

RESEARCH

Open Access

p38 β MAPK upregulates atrogen1/MAFbx by specific phosphorylation of C/EBP β

Guohua Zhang and Yi-Ping Li*

Abstract

Background: The p38 mitogen-activated protein kinases (MAPK) family plays pivotal roles in skeletal muscle metabolism. Recent evidence revealed that p38 α and p38 β exert paradoxical effects on muscle protein homeostasis. However, it is unknown why p38 β , but not p38 α , is capable of mediating muscle catabolism via selective activation of the C/EBP β that upregulates atrogen1/MAFbx.

Methods: Tryptic phosphopeptide mapping was carried out to identify p38 α - and p38 β -mediated phosphorylation sites in C/EBP β . Chromosome immunoprecipitation (ChIP) assay was used to evaluate p38 α and p38 β effect on C/EBP β binding to the atrogen1/MAFbx promoter. Overexpression or siRNA-mediated gene knockdown of p38 α and p38 β , and site-directed mutagenesis or knockout of C/EBP β , were used to analyze the roles of these kinases in muscle catabolism in C2C12 myotubes and mice.

Results: Cellular expression of constitutively active p38 α or p38 β resulted in phosphorylation of C/EBP β at multiple serine and threonine residues; however, only p38 β phosphorylated Thr-188, which had been known to be critical to the DNA-binding activity of C/EBP β . Only p38 β , but not p38 α , activated C/EBP β -binding to the atrogen1/MAFbx promoter. A C/EBP β mutant in which Thr-188 was replaced by alanine acted as a dominant-negative inhibitor of atrogen1/MAFbx upregulation induced by either p38 β or Lewis lung carcinoma (LLC) cell-conditioned medium (LCM). In addition, knockdown of p38 β specifically inhibited C/EBP β activation and atrogen1/MAFbx upregulation induced by LCM. Finally, expression of active p38 β in mouse tibialis anterior specifically induced C/EBP β phosphorylation at Thr-188, atrogen1/MAFbx upregulation and muscle mass loss, which were blocked in C/EBP β -null mice.

Conclusions: The α and β isoforms of p38 MAPK are capable of recognizing distinct phosphorylation sites in a substrate. The unique capacity of p38 β in mediating muscle catabolism is due to its capability in phosphorylating Thr-188 of C/EBP β .

Keywords: Cachexia, E3 protein, Gene regulation, DNA-binding, Thr-188

Background

The p38 mitogen-activated protein kinases (MAPK) family plays a pivotal role in skeletal muscle by mediating diverse cellular activities, and interestingly, some of which result in paradoxical effects. For example, p38 mediates both insulin stimulation of glucose uptake [1] and TNF- α stimulation of insulin resistance [2] in muscle. In the context of skeletal muscle protein homeostasis, p38 responds to both catabolic (lipopolysaccharide, cytokines, and ROS) [3-5] and anabolic (insulin and

exercise) stimuli [6]. On one hand, p38 stimulates muscle satellite cell proliferation [7] and differentiation [8], which increases muscle mass; on the other hand, p38 stimulates muscle protein degradation leading to muscle atrophy [3-5]. Intriguingly, p38 has the capacity to activate different protein substrates depending on the cellular environment [7]. It is of great interest to understand how the p38 MAPK family is able to mediate the discrete and sometimes opposing effects in response to diverse physiological and pathological stimuli. The family of MAPK is composed of at least four members (α , β , γ and δ), which enable the transduction of a variety of extracellular signals into distinct nuclear responses

* Correspondence: yi-ping.li@uth.tmc.edu

Department of Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, 6431 Fannin Street, Houston, TX 77030, USA

[9-11]. The α , β , and γ isoforms are found in muscle cells. Recently, it became clear that p38 α MAPK plays an essential role in myogenic differentiation [12,13]. On the other hand, p38 γ MAPK appears to regulate the expansion of myogenic precursor cells [14], endurance exercise-induced mitochondrial biogenesis and angiogenesis [15], as well as glucose uptake [16]. But, little was known about the function of p38 β until our most recent discovery of its role in regulating the atrogin1/MAFbx gene [17].

Cachexia, a wasting disease characterized by loss of muscle mass with or without loss of fat mass, is frequently associated with such diseases as cancer, sepsis, AIDS, congestive heart failure, diabetes, chronic renal failure and chronic obstructive pulmonary disease (COPD). Cachexia is distinct from starvation-, disuse-, aging-, primary depression-, malabsorption- and hyperthyroidism-induced muscle mass loss and is associated with increased morbidity and mortality [18,19]. The prominent clinical feature of cachexia is weight loss with anorexia, inflammation, insulin resistance, and increased muscle protein breakdown. Increased muscle protein breakdown in cachexia is at least partially due to accelerated muscle proteolysis by the ubiquitin-proteasome pathway, a common pathway of muscle mass loss due to pathological as well as physiological causes [20]. However, the signaling mechanism of the activation of the ubiquitin-proteasome pathway in cachexia appears to be different from that of physiological muscle atrophy. It is well established that a depression in AKT activity activates FoxO1/3 transcription factors, which upregulates two key ubiquitin ligases (E3 proteins), atrogin1/MAFbx and MuRF1, in animal models of physiological muscle atrophy caused by fasting, denervation and disuse [21-23]. In animal models of cachexia, however, AKT is often activated, which leads to the inactivation of FoxO1/3 [4,5,24]. In fact, it has been shown in animal models of cachexia that upregulation of MuRF1 is mediated by NF- κ B [25], and upregulation of atrogin1/MAFbx is mediated by p38 MAPK [4,5].

We showed most recently that among the known p38 MAPK isoforms only p38 β MAPK is capable of upregulating atrogin1/MAFbx via the activation of transcription factor C/EBP β in response to tumor cell-conditioned medium. In addition, we demonstrated that p38 β MAPK upregulation of atrogin1/MAFbx is independent of the AKT-FoxO1/3 signaling pathway [17]. Thus, p38 β emerged as a key mediator and a specific therapeutic target of cachexia. Notwithstanding, why p38 β is uniquely capable of activating C/EBP β among the p38 isoforms is unknown. The current study is designed to address the mechanism through which p38 β specifically activates C/EBP β in the context of tumor-induced cachexia. We demonstrate that while p38 β shares some

common phosphorylation sites with p38 α , it specifically phosphorylates the Thr-188 residue of C/EBP β , which activates C/EBP β binding to the atrogin1/MAFbx promoter and upregulates this gene in response to a tumor.

Methods

Tryptic phosphopeptide mapping was carried out to identify p38 α - and p38 β -mediated phosphorylation sites in C/EBP β . ChIP assay was used to evaluate p38 α and p38 β effect on C/EBP β -binding to the atrogin1/MAFbx promoter. Overexpression or siRNA-mediated gene knockdown of p38 α and p38 β , and site-directed mutagenesis or knockout of C/EBP β , were used to analyze the roles of these kinases in muscle catabolism in C2C12 myotubes and mice.

Tryptic phosphopeptide mapping

HEK293T cells (American Type Culture Collection, Manassas, VA, USA) cultured in 150 mm culture plates that were ~50% confluent were co-transfected with a plasmid encoding LAP with a FLAG tag (Addgene) and a plasmid encoding constitutively active p38 α or p38 β [26] (10 μ g each) using deacylated polyethylenimine (PEI) 22000 [27], a gift from Dr. Guangwei Du (University of Texas Health Science Center at Houston, Houston, TX, USA). The cell culture medium was replaced with fresh medium at 24 h. Cells were lysed in RIPA buffer (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.1% SDS, 2 mM phenylmethylsulfonylfluoride (PMSF), 0.5% sodium deoxycholate, 1 mM NaF, 1/100 protease inhibitor cocktail, and 1/100 phosphatase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) after an additional incubation of 24 h. LAP in cell lysates was precipitated using FLAG-M2 magnetic beads (Sigma-Aldrich) and subjected to 10% SDS-PAGE. The gel was then stained with Coomassie Blue R-250. The LAP band was cut out and subjected to tryptic phosphopeptide mapping conducted by Taplin Mass Spectrometry Facility at Harvard Medical School using an LTQ-Orbitrap mass spectrometer (Thermo Electron, West Palm Beach, FL, USA).

Myogenic cell culture and transfection

Murine C2C12 myoblasts (American Type Culture Collection, Manassas, VA, USA) were cultured in growth medium (DMEM supplemented with 10% fetal bovine serum) at 37°C under 5% CO₂. At approximately 85 to 90% confluence, myoblast differentiation was induced by incubation for 96 h in differentiation medium (DMEM supplemented with 4% heat-inactivated horse serum) to form myotubes. Plasmids encoding constitutively active p38 isoforms [26] or a C/EBP β mutant (p3xFlag-CMV-10-LAP-T188A) were transfected into C2C12 myoblasts of 50% confluence at 1 μ g/well in six-well plates using

deacylated polyethylenimine (PEI) 22000. At 24 h we induced the cells to differentiate by switching them to differentiation medium. When indicated, myotubes were treated with Lewis lung carcinoma cell (National Cancer Institute, Bethesda, MD, USA)-conditioned medium (LCM, 25% final volume) [17] or directly harvested. Cell lysate was prepared using the RIPA buffer for further analyses.

Chromosome immunoprecipitation assay

Chromosome immunoprecipitation (ChIP) assay was performed as previously described [17].

Generation of expression vector for a C/EBP β mutant (LAP-T188A)

A plasmid encoding C/EBP β in which Thr-188 was replaced by alanine in the pCDNA3 vector (a gift from Dr. Qi-Qun Tang of Fudan University, Shanghai, China) was digested at BamHI and EcoRI restriction sites to release the cDNA insert. That insert was then subcloned into the p3xFlag-CMV-10 vector (Sigma-Aldrich) at the same restriction sites to generate plasmid p3xFlag-CMV-10-LAP-T188A.

Western blot analysis

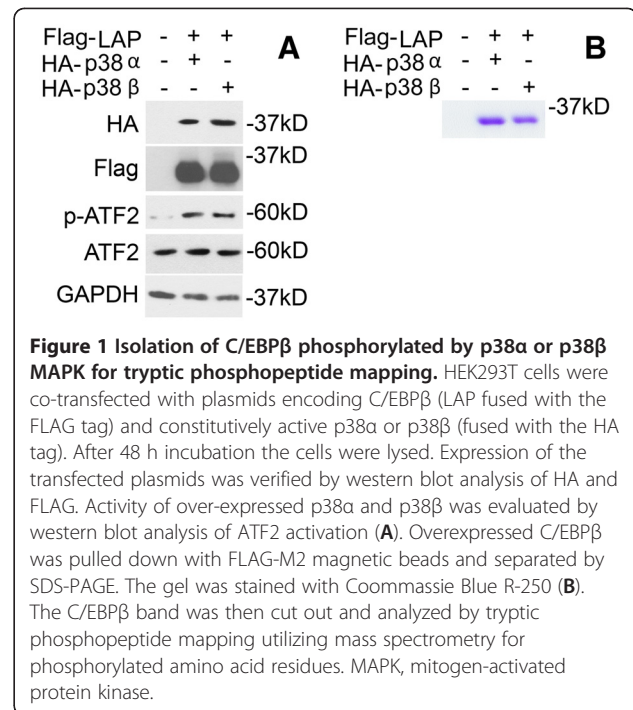
Cell and muscle lysate were prepared and western blot analysis was carried out as described previously [17]. Antibodies for total and/or phosphorylated ATF2, FoxO1 (Thr-24)/FoxO3a (Thr-32) and C/EBP β phosphorylated at Thr-188 were from Cell Signaling Technology (Danvers, MA, USA). Antibody for atrogen1/MAFbx was from ECM Biosciences (Versailles, KY, USA). Antibodies to C/EBP β (H-7) were from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibody to the HA tag was from Covance (Princeton, NJ, USA). Data were normalized to GAPDH.

Gene knockdown by siRNA

p38 α -specific siRNA (5'-CUCCUUUACUAUCUUUCUCAA-3') and p38 β -specific siRNA (5'-GUCCUGAGGUUCUAGCAAAdTdT-3') were synthesized by Sigma-Aldrich and transfected into C2C12 myoblasts by electroporation (5 μ g/1 \times 10⁷ cells) with the Nucleofector system (Lonza, Walkersville, MD, USA), according to the manufacturer's protocol. Control siRNA was obtained from Ambion (Austin, TX, USA). Differentiation was induced 24 h after transfection.

Animal use

Experimental protocols were approved in advance by the institutional Animal Welfare Committee at the University of Texas Health Science Center at Houston. C/EBP β ^{-/-} mice in C57BL/6 background were bred from C/EBP β ^{-/+} mice generated by Dr. Peter Johnson of NCI



[28]. While the mice were under anesthesia, plasmids encoding constitutively active p38 α or p38 β were injected into the tibialis anterior (TA) of the right leg for each mouse (100 μ g in 50 μ l), and the empty vector pCDNA3.1 was injected into the left leg as control. Immediately after plasmid injection, TA was electroporated by applying square-wave electrical pulses (100 V/cm) eight times with an electrical pulse generator (Model 830, BTX) at a rate of one pulse per second, with each pulse being 20 ms in duration, through a pair of stainless steel needles that were 5 mm apart. The above transfection procedure was repeated in 7 days. In another 7 days, the mice were sacrificed and TAs were collected for analysis.

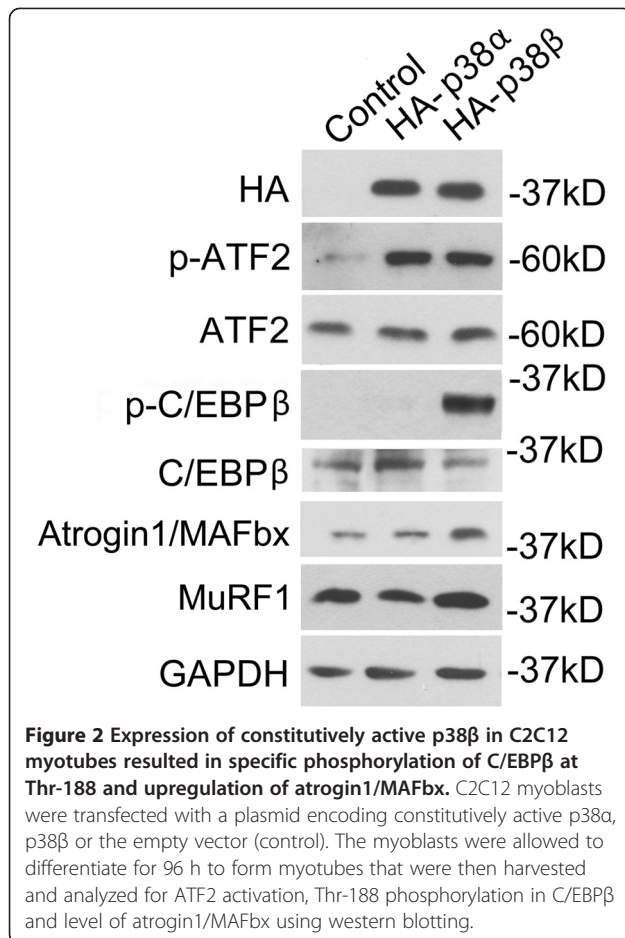
Statistical analysis

Data were analyzed with one-way ANOVA or student *t* test using the SigmaStat software (Systat Software, Point Richmond, CA, USA) as indicated. When applicable,

Table 1 Expression of constitutively active p38 α or p38 β resulted in the phosphorylation of diverse amino acid residues in C/EBP β

Kinase	Phosphorylated amino acid residues in C/EBP β
p38 α	Ser110, Tyr108
p38 β	Ser182, Ser183, Ser190, Thr188
p38 α /p38 β	Ser64, Ser184, Ser222, Ser276

The C/EBP β bands cut out from SDS-PAGE described in Figure 1B were analyzed by tryptic phosphopeptide mapping utilizing mass spectrometry. Identified phosphorylated amino acid residues in C/EBP β are listed.

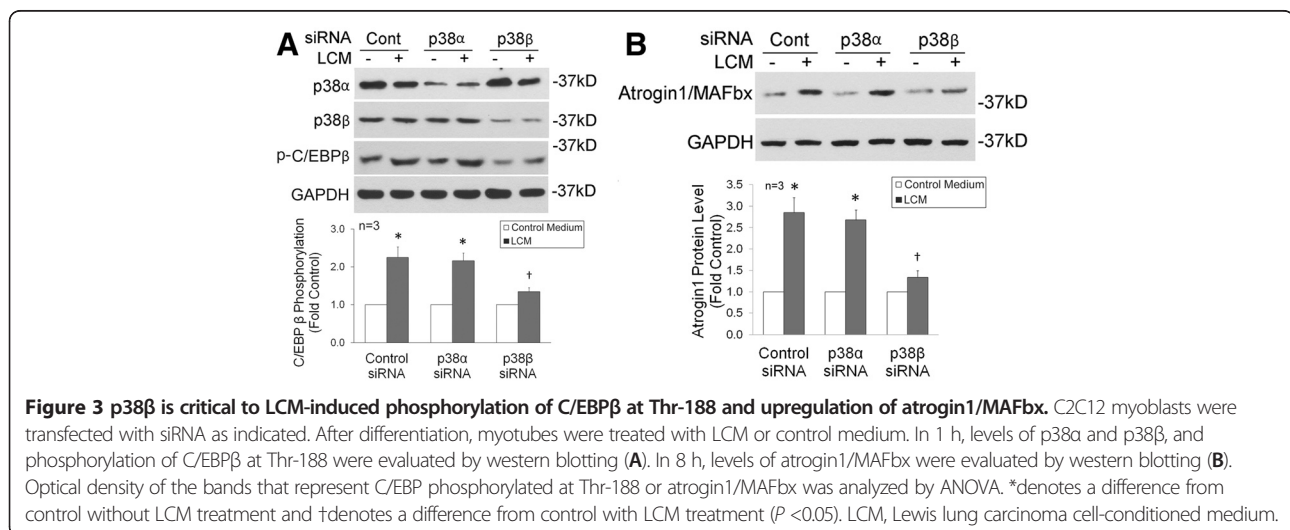


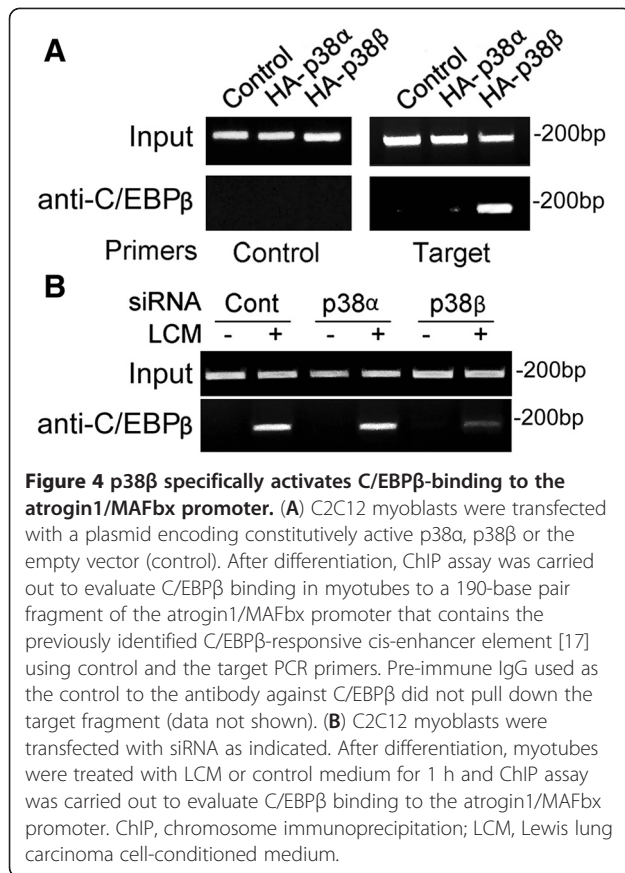
control samples from independent experiments were normalized to a value of 1 without showing variations (actual variations were within a normal range). A *P* value <0.05 was considered to be statistically significant. Data are presented as the mean ± S.E.

Results

p38β MAPK specifically phosphorylates the Thr-188 residue of C/EBPβ

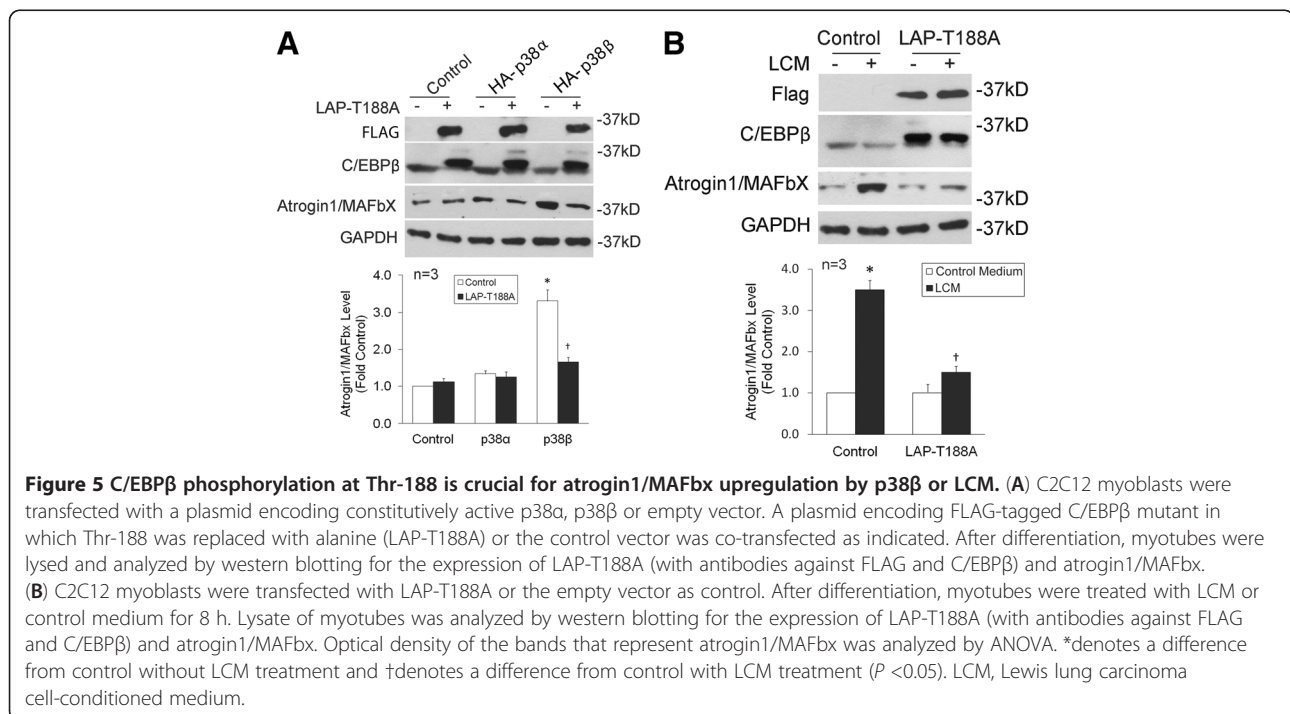
C/EBPβ is a transcription factor that is normally repressed due to the intrinsic repression of its DNA-binding and transactivation functions [29,30]. The DNA-binding function of C/EBPβ is activated by sequential phosphorylation of specific amino acid residues by multiple kinases [31-33]. C/EBPβ was previously shown to be a p38 substrate *in vitro* [34]. Recently, we showed that p38 interacts with and phosphorylates C/EBPβ in C2C12 myotubes. In addition, while the p38α/β inhibitor SB202190 blocks tumor-induced atrogenin1/MAFbx upregulation, only p38β is capable of upregulating atrogenin1/MAFbx via the activation of C/EBPβ [17]. To investigate why p38β, but not p38α, has the capacity for activating C/EBPβ we set out to investigate the phosphorylation sites in C/EBPβ that are targeted by p38α and p38β. Plasmids encoding C/EBPβ (LAP fused with the FLAG tag) and constitutively active p38α or p38β (fused to HA) were co-transfected into HEK293T cells. At 48 h expression of the transfected plasmids was verified by western blot analysis of HA (p38 MAPKs) and FLAG (LAP) in the cell lysate. Activation of p38 substrate ATF2 (via phosphorylation) by expressed p38 MAPKs was also evaluated by western blot analysis (Figure 1A). Overexpressed C/EBPβ was pulled down with FLAG-M2 magnetic beads and separated with SDS-PAGE (Figure 1B). The C/EBPβ band was cut out from the gel and analyzed by tryptic phosphopeptide mapping utilizing mass spectrometry (for original reports see Additional file 1: Table S1 and Additional file 2: Table S2). Six phosphorylated amino acid residues were identified in C/EBPβ that was co-expressed with active p38α and eight phosphorylated amino acid residues were identified in C/EBPβ that was co-expressed with active p38β. The two p38 MAPK

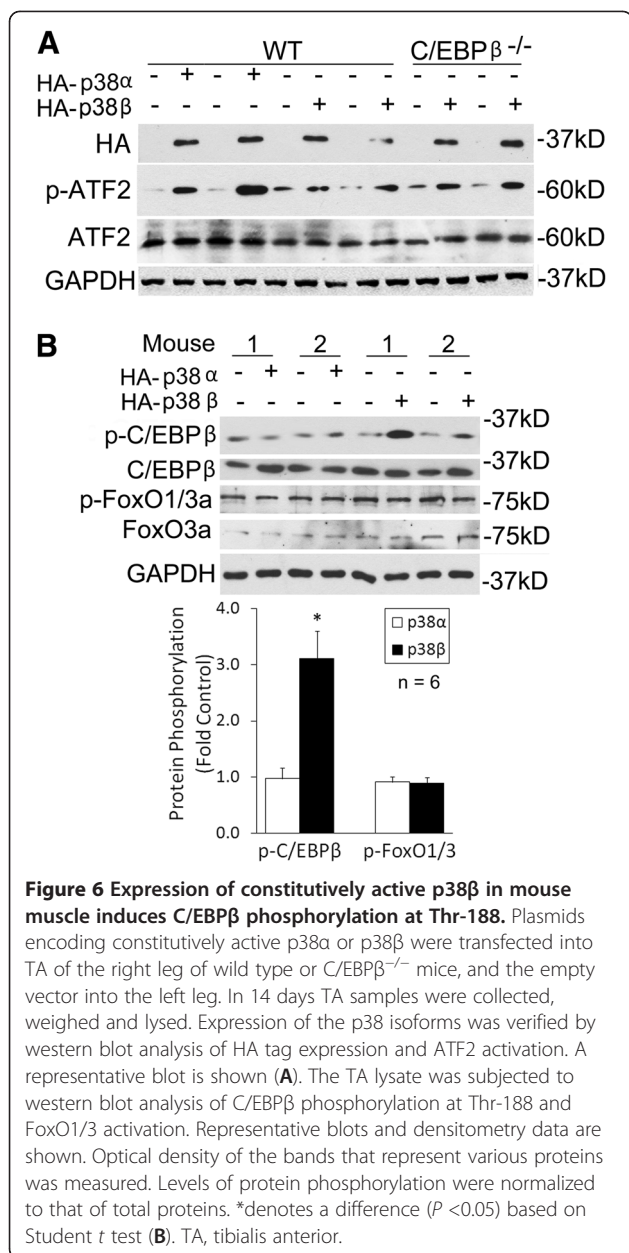




isoforms shared four common phosphorylation sites (Ser-64, Ser-184, Ser-222, and Ser-276). On the other hand, p38 α uniquely phosphorylated Ser-110 and Tyr-108, while p38 β uniquely phosphorylated Ser-182, Ser-183, Ser-190 and Thr-188 (Table 1). Among the unique amino acid residues phosphorylated by p38 β , Thr-188 is known to be crucial for the activation of C/EBP β binding to its targeted DNA sequence [31-33].

To verify whether p38 β specifically mediates the phosphorylation of Thr-188 of C/EBP β in muscle cells, we transfected C2C12 myoblasts with plasmids encoding active p38 α or active p38 β and used empty vector as the control. Although constitutively active p38 α appeared to accelerate differentiation during the early stage of differentiation, at 96 h of differentiation, there was no visible difference in the myotubes formed compared with control myotubes and active p38 β -expressing myotubes. After differentiation, lysate of myotubes was evaluated by western blot analysis of expression of the HA tag that fused to active p38 α or p38 β , ATF2 activation and C/EBP β phosphorylation at Thr-188. As shown in Figure 2, although both of the active p38 isoforms activated ATF2, only the expression of active p38 β resulted in C/EBP β phosphorylation at Thr-188 and upregulation of atrogin1/MAFbx. Therefore, the specific phosphorylation of Thr-188 by p38 β may be the key to its specific activation of C/EBP β .





p38β MAPK-mediated Thr-188 phosphorylation activates C/EBPβ binding to the atrogin1/MAFbx promoter and upregulates atrogin1/MAFbx in C2C12 myotubes

To verify whether p38β is critical to Thr-188 phosphorylation and atrogin1/MAFbx upregulation induced by cachectic tumor cells, siRNA-mediated mRNA knockdown was carried out. We observed that knockdown of the p38β expression, but not the p38α expression, blocked Lewis lung carcinoma cell-conditioned medium (LCM)-induced Thr-188 phosphorylation (Figure 3A) and atrogin1/MAFbx upregulation (Figure 3B). Therefore, p38β is indeed a key mediator of Thr-188 phosphorylation and atrogin1/MAFbx upregulation by Lewis lung carcinoma.

Previously, we observed that MAPK kinase 6 (MKK6) activates C/EBPβ binding to the C/EBPβ-responsive enhancer in the atrogin1/MAFbx promoter via the activation of p38 [17]. To investigate whether p38β specifically activates C/EBPβ binding to this C/EBPβ-responsive enhancer, we conducted the ChIP assay. We observed in C2C12 myotubes that the expression of active p38β, but not active p38α, activated C/EBPβ binding to the atrogin1/MAFbx promoter region containing the C/EBPβ-responsive enhancer (Figure 4A). Conversely, siRNA-mediated knockdown of the p38β gene, but not the p38α gene, blocked LCM-induced activation of C/EBPβ binding to the atrogin1/MAFbx promoter (Figure 4B). Thus, we conclude that p38β specifically activates C/EBPβ binding to its targeted DNA motif.

To evaluate whether C/EBPβ phosphorylation at Thr-188 is critical to p38β-mediated upregulation of atrogin1/MAFbx, a plasmid encoding a C/EBPβ mutant in which Thr-188 was replaced by alanine (C/EBPβ-T188A) was transfected into C2C12 myoblasts along with a plasmid encoding active p38α or active p38β. After differentiation, levels of atrogin1/MAFbx in myotubes that expressed active p38β, but not active p38α, were elevated. The elevation of atrogin1/MAFbx was blocked in myotubes co-expressing the C/EBPβ-T188A mutant (Figure 5A). To evaluate whether tumor-induced atrogin1/MAFbx upregulation requires the phosphorylation of C/EBPβ at Thr-188, the plasmid encoding C/EBPβ-T188A was transfected into C2C12 myoblasts. After differentiation, myotubes were treated with LLC cell-conditioned medium (LCM). We observed that LCM upregulation of atrogin1/MAFbx was inhibited in myotubes that overexpress the C/EBPβ-T188A mutant (Figure 5B). Therefore, p38β-mediated C/EBPβ phosphorylation at Thr-188 is crucial for atrogin1/MAFbx upregulation by LLC cells.

p38β MAPK specifically induces atrogin1/MAFbx upregulation and muscle mass loss in mice via C/EBPβ

We previously showed that C/EBPβ is essential for the atrogin1/MAFbx upregulation and muscle mass loss in Lewis lung carcinoma (LLC)-tumor bearing mice [17]. To evaluate whether activation of p38β *in vivo* specifically induces C/EBPβ phosphorylation at Thr-188, atrogin1/MAFbx upregulation and muscle mass loss, the plasmids encoding constitutively active p38α or p38β were transfected into the tibialis anterior (TA) of wild type or C/EBPβ^{-/-} mice with the empty vector as control. At 14 days, the mice were sacrificed and the excised TAs were analyzed. Expression of the HA tag that fused to p38α or p38β and activation of the p38 substrate ATF2 were evaluated by western blot analysis to confirm the expression of active p38α and p38β (Figure 6A). Expression of active p38β, but not active p38α, resulted in phosphorylation of C/EBPβ at Thr-

188 (Figure 6B). Because the atrogin1/MAFbx promoter also contains FoxO1/3-responsive cis-elements that are regulated by AKT [21], we evaluated whether p38 β affected FoxO1/3 activity. Expression of active p38 β did not alter the phosphorylation state of FoxO1/3 (Figure 6B). Therefore, p38 β does not affect FoxO1/3 activity. As shown in Figure 7A, expression of active p38 β resulted in atrogin1/MAFbx upregulation in the TA of wild type mice. However, active p38 β failed to upregulate atrogin1/MAFbx in C/EBP β ^{-/-} mice. Finally, expression of active p38 β , but not active p38 α , induced TA weight loss by 14% in wild type mice. In contrast, active p38 β expression did not alter TA weight in C/EBP β ^{-/-} mice (Figure 7B). Therefore, *in vivo* activation of p38 β induces atrogin1/MAFbx upregulation and muscle mass loss via the activation of C/EBP β .

Discussion

The selective activation of substrates by various p38 MAPK isoforms was previously attributed to preferential activation of the isoforms by specific MAPK kinase as well as compartmentalization of the isoforms. For example, while p38 α is activated by MKK3, MKK6 and MKK4, p38 β is activated by MKK6 [10,35]. However, these may not explain the specific activation of C/EBP β by p38 β , because both p38 α and p38 β are present in the nucleus and activated by MKK6. The current study demonstrates that the selective activation of substrates by p38 MAPK isoforms is further realized by their recognition of specific phosphorylation sites within a substrate.

Particularly, we show that p38 β specifically mediates the phosphorylation of C/EBP β required for the activation of its binding to the atrogin1/MAFbx promoter. Previous studies indicated that phosphorylation of Thr-

188 in C/EBP β by ERK1/2 MAPK [31] or cdk2/cyclinA [32] primes C/EBP β for subsequent phosphorylation on Ser-184 or Thr-179 by glycogen synthase kinase 3 β (GSK3 β), which activates the DNA-binding and transactivation functions of C/EBP β [33]. Our data presented here demonstrate that p38 β has the unique capability of mediating dual phosphorylation at both Thr-188 and Ser-184, resulting in the activation of C/EBP β binding to the atrogin1/MAFbx promoter. In contrast, p38 α that mediates phosphorylation of Ser-184 but not Thr-188 is unable to activate C/EBP β binding to the atrogin1/MAFbx promoter. Because GSK3 β was previously shown inactivated by p38 MAPK-mediated phosphorylation [36], it is unlikely that GSK3 β mediates C/EBP β phosphorylation at Ser-184 in response to p38 α and p38 β activation.

In the present study we also present the first evidence that overexpression of active p38 β in muscle induces muscle catabolism, demonstrating a direct effect of p38 β on muscle catabolism *in vivo*. Previous studies involving systemic activation of p38 in tumor-bearing mice [17] or septic mice [4] did not allow such a conclusion.

Although p38 β is widely distributed in various tissues [37] its function is largely unknown, especially when it is compared to p38 α , which is not only responsible for the known roles of p38 in inflammatory responses [38,39] but also in the regulation of myogenesis [12,13]. The present study demonstrates that activation of p38 β , not p38 α , induces atrogin1/MAFbx upregulation and muscle mass loss via specific phosphorylation of C/EBP β , which explains at the molecular level why p38 is capable of playing the seemingly opposing roles in muscle protein homeostasis (promoting myogenesis *versus* promoting muscle catabolism). Further, these data support p38 β as a selective therapeutic target of cachexia. Because

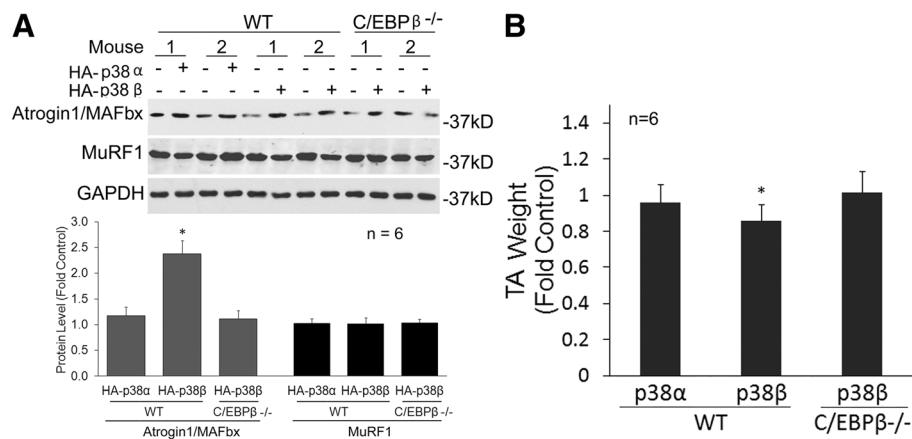


Figure 7 Expression of constitutively active p38 β in mouse muscle induces atrogin1/MAFbx upregulation and muscle mass loss in a C/EBP β -dependent manner. The TA lysate derived from Figure 6 was further analyzed for level of atrogin1/MAFbx by western blotting. Representative blots and densitometry data are shown (A). The weight of TA transfected with a plasmid encoding an active p38 isoform was compared to that of TA transfected with empty vector (control) from the same mouse (B). *denotes a difference ($P < 0.05$) based on ANOVA. TA, tibialis anterior.

C/EBP β is activated by a number of kinases and regulates a wide variety of genes [40], it may not be suitable as a drug target. On the other hand, p38 β has few known functions, therefore, specific inhibitors of p38 β MAPK would be highly desirable for the intervention of cancer cachexia. Unfortunately, only p38 α/β -dual or p38 α -specific inhibitors are available at the present time.

Because p38 β is highly expressed in the heart [37], it may also regulate the protein homeostasis in heart muscle via influencing C/EBP β -regulated atrogen1/MAFbx expression. Consistent to this notion, it has been shown recently that exercise induces a reduction in C/EBP β in cardiomyocytes, which mediates cardiomyocyte hypertrophy [41]. In addition, the transactivation activity of C/EBP β is suppressed by insulin, an anabolic hormone [42]. Therefore, diverse signaling pathways that regulate protein homeostasis in striated muscles may converge upon C/EBP β .

Conclusions

The present study demonstrates that the α and β isoforms of p38 MAPK recognize distinct phosphorylation sites in a substrate. p38 β MAPK has the unique capacity to mediate the dual phosphorylation of Thr-188 and Ser-184 in C/EBP β , thereby, activating this transcription factor and inducing muscle catabolism. Therefore, p38 β MAPK should be considered as a therapeutic target for cachexia.

Additional files

Additional file 1: C/EBP β Phosphorylation Sites By p38 α .

Additional file 2: C/EBP β Phosphorylation Sites By p38 β .

Abbreviations

ChIP: Chromosome immunoprecipitation; COPD: Chronic obstructive pulmonary disease; DMEM: Dulbecco's modified Eagle's medium; LCM: Lewis lung carcinoma cell-conditioned medium; LLC: Lewis lung carcinoma; MAPK: Mitogen-activated protein kinase; MKK6: MAPK kinase 6; NF- κ B: Nuclear factor- κ B; PCR: Polymerase chain reaction; siRNA: Small interfering RNA; TA: Tibialis anterior; TNF- α : tumor necrosis factor- α .

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

GZ performed the experiments, analyzed the data, and generated the figures. Y-PL designed the research and wrote the article. Both authors read and approved the final manuscript.

Acknowledgements

This study was supported by an R01 grant from National Institute of Arthritis and Musculoskeletal and Skin Diseases to Y-P Li (AR052511). We thank Peter Johnson of National Cancer Institute for sharing the C/EBP β ^{-/-} mice, David Engelberg of Hebrew University for sharing plasmids encoding the constitutively active mutants of p38 MAPK isoforms, and Qi-Qun Tang of Fudan University for sharing plasmid encoding C/EBP β mutant pcDNA3-LAP-T188A.

Received: 9 August 2012 Accepted: 21 September 2012
Published: 9 October 2012

References

1. Tremblay F, Dubois MJ, Marette A: Regulation of GLUT4 traffic and function by insulin and contraction in skeletal muscle. *Front Biosci* 2003, **8**:d1072-d1084.
2. de Alvaro C, Teruel T, Hernandez R, Lorenzo M: Tumor necrosis factor alpha produces insulin resistance in skeletal muscle by activation of inhibitor kappaB kinase in a p38 MAPK-dependent manner. *J Biol Chem* 2004, **279**:17070-17078.
3. Li YP, Chen Y, John J, Moylan J, Jin B, Mann DL, Reid MB: TNF-alpha acts via p38 MAPK to stimulate expression of the ubiquitin ligase atrogen1/MAFbx in skeletal muscle. *FASEB J* 2005, **19**:362-370.
4. Doyle A, Zhang G, Abdel Fattah EA, Eissa NT, Li YP: Toll-like receptor 4 mediates lipopolysaccharide-induced muscle catabolism via coordinate activation of ubiquitin-proteasome and autophagy-lysosome pathways. *FASEB J* 2011, **25**:99-110.
5. McClung JM, Judge AR, Powers SK, Yan Z: p38 MAPK links oxidative stress to autophagy-related gene expression in cachectic muscle wasting. *Am J Physiol Cell Physiol* 2010, **298**:C542-C549.
6. Long YC, Widegren U, Zierath JR: Exercise-induced mitogen-activated protein kinase signalling in skeletal muscle. *Proc Nutr Soc* 2004, **63**:227-232.
7. Jones NC, Tyner KJ, Nibarger L, Stanley HM, Cornelison DD, Fedorov YV, Olwin BB: The p38alpha/beta MAPK functions as a molecular switch to activate the quiescent satellite cell. *J Cell Biol* 2005, **169**:105-116.
8. Lluís F, Perdiguer E, Nebreda AR, Munoz-Canoves P: Regulation of skeletal muscle gene expression by p38 MAP kinases. *Trends Cell Biol* 2006, **16**:36-44.
9. Remy G, Risco AM, Inesta-Vaquera FA, Gonzalez-Teran B, Sabio G, Davis RJ, Cuenda A: Differential activation of p38MAPK isoforms by MKK6 and MKK3. *Cell Signal* 2010, **22**:660-667.
10. Enslin H, Raingeaud J, Davis RJ: Selective activation of p38 mitogen-activated protein (MAP) kinase isoforms by the MAP kinase kinases MKK3 and MKK6. *J Biol Chem* 1998, **273**:1741-1748.
11. Loesch M, Chen G: The p38 MAPK stress pathway as a tumor suppressor or more? *Front Biosci* 2008, **13**:3581-3593.
12. Perdiguer E, Ruiz-Bonilla V, Gresh L, Hui L, Ballestar E, Sousa-Victor P, Baeza-Raja B, Jardi M, Bosch-Comas A, Esteller M, Caelles C, Serrano AL, Wagner EF, Munoz-Canoves P: Genetic analysis of p38 MAP kinases in myogenesis: fundamental role of p38alpha in abrogating myoblast proliferation. *EMBO J* 2007, **26**:1245-1256.
13. Palacios D, Mozzetta C, Consalvi S, Caretti G, Saccone V, Proserpio V, Marquez VE, Valente S, Mai A, Forcales SV, Sartorelli V, Puri PL: TNF/p38alpha/polycomb signaling to Pax7 locus in satellite cells links inflammation to the epigenetic control of muscle regeneration. *Cell Stem Cell* 2010, **7**:455-469.
14. Gillespie MA, Le Grand F, Scime A, Kuang S, von Maltzahn J, Seale V, Cuenda A, Ranish JA, Rudnicki MA: p38-(gamma)-dependent gene silencing restricts entry into the myogenic differentiation program. *J Cell Biol* 2009, **187**:991-1005.
15. Pogożelski AR, Geng T, Li P, Yin X, Lira VA, Zhang M, Chi JT, Yan Z: p38gamma mitogen-activated protein kinase is a key regulator in skeletal muscle metabolic adaptation in mice. *PLoS One* 2009, **4**:e7934.
16. Ho RC, Alcazar O, Fujii N, Hirshman MF, Goodyear LJ: p38gamma MAPK regulation of glucose transporter expression and glucose uptake in L6 myotubes and mouse skeletal muscle. *Am J Physiol Regul Integr Comp Physiol* 2004, **286**:R342-R349.
17. Zhang G, Jin B, Li YP: C/EBPbeta mediates tumour-induced ubiquitin ligase atrogen1/MAFbx upregulation and muscle wasting. *EMBO J* 2011, **30**:4323-4335.
18. Evans WJ, Morley JE, Argiles J, Bales C, Baracos V, Guttridge D, Jatoi A, Kalantar-Zadeh K, Lochs H, Mantovani G, Marks D, Mitch WE, Muscaritoli M, Najand A, Ponikowski P, Rossi Fanelli F, Schambelan M, Schols A, Schuster M, Thomas D, Wolfe R, Anker SD: Cachexia: a new definition. *Clin Nutr* 2008, **27**:793-799.
19. Tisdale MJ: Mechanisms of cancer cachexia. *Physiol Rev* 2009, **89**:381-410.
20. Lecker SH, Goldberg AL, Mitch WE: Protein degradation by the ubiquitin-proteasome pathway in normal and disease states. *J Am Soc Nephrol* 2006, **17**:1807-1819.
21. Sandri M, Sandri C, Gilbert A, Skurk C, Calabria E, Picard A, Walsh K, Schiaffino S, Lecker SH, Goldberg AL: Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogen-1 and cause skeletal muscle atrophy. *Cell* 2004, **117**:399-412.
22. Stitt TN, Drujan D, Clarke BA, Panaro F, Timofeyeva Y, Kline WO, Gonzalez M, Yancopoulos GD, Glass DJ: The IGF-1/PI3K/Akt pathway prevents expression of muscle atrophy-induced ubiquitin ligases by inhibiting FOXO transcription factors. *Mol Cell* 2004, **14**:395-403.

23. Sacheck JM, Hyatt JP, Raffaello A, Jagoe RT, Roy RR, Edgerton VR, Lecker SH, Goldberg AL: **Rapid disuse and denervation atrophy involve transcriptional changes similar to those of muscle wasting during systemic diseases.** *FASEB J* 2007, **21**:140–155.
24. Penna F, Bonetto A, Muscaritoli M, Costamagna D, Minero VG, Bonelli G, Rossi Fanelli F, Baccino FM, Costelli P: **Muscle atrophy in experimental cancer cachexia: is the IGF-1 signaling pathway involved?** *Int J Cancer* 2010, **127**(7):1706–1717.
25. Cai D, Frantz JD, Tawa NE Jr, Melendez PA, Oh BC, Lidov HG, Hasselgren PO, Frontera WR, Lee J, Glass DJ, Shoelson SE: **IKKbeta/NF-kappaB activation causes severe muscle wasting in mice.** *Cell* 2004, **119**:285–298.
26. Askari N, Beenstock J, Livnah O, Engelberg D: **p38 is active in vitro and in vivo when monophosphorylated on Thr180.** *Biochemistry* 2009, **48**:2497–2504.
27. Thomas M, Lu JJ, Ge Q, Zhang C, Chen J, Klivanov AM: **Full deacylation of polyethylenimine dramatically boosts its gene delivery efficiency and specificity to mouse lung.** *Proc Natl Acad Sci USA* 2005, **102**:5679–5684.
28. Sterneck E, Tessarollo L, Johnson PF: **An essential role for C/EBPbeta in female reproduction.** *Genes Dev* 1997, **11**:2153–2162.
29. Williams SC, Baer M, Dillner AJ, Johnson PF: **CRP2 (C/EBP beta) contains a bipartite regulatory domain that controls transcriptional activation, DNA binding and cell specificity.** *EMBO J* 1995, **14**:3170–3183.
30. Lee S, Miller M, Shuman JD, Johnson PF: **CCAAT/Enhancer-binding protein beta DNA binding is auto-inhibited by multiple elements that also mediate association with p300/CREB-binding protein (CBP).** *J Biol Chem* 2010, **285**:21399–21410.
31. Tang QQ, Gronborg M, Huang H, Kim JW, Otto TC, Pandey A, Lane MD: **Sequential phosphorylation of CCAAT enhancer-binding protein beta by MAPK and glycogen synthase kinase 3beta is required for adipogenesis.** *Proc Natl Acad Sci USA* 2005, **102**:9766–9771.
32. Li X, Kim JW, Gronborg M, Urlaub H, Lane MD, Tang QQ: **Role of cdk2 in the sequential phosphorylation/activation of C/EBPbeta during adipocyte differentiation.** *Proc Natl Acad Sci USA* 2007, **104**:11597–11602.
33. Kim JW, Tang QQ, Li X, Lane MD: **Effect of phosphorylation and S-S bond-induced dimerization on DNA binding and transcriptional activation by C/EBPbeta.** *Proc Natl Acad Sci USA* 2007, **104**:1800–1804.
34. Engelman JA, Lisanti MP, Scherer PE: **Specific inhibitors of p38 mitogen-activated protein kinase block 3T3-L1 adipogenesis.** *J Biol Chem* 1998, **273**:32111–32120.
35. Coulthard LR, White DE, Jones DL, McDermott MF, Burchill SA: **p38(MAPK): stress responses from molecular mechanisms to therapeutics.** *Trends Mol Med* 2009, **15**:369–379.
36. Thornton TM, Pedraza-Alva G, Deng B, Wood CD, Aronshtam A, Clements JL, Sabio G, Davis RJ, Matthews DE, Doble B, Rincon M: **Phosphorylation by p38 MAPK as an alternative pathway for GSK3beta inactivation.** *Science* 2008, **320**:667–670.
37. Jiang Y, Chen C, Li Z, Guo W, Gegner JA, Lin S, Han J: **Characterization of the structure and function of a new mitogen-activated protein kinase (p38beta).** *J Biol Chem* 1996, **271**:17920–17926.
38. O'Keefe SJ, Mudgett JS, Cupo S, Parsons JN, Chartrain NA, Fitzgerald C, Chen SL, Lowitz K, Rasa C, Visco D, Luell S, Carballo-Jane E, Owens K, Zaller DM: **Chemical genetics define the roles of p38alpha and p38beta in acute and chronic inflammation.** *J Biol Chem* 2007, **282**:34663–34671.
39. Sicard P, Clark JE, Jacquet S, Mohammadi S, Arthur JS, O'Keefe SJ, Marber MS: **The activation of p38 alpha, and not p38 beta, mitogen-activated protein kinase is required for ischemic preconditioning.** *J Mol Cell Cardiol* 2010, **48**:1324–1328.
40. Ramji DP, Foka P: **CCAAT/enhancer-binding proteins: structure, function and regulation.** *Biochem J* 2002, **365**:561–575.
41. Bostrom P, Mann N, Wu J, Quintero PA, Plovie ER, Panakova D, Gupta RK, Xiao C, MacRae CA, Rosenzweig A, Spiegelman BM: **C/EBPbeta controls exercise-induced cardiac growth and protects against pathological cardiac remodeling.** *Cell* 2010, **143**:1072–1083.
42. Guo S, Cichy SB, He X, Yang Q, Ragland M, Ghosh AK, Johnson PF, Unterman TG: **Insulin suppresses transactivation by CAAT/enhancer-binding proteins beta (C/EBPbeta). Signaling to p300/CREB-binding protein by protein kinase B disrupts interaction with the major activation domain of C/EBPbeta.** *J Biol Chem* 2001, **276**:8516–8523.

doi:10.1186/2044-5040-2-20

Cite this article as: Zhang and Li: p38 β MAPK upregulates atrogin1/MAFbx by specific phosphorylation of C/EBP β . *Skeletal Muscle* 2012 2:20.

Submit your next manuscript to BioMed Central and take full advantage of:

- **Convenient online submission**
- **Thorough peer review**
- **No space constraints or color figure charges**
- **Immediate publication on acceptance**
- **Inclusion in PubMed, CAS, Scopus and Google Scholar**
- **Research which is freely available for redistribution**

Submit your manuscript at
www.biomedcentral.com/submit

