UBE2B is implicated in myofibrillar protein loss in catabolic C2C12 myotubes

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Introduction

Muscle atrophy prevails in numerous diseases (cancer cachexia, renal failure, infections, etc.) and mainly results from elevated proteolysis. This largely contributes to increased health care costs. Finding new strategies to prevent muscle wasting is a major clinical challenge and requires information on the precise mechanisms of contractile proteins breakdown . Actin is a crucial component of skeletal muscle. Indeed, actin is a contractile protein and the most abundant protein in skeletal muscle (> 30%). The ubiquitin proteasome system (UPS) degrades some myofibrillar proteins. The targeting of UPS substrates by a ubiquitin chain (Ub) is achieved by an enzymatic cascade E1, E2, E3. UPS substrates are recognized by E3 enzymes while in most cases E2 enzymes define the type of Ub chains and thus whether the substrate is degraded or not by the proteasome. We previously found that the levels of myofibrillar actin were controlled by the muscle-specific E3 ligase MuRF1. In this work, we wanted to identify the E2s potentially involved in myofibrillar protein degradation in catabolic C2C12 myotubes.

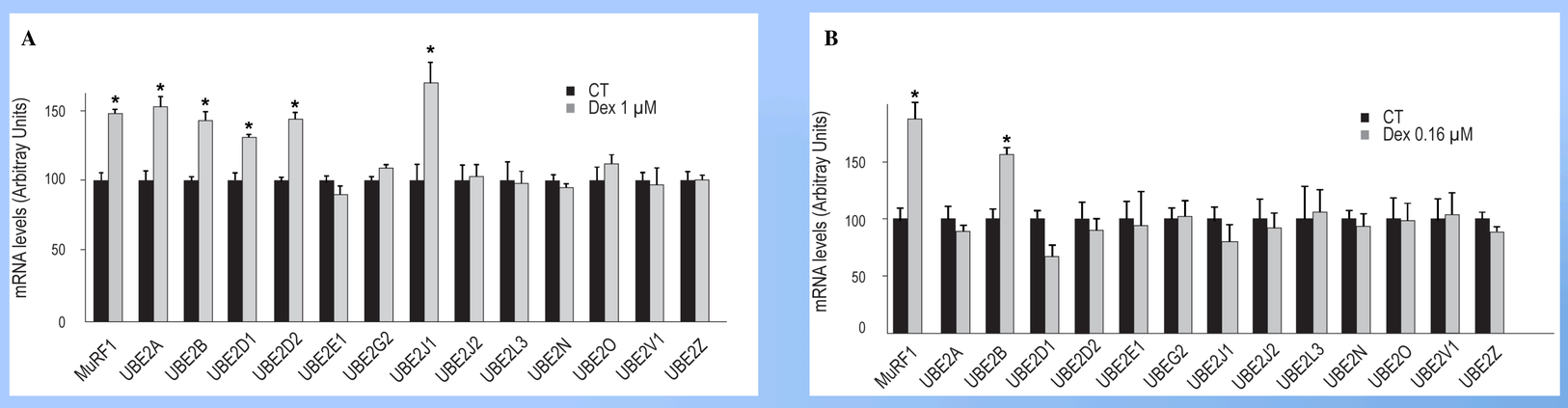

Figure 1: UBE2B is the only E2 enzyme overexpressed in moderately catabolic C2C12 myotubes (0.16 µM Dexamethasone, Dex)

Based on published data, we selected the E2 enzymes known to be highly present at the mRNA level in skeletal muscles and/or overexpressed during different catabolic situations. Using fa-C2C12 myotubes that stably express flag-actin, we assayed the mRNA levels of these enzymes and used the E3 ligase MuRF1 as a positive control regarding the development of a catabolic situation. Dex treatment is known to induce actin and myosin degradation in both myotubes and rodent muscles. (A) Using 1 µM Dex, a commonly used concentration for C2C12 myotubes, we found that UBE2A, B, D1, D2 and J1 mRNA levels were enhanced, suggesting that these E2s might be important for myofibrillar protein degradation in myotubes. (B) Interestingly, a milder catabolic situation (0.16 µM Dex) only induced an overexpression of UBE2B mRNAs together with the E3 ligase MuRF1. These data suggest that UBE2B is more responsive to catabolic situations than the other E2s tested.
*, statistically different from controls, $P < 0.05$, n = 5-6.

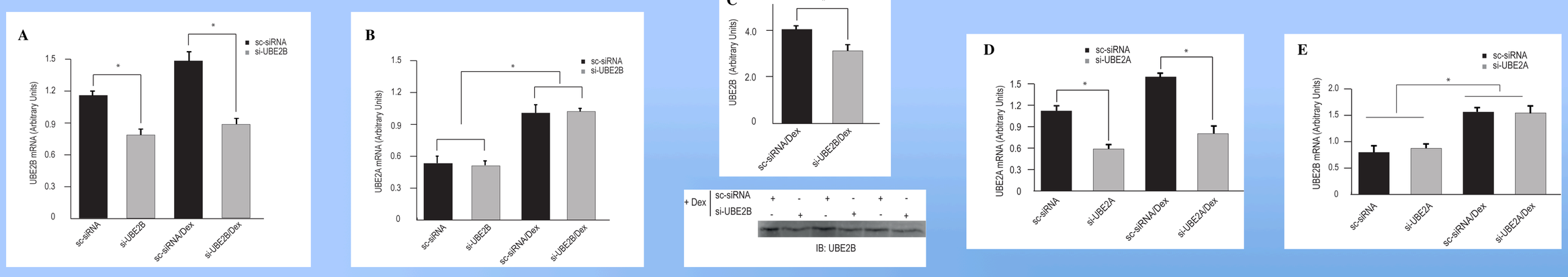

Figure 2: UBE2B knockdown selectively depressed UBE2B mRNA levels in both control and Dex-treated C2C12 myotubes without affecting the isoform UBE2A

To further investigate the importance of UBE2B for the establishment of a catabolic situation, we performed a knockdown in fa-C2C12 myotubes treated or not with 1 µM Dex (48 h). siRNA transfection was performed with either a negative control (sc-siRNA), a siRNA targeting UBE2B (siUBE2B) or the isoform UBE2A (si-UBE2A). mRNA levels were determined 48 h post transfection. (A) Following the knockdown, we typically obtained a 40-50% decrease in UBE2B in either control or Dex-treated fa-C2C12. (B) The isoform UBE2A possesses 75% homology at the mRNA level (96% at the protein level) with UBE2B and one hypothesis is that these isoforms may have redundant functions. We thus tested whether UBE2B knockdown modified UBE2A mRNA levels. In our conditions, we did not find any modification of UBE2A mRNA in both control and Dex-treated fa-C2-C12. (C) Immunoblotting revealed that UBE2B was also depressed at the protein level. (D) Similarly, UBE2A knockdown was efficient and did not affect UBE2B mRNA levels (E). *, statistically different from controls, $P < 0.05$, n = 5-6.

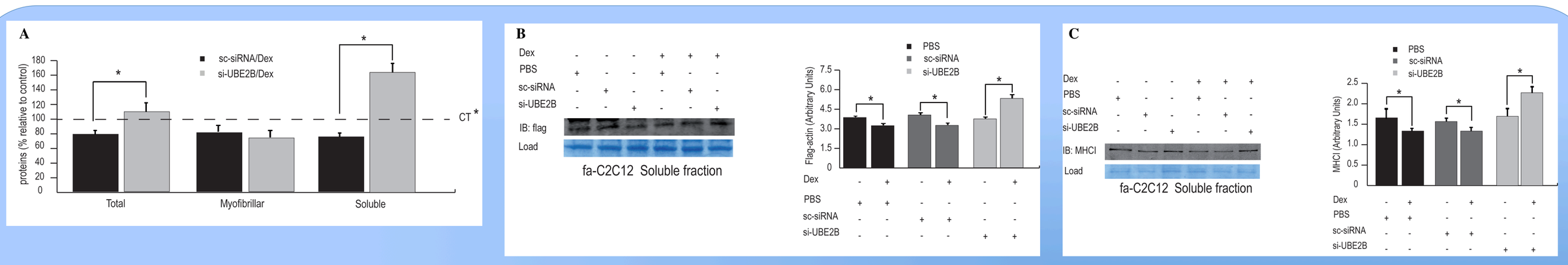

Figure 3: UBE2B knockdown induced an accumulation of soluble proteins in Dex-treated C2C12 myotubes

(A) Dex treatment (1 µM, 48 h) depressed total protein content in fa-C2C12 myotubes (black bars) when compared to untreated control cells (CT, dashed line) with a similar contribution of myofibrillar and soluble fractions. UBE2B knockdown induced an accumulation of total proteins in Dex-treated fa-C2C12 myotubes (grey bars) when compared to control-transfected cells (sc-siRNA). This accumulation was only due to an increase in the soluble fraction. (B) Dex induced a decrease in flag-actin content in myotubes treated either with PBS (black bars) or sc-siRNA (negative control, dark grey bars). Interestingly, actin accumulated when UBE2B was knocked down (grey bars). Loading was controlled by staining the blot with the FastStain kit (Agrobio) and densitometric analysis was used to correct for uneven loading. A portion of the colored gel is shown (Load). (C) A similar accumulation was also observed for myosin heavy chain (MHCI) in UBE2B knockdown myotubes, indicating that UBE2B is involved (directly or indirectly) in the processing of myofibrillar proteins, and that this accumulation is only observed in the soluble fraction. *, statistically different from controls, $P < 0.05$, n = 5-6.

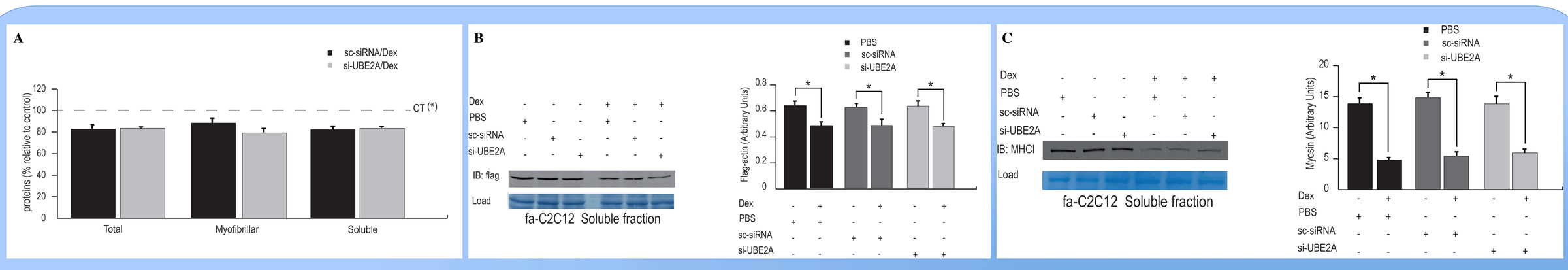

Figure 4: UBE2A knockdown did not impact protein losses in Dex-treated C2C12 myotubes

As others hypothesized that UBE2A might posses redundant functions with UBE2B, we performed a knockdown directed against UBE2A. (A) We found again that Dex induced a decrease in total, myofibrillar and soluble protein content (black bars) when compared to controls (CT, dashed line). By contrast with UBE2B, UBE2A knockdown did not favor protein accumulation (grey bars). (B and C) In accordance with the lack of accumulation of proteins, both flag-actin and MHCI levels were depressed upon Dex treatment and this was not modified by UBE2A knockdown. *, statistically different from controls, $P < 0.05$, n = 5-6.

Conclusion

A limited number of E2 enzymes is overexpressed at the mRNA level in Dex-treated (1 µM, 48 h) fa-C2C12 myotubes, suggesting that these E2s may play important roles in muscle wasting during catabolic conditions. Surprisingly, only UBE2B was sensitive to mild catabolic conditions (Dex 0.16 µM). We found that UBE2B was implicated, either directly or indirectly, in the removing of myofibrillar proteins like actin (flag-actin here) and MHCI, but surprisingly this was limited to the soluble fraction. It should be emphasized that in our conditions, loosely attached myofibrils were present in the soluble fraction, so that we can not distinguish between this protein pool and individual myofibrillar proteins. We also demonstrated that UBE2A and UBE2B do not share identical functions and that at least part of their targets are not redundant. This is in agreement with previous data that showed that UBE2B loss was not compensated by UBE2A in testis, despite the fact that UBE2A has the theoretical capacity for conjugating Ub with high efficiency. Future work will have to precise the mechanism by which UBE2B modify myofibrillar protein levels in the soluble fraction together with the exact pool influenced by UBE2B (individual proteins vs. loosely attached myofilaments). Another crucial point will be to determine which E3 is implicated with UBE2B for myofibrillar protein degradation and whether this action is direct or indirect. While Ubr E3 ligases are known to work with UBE2B in skeletal muscles, they have not been implicated in myofibrillar protein targeting so far.