mean ± standard deviation. **D**) Reduced (CK-/CD45-) cell counts in Syrosingopine-treated samples from NSCLC patients (p=0.3185) compared to control. **E)** Reduced MCT1-positive cell counts in Syrosingopine-treated samples from NSCLC patients (p=0.0572) compared to control (** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$).

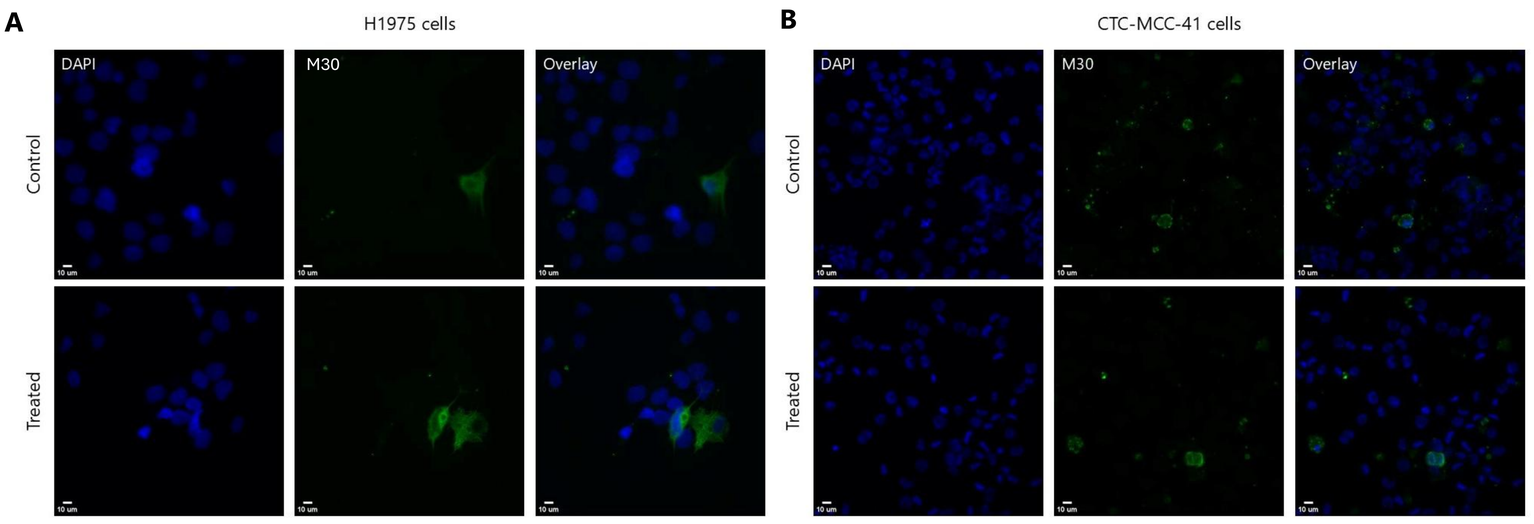

***Figure 2*. Detection of apoptosis after Syrosingopine treatment on cancer cells. A)** Immunofluorescence staining of M30 in H1975 cells. M30 is shown in green and nuclei (DAPI) in blue; overlay images are also presented. Scale bar: 10 μm. **B)** Immunofluorescence staining of M30 in CTC-MCC-41 cells. M30 is shown in green and nuclei (DAPI) in blue; overlay images are also presented. Scale bar: 10 μm.

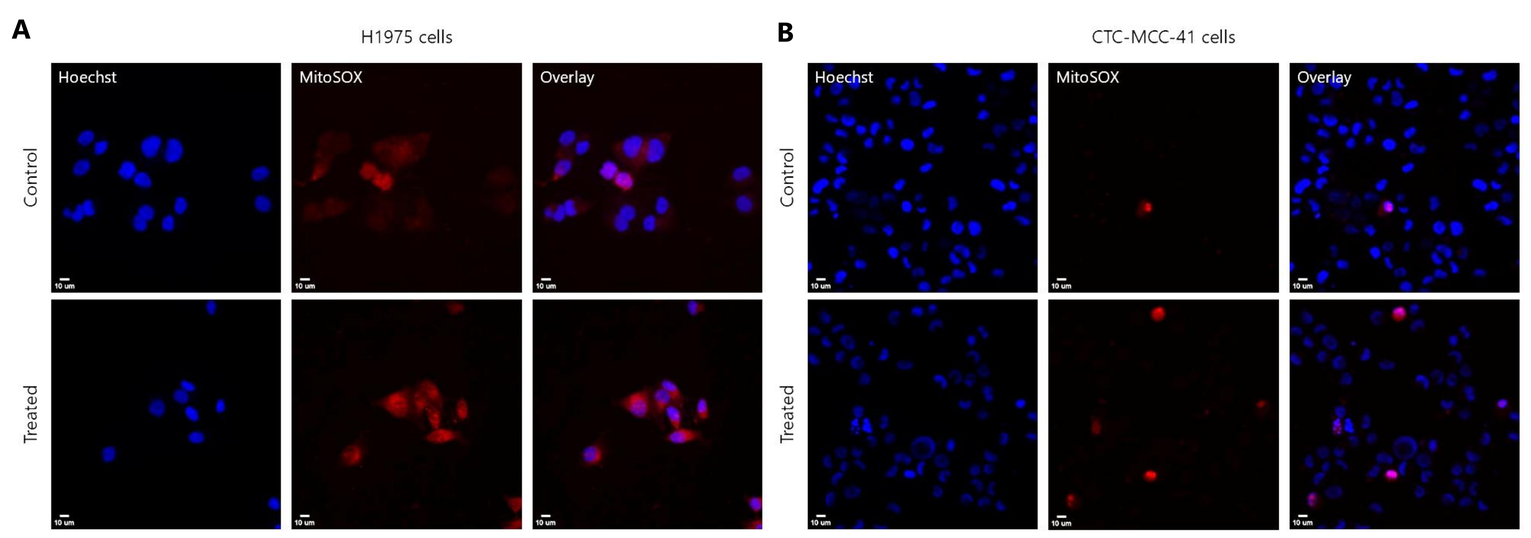

***Figure 3*. Detection of mtROS levels after Syrosingopine treatment on cancer cells. A)** Detection of MitoSOX Red in H1975 cells. MitoSOX Red–positive cells are shown in red and nuclei (Hoechst) in blue; overlay images are also presented. Scale bar: 10 μm. **B)** Detection of MitoSOX Red in CTC-MCC-41 cells. MitoSOX Red–positive cells are shown in red and nuclei (Hoechst) in blue; overlay images are also presented. Scale bar: 10 μm.

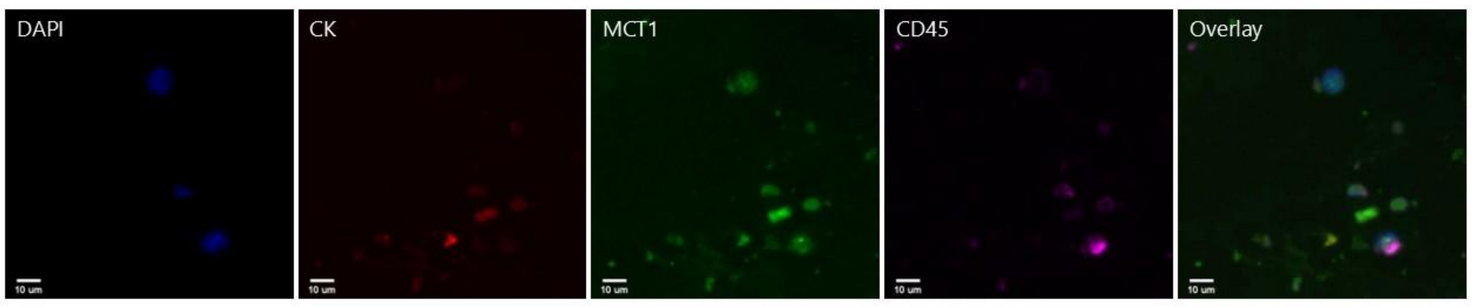

***Figure 4*. MCT1 expression in a (CK-/CD45-) cell derived from an NSCLC patient.** CK is shown in red colour, MCT1 in green and CD45 in purple colour. Nuclei were stained with DAPI (blue); overlay image is also presented. Scale bar: 10 μm.

Conclusions

- ✓ **Dual MCT1/MCT4 inhibition reduced cell viability, and increased apoptosis in NSCLC cells and CTCs**
- ✓ **Targeting MCTs potentially influence cancer cell survival and metastasis through metabolic and oxidative stress mechanisms related to increased mtROS levels**
- ✓ **MCT1 and MCT4 may serve as potential prognostic biomarkers and therapeutic targets for NSCLC patients**
- ✓ **Further experiments in NSCLC patients' CTCs are warranted to validate clinical relevance of these molecules**

References

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Acknowledgements

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