Dissecting the possible role of p97 in muscle wasting during cancer cachexia

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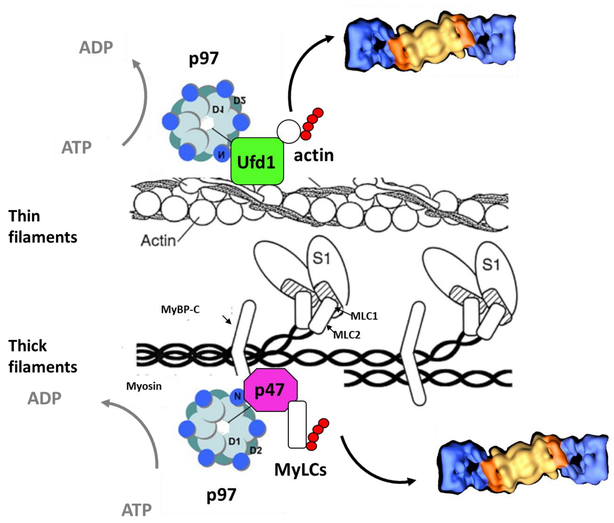
PhD STUDENTS MEETING

Overview

- ✓ The p97/VCP ATPase complex facilitates the extraction and degradation of ubiquitinated proteins from larger structures.



- ✓ It was clearly demonstrated that p97 is essential for the loss of muscle mass upon denervation as well as food deprivation[1, 2, 3].
- ✓ Since cancer-induced muscle wasting shares similar mechanisms as that during denervation or fasting [4], we tested if p97 is involved also in muscle loss during cancer (i.e. cachexia).

Introduction

Half of patients with malignancies develops muscle wasting (i.e. cachexia) and aerobic exercise ameliorates their prognosis. The p97/VCP ATPase complex facilitates the rapid degradation of myofibrillar proteins during muscle atrophy caused by denervation or fasting. The aim of this study was to investigate if p97 plays a role also during cancer cachexia and if it is modulated by physical exercise.

Methods

To induce cachexia in mice, we injected subcutaneously colon adenocarcinoma (C26) or Lewis Lung Carcinoma (LLC) cells. To understand if aerobic exercise improves cancer cachexia through p97 modulation in muscle, C26-bearing mice were run on treadmill for 5 days at 12 m/min and +15° inclination for 45 min/day. By Q-PCR, we measured the expression of p97 and its main adaptor proteins (Ufd1, Ufd2, p47) in cachectic Tibialis Anterior (TA) muscle. In vitro we performed luciferase assay to test the possible effect of p97 or its dominant negative mutant (DNp97) on MuRF1 signalling.

Results

In vivo, we found that the mRNA levels of p97 and its interactors p47, Ufd1 and Ufd2 were induced in TA muscle during advanced cachexia of C26-bearing mice. UFD2 expression is enhanced even earlier (Figure 1).

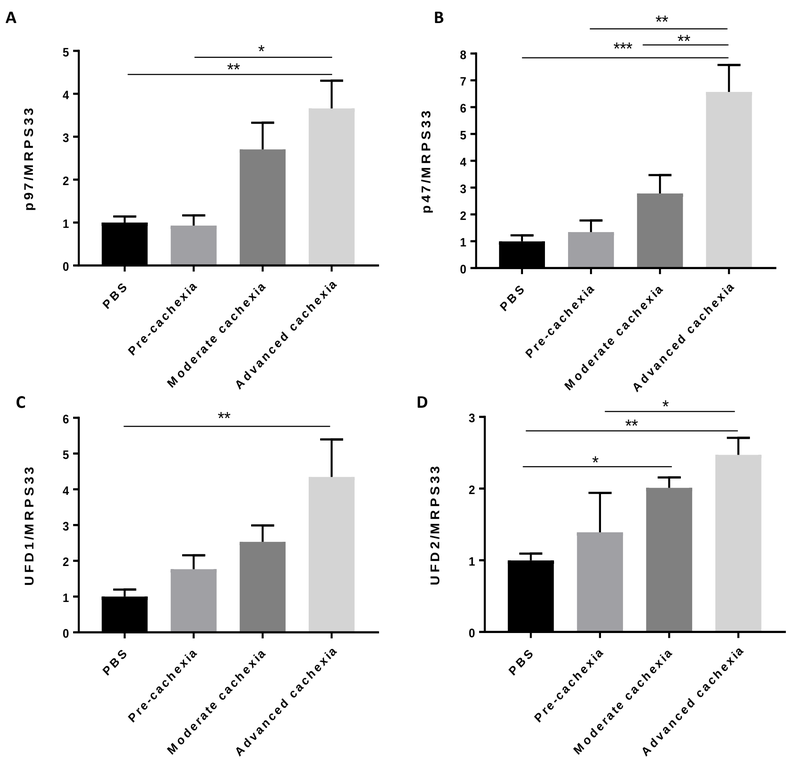


Figure 1: Tibialis anterior of C26-bearing mice, during pre-, moderate and advanced cachexia, were analyzed by qPCR for p97 (A) p47 (B), UFD1 (C) and UFD2 (D) expression. Fold change is shown. MRPS33 was used as housekeeping gene. PBS-treated mice were used as control. Pre-cachexia (body weight loss, BWL≤14%); moderate cachexia (14%<BWL<20%); severe cachexia (BWL≥20%). All results are plotted as mean ± SEM. * p<0.05, ** p<0.01, *** p<0.001; one-way ANOVA with post hoc Tukey's multiple comparisons test.

Interestingly, treadmill exercise had no effect on tumor growth in C26-bearing mice but protected them from muscle loss and rescued C26-induced upregulation of p97. No reduction was observed in C26-induced expression levels of p97 and its adaptors upon running (Figure 2).

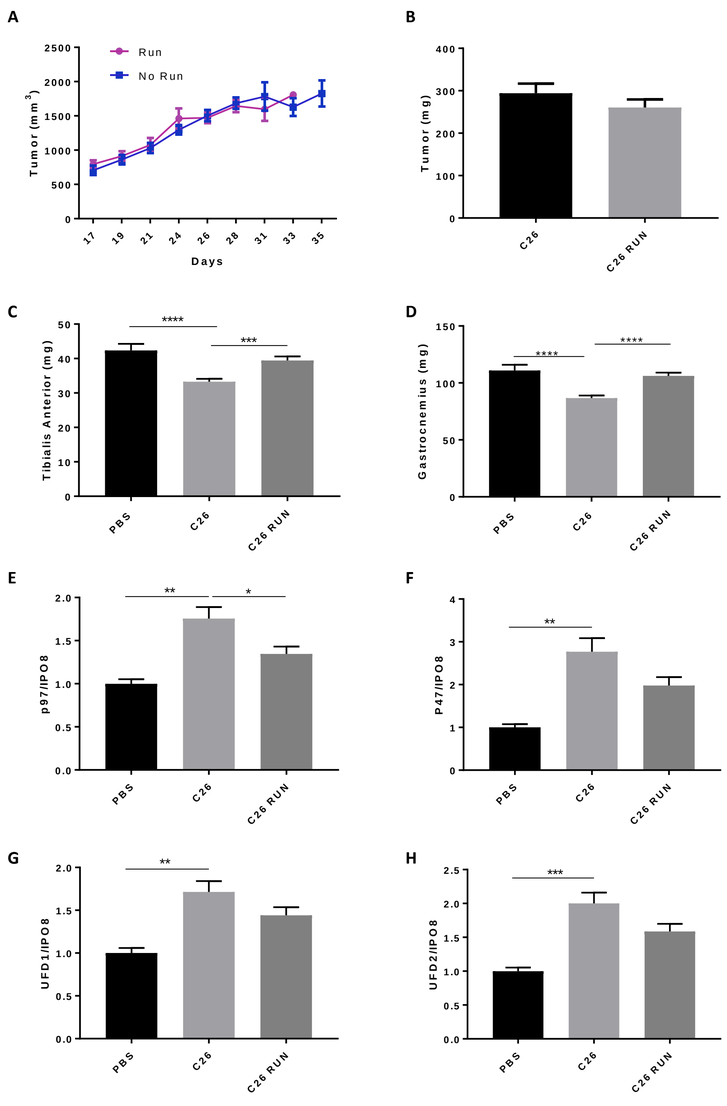


Figure 2: Mice were sacrificed when they lost 20% of their body weights for 72 hours and/or showed signs of distress. Tumor size, measured manually with caliper, are plotted over time, after 17 days from tumor injection (A). Tumor weights are shown (B). TA and gastrocnemius weights are fully preserved in C26-bearing mice in the running schedule (C26 RUN)(C,D). gastrocnemius of C26-bearing mice, and C26 RUN-mice, were analyzed by qPCR for p97 (A) p47 (B), UFD1 (C) and UFD2 (D) expression. Fold change is shown. IPO8 was used as housekeeping gene. PBS-treated mice were used as control. All results are plotted as mean ± SEM. * p<0.05, ** p<0.01, *** p<0.001; one-way ANOVA with post hoc Tukey's multiple comparisons test.

As expected, MuRF1 was induced in dexamethasone-stimulated myoblasts. Surprisingly, we found that, other than WTp97, exogenous DNp97 also slightly upregulated MuRF1 signalling (Figure 3).

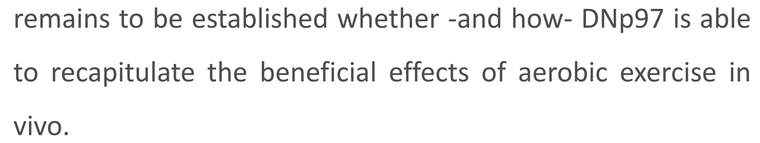


Figure 3: Analysis of MuRF1 signal induction in myoblasts transfected with MuRF1 FLuc and GFP plasmids, and treated for 24h with Dexamethasone (DEXA, 10µM) (A). Analysis of Murf-1 signal induction in myoblasts co-transfected with Murf-1 FLuc, GFP, p97, and dominant negative p97 (DNp97). 1:10 of p97 was also co-transfected with GFP (P97 1:10 GFP). Renilla luciferase plasmid was used as control. Results are expressed as mean ± SEM. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001; unpaired t-test (A) and one-way ANOVA with post hoc Tukey's multiple comparisons test (B).

Conclusions

Our preliminary data suggest that p97/VCP ATPase may play a role in muscle wasting also during cancer in mice but it remains to be established whether -and how- DNp97 is able to recapitulate the beneficial effects of aerobic exercise in vivo.

Acknowledgements

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References

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