May the post-translational process of succination be involved in cardiotoxicity?

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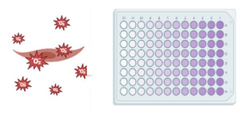
Introduction

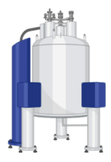
The small ubiquitin-like modifier 1 (SUMO1) plays an important role in the regulation of the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a), which is responsible of facilitating the reuptake of Ca^{2+} in the endoplasmic reticulum after cardiac contraction. Consequently, any modification in SUMO1 could lead to the modification of SERCA2a's activity/stability , hence in cardiotoxicity. In this context, previous in-house data showed that SUMO1 could be a target of succination, an irreversible post-translational modification resulting from the interaction of fumarate with reactive cysteine thiols present in SUMO1. This study aims to understand the impact of SUMO1 succination on SERCA2a SUMOylation, with the hope to better understanding heart physiopathology.

Aims and methods

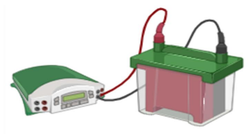
1. **Succination model development using AC16 cardiomyocytes**



2. **Cell viability and oxidative stress investigation**

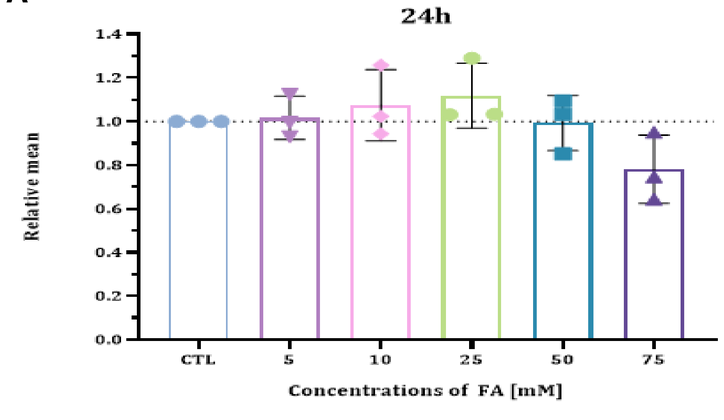


3. **Model characterization using ^{1}H-NMR spectrometry**



4. **Model characterization using Western blotting (on cell extract and on bench assay using a SUMOylation kit)**

Results



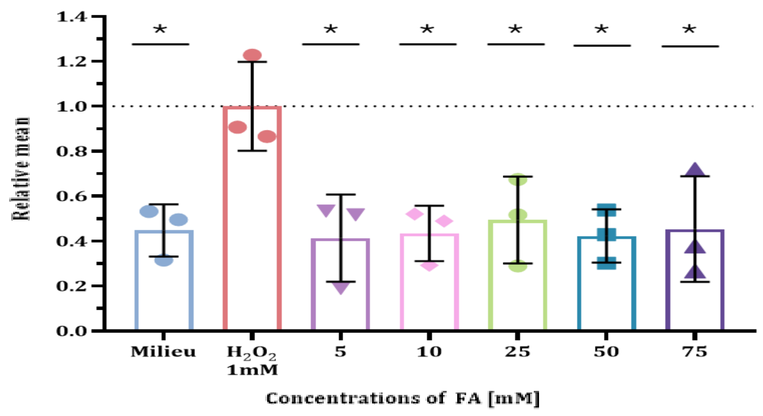


Figure 1 : Effect of Fumaric Acid (FA) treatment on cell viability and possible oxidative stress induction in AC16 cardiomyocytes. A. Relative mean of viability of control cells (negative control) *vs* FA treated cells, using the crystal violet assay. **B.** Relative mean of oxidative stress in H_2O_2 group (Positive control) *vs* FA treated cells. *Data are presented as means; each point represents the mean of independent biological replicates, pipetted 6X, ± SD. Control group is represented at 1 (A. CTL; B. H_2O_2).Statistical analysis were performed by one-way ANOVA followed by Tukey post-test. * p ≤ 0.05.*

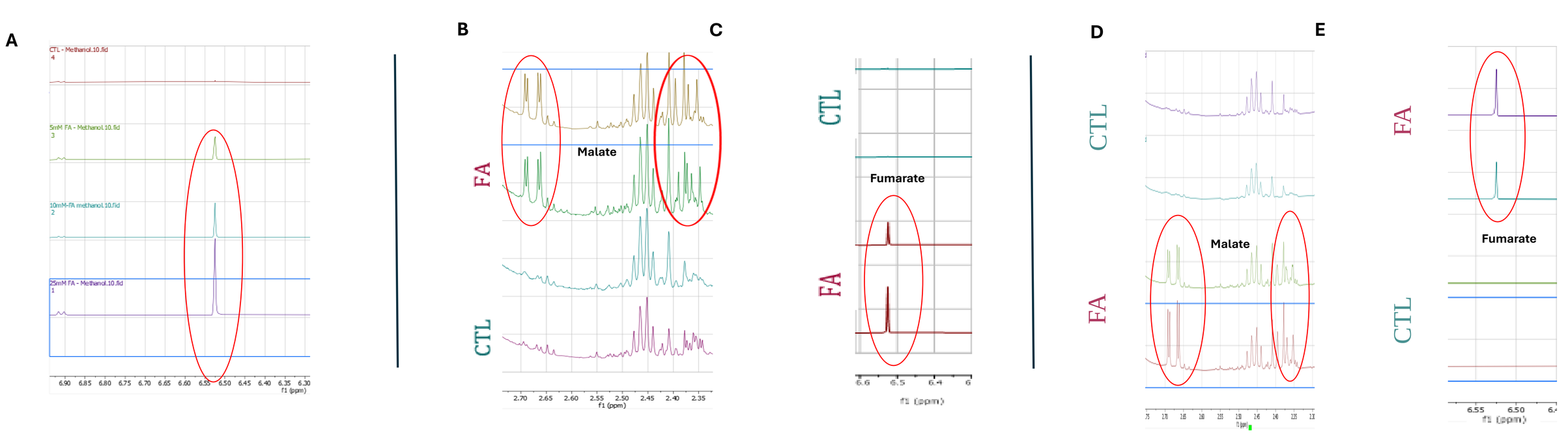
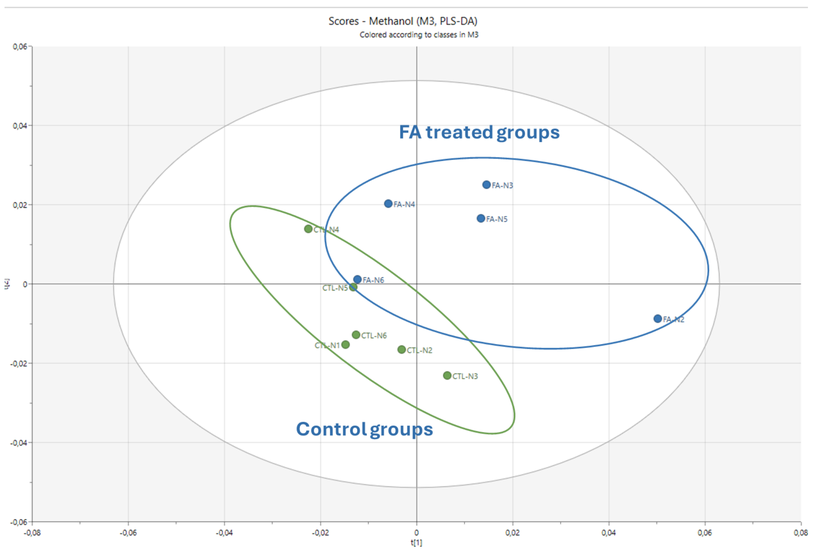


Figure 2: 1H NMR results section. A. Polar cell extract results showing the elevation in the concentration of Fumarate following the addition of FA concentrations raging from 5 to 25mM in comparison to the control group (untreated group). **B.** Extracellular media spectra, showing an elevation in malate metabolite in the treated groups (in red) following the addition of 10mM of Fumaric acid to the cells media in comparison to the non-treated groups. **C** Polar cell extract spectra, showing an elevation in Fumarate in the treated groups (in red) following the addition of 10mM Fumaric acid in comparison to the non treated groups. **D.** Extracellular media spectra, showing an elevation in malate in the treated groups (in red) following the addition of 25mM of Fumaric acid to the cells media in comparison to the non-treated groups. **E** Polar cell extract spectra, showing an elevation in Fumarate in the treated groups (in red) following the addition of 25mM Fumaric acid in comparison to the non treated groups.



PLS-DA : R2X[1] = 0,462; R2X[2] = 0,181; Ellipse: Hotelling's T2 (95%)

Figure3: Score plot of PLS-DA showing the separation of the treated groups (in blue) from the Control groups (in green).

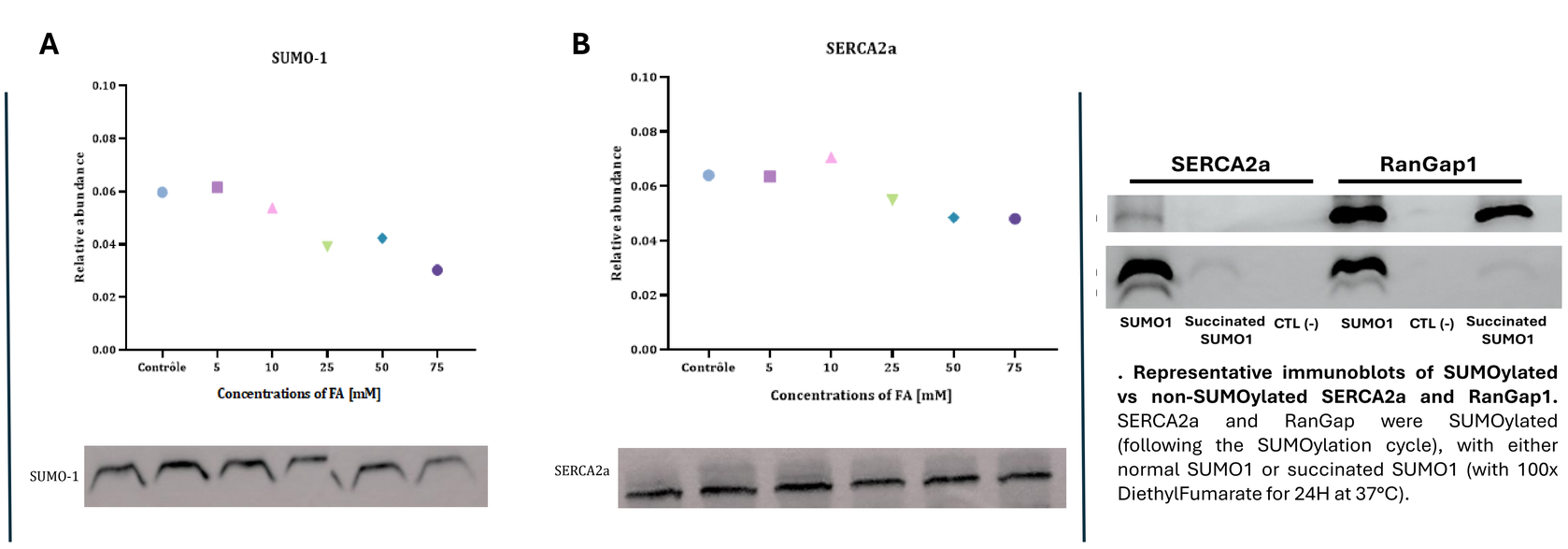


Figure 4: Representative immunoblots of SUMO1 (A) and SERCA2a (B) and quantification of protein abundance in AC16 cardiomyocytes (Succination model) in control group and in FA treated groups. *The graphics are a simple representation of the immunoblots normalized on ponceau, one biological replicate (n=1).*

. Representative immunoblots of SUMOylated vs non-SUMOylated SERCA2a and RanGap1. SERCA2a and RanGap were SUMOylated (following the SUMOylation cycle), with either normal SUMO1 or succinated SUMO1 (with 100x DiethylFumarate for 24H at 37°C).

Conclusion

Preliminary ^{1}H-NMR results suggest the occurrence of succination in treated cells starting at a concentration of 10mM of FA. Though western blot results were not significant, they suggested a tendency of decrease in the abundance of both SUMO1 and SERCA2a especially at higher concentrations of FA (consecrations starting 10mM). In addition, the SUMOylation/Succination reaction assay confirms the decrease of the activity of SUMO1 in SUMOylation both SERCA2a and RanGap1 due to their succination. In conclusion, understanding the impact of SUMO1 succination on SERCA2a SUMOylation, could reveal a new regulatory mechanism in cardiac function, and this might allow a better understanding of heart physiopathology.

References

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