Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1629–1634
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Overexpression of the elongation factor 1A1 relates to muscle proteolysis and proapoptotic p66$^{(ShcA)}$ gene transcription in hypercatabolic trauma patients

Alessandra Bosutti[a,1], Bruna Scaggiante[b,*,1], Gabriele Grassi[a], Gianfranco Guarnieri[a], Gianni Biolo[a]

[a]*Division of Internal Medicine, Department of Clinical, Morphological and Technological Sciences, University of Trieste, 34127 Trieste, Italy*
[b]*Department of Biochemistry, Biophysics, and Macromolecular Chemistry, University of Trieste, 34127 Trieste, Italy*

Received 1 November 2006; accepted 6 July 2007

Abstract

The eukaryotic elongation factors (eEF1A2 and eEF1A1) play a key role in translation of messenger RNA (mRNA) to protein. In skeletal muscle of healthy humans, EEF1A2 is overexpressed and selected over EEF1A1. In cellular stress models, muscle EEF1A1 expression increased and was associated with apoptosis and catabolism. We have determined mRNA levels of EEF1A1 and EEF1A2, as well as those of other proapoptotic genes, such as p66$^{(ShcA)}$ and c-MYC, in skeletal muscle of severely traumatized patients and healthy volunteers. Muscle protein kinetic was determined by stable isotopes and the arteriovenous technique. The patients were in a hypercatabolic condition because the rate of muscle proteolysis exceeded that of synthesis. Mean mRNA levels of EEF1A1 and EEF1A2 were 165- and 29-fold greater ($P < .01$) in patients than in the control group, respectively. Mean p66$^{(ShcA)}$ mRNA levels were 3-fold greater ($P < .05$) in patients than in the controls. In contrast, c-MYC mRNA levels were not significantly different in patients and healthy controls. In patients, muscle mRNA levels of EEF1A1 and p66$^{(ShcA)}$ directly correlated ($P < .05$) with the rate of proteolysis ($R = 0.901$ and $R = 0.826$, respectively). This is in agreement with a reduction in actin and tubulin protein content, both markers of cytoskeletal and sarcomeric disorganization, and with an increased poly(adenosine diphosphate–ribose) polymerase cleavage, a marker of apoptosis. In conclusion, in hypercatabolic traumatized patients, an up-regulation of muscle EEF1A1 and p66$^{(ShcA)}$ relates to proteolysis rate, suggesting an involvement of these genes in muscle catabolic response.

1. Introduction

Systemic stress response and muscle wasting are common features in severely traumatized patients. The rate of muscle proteolysis is greatly accelerated and not balanced by the rate of protein synthesis [1]. Several studies have indicated that muscle catabolism is associated with an increased expression and activity of critical components of proteolytic and apoptotic systems [2-4] Close relationships between proteolytic pathways and apoptotic response in human skeletal muscle have been demonstrated [2,3]. However, the molecular mechanisms underlining these processes remain to be elucidated.

We selected the eukaryotic elongation factors (EEF1A), p66$^{(ShcA)}$, and c-MYC as candidate genes to understand the relationships between muscle proteolysis and apoptosis in skeletal muscle of hypercatabolic trauma patients [5-7]. The eEF1A proteins play a key role for the translation machinery. After codon-anticodon recognition, they carry each aminoacyl–transfer RNA complex to the A site of ribosome [8]. In mammals, the eEF1A proteins are encoded by the EEF1A1 and EEF1A2 genes mapped on different chromosomes. The coding sequence of the 2 genes has a 78% homology, with a protein identity of 92% [9]. In human tissues, EEF1A1 is ubiquitously expressed, with the exception of skeletal muscle, heart, and brain, where it progressively declines at the early phases of development [9-11]. In adult muscle, EEF1A2 takes over the EEF1A1-specific function for protein synthesis [12]. In aging rats, muscle EEF1A1 transcription increases, reverting the eEF1A1/eEF1A2 ratio

* Corresponding author. Tel.: +39 0405583678; fax: +39 0405583691.
E-mail address: bscaggiante@units.it (B. Scaggiante).
[1] These 2 authors must both be considered as first author.

0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.07.003

[13]. In contrast to the rat model, in humans, there is no increase of EEF1A1 messenger RNA (mRNA)/protein in the late phases of life [14]. In cultured myotubes, EEF1A1 expression was associated with increased apoptosis, whereas EEF1A2 was associated with antiapoptotic conditions and cell proliferation [15]. Thus, the changes in the expression levels of these 2 elongation factors related to pro- and antiapoptotic responses [15,16] could be informative on the molecular events underlying accelerated catabolism in skeletal muscle of traumatized patients. The $p66^{(ShcA)}$ gene is an adaptor protein arising from alternative splicing of the primary ShcA transcript involved in mitogenic, survival, and cytokine signaling pathways [6]. In particular, $p66^{(ShcA)}$ is a crucial mediator of the response for the elevation of intracellular pro-oxidants, mitochondrial permeability transition, oxidation, and release of cytochrome *c* [6]. The increase in $p66^{(ShcA)}$ mRNA levels has been demonstrated to be significantly related to apoptosis and stress response in cultured cells [6,17] and to aging in rats [18]. In human muscle, its role in the pathological status, such as trauma, has not yet been investigated. The oncogene c-MYC is an essential mediator of cell growth and proliferation [7,19]. Overexpression of c-MYC may increase the cellular reactive oxygen species production [7,19] and the cytochrome *c* release from mitochondria, leading to mitochondrial membrane destabilization and apoptosis [7,19]. In humans, an upregulation of muscle c-MYC has been observed in the acute quadriplegic myopathy often experienced by patients with severe systemic critical illness [20].

To understand the relationships between proteolysis and apoptotic response in skeletal muscle of hypercatabolic trauma patients, we have determined the mRNA levels of EEF1A2, EEF1A1, $p66^{(ShcA)}$, and c-MYC genes in biopsy specimens and related with the rate of muscle proteolysis measured by stable isotopes and the leg arteriovenous technique. Moreover, the protein levels of actin and tubulin, both markers of cytoskeletal and sarcomeric disorganization, were evaluated [21-23]. Finally, the cleavage status of poly(adenosine diphosphate–ribose) polymerase (PARP) protein, a known marker of apoptosis [24], was studied. Results of muscle protein kinetics have been previously published [25,26].

2. Material and methods

2.1. Patients and experimental protocol

Seven adult patients (5 men, 2 women; age, 38 ± 6 years; weight, 72 ± 43 kg; height, 171 ± 3 cm; body mass index, 24 ± 0.8 kg/m^2) with multiple injuries (Acute Physiology and Chronic Health Evaluation II score, 15 ± 1) were studied between days 7 and 12 after admission to the intensive care unit of the University Hospital of Cattinara, Trieste, Italy. Informed consent was obtained from the patients' close relatives. The protocol was approved by the competent hospital authority. All patients received continuous combined intravenous (80% of total energy) and enteral (20% of total energy) nutrition providing 35 kcal/(kg d) with 250 mg nitrogen/(kg d). Nutrient administration was constant during the study and for at least 1 day before the study.

2.2. Healthy controls

The control group consisted of 5 male healthy subjects matched for age (40 ± 3 years) and body mass index (24 ± 1 kg/m^2) to trauma patients. Informed consent was also obtained from these subjects. Only male subjects were available as normal healthy controls, and they were enrolled because no difference between men and women on catabolic trauma conditions was observed.

2.3. Muscle protein synthesis and proteolysis calculation

Rates of muscle protein synthesis and degradation were determined as rate of intracellular phenylalanine disposal and appearance by the isotope dilution technique during primed, continuous infusion of L-[ring-$^2H_5$] phenylalanine (Mass Trace, Woburn, MA), as previously described [1,25,26]. Blood samples from the femoral artery and vein and muscle biopsies by a Bergström needle (Stille, Stockholm, Sweden) from the vastus lateralis muscle were taken to measure steady-state values of the phenylalanine concentration and enrichment by gas chromatography/mass spectrometry (Finnigan MAT, Bremen, Germany). Leg blood flow was measured by the dye-dilution technique using indocyanine green (Infracyanine; SERB, Paris, France). Calculations are described in our previous report [1,25,26]. Biopsies of the vastus lateralis muscle were also obtained from the healthy controls. Protein kinetics was not determined in the control group.

2.4. RNA extraction and quantification

Total RNA was extracted from muscle samples and reverse transcribed as previously described [25-27]. The RNA quality was checked by Bioanalyzer 2100 expert assay (Agilent 2100), and all samples selected showed an RNA integrity number between 2.9 and 5.7.

Real-time polymerase chain reaction (PCR) was performed using the SYBR Green dye chemistry in singleplex reactions and the 7900/HT Sequence Detection System (Applied Biosystems-Appelera Corp, USA). Regions of EEF1A1, EEF1A2, $p66^{(ShcA)}$, c-MYC, and 28S transcripts were chosen with particular attention to obtain the specific amplicons without significant contaminant bands or genomic products. The specificity of the obtained amplicons was checked by digesting the PCR product with appropriate restriction endonucleases (data not shown). Furthermore, samples were treated with deoxyribonuclease before performing reverse transcription to completely exclude the possibility that amplicons of EEF1A1 could be derived from nonactively transcribed retropseudogenes [28]. Although the coding regions of EEF1A genes are very similar, the introns, untranslated regions, and promoters are highly divergent. For complementary DNA (cDNA) amplifications, the following primers were used: EEF1A1 sense 5′-

AACATTGTCGTCATTGGACA-3′ (nucleotides 1606-1625) and EEF1A1 antisense 5′-ACTTGCTGGTCTCAAATTTC-3′ (nucleotides 2181-2200), GenBank accession no. J04617; EEF1A2 sense 5-'GCCACCGTCAATAGGTGGAC-3' (nucleotides 2073-2092) and EEF1A2 antisense 5′-TGATGTGGGTCTTCTCCTTG-3′ (nucleotides 3438-3457), GenBank accession no. AF163763; C-MYC sense 5′-AAGCTCGTCTCAGAGAAGCT-3′ (nucleotides 996-1015) and C-MYC antisense 5′-CTTCTTGTTCCTCCTCAGAG-3′ (nucleotides 1322-1341), GenBank accession no. NM_002467.3; $p66^{(ShcA)}$ sense 5′-CACGGGAGCTTTGTCAATAAGC-3′ (nucleotides 409-430) and $p66^{(ShcA)}$ antisense 5′-CCCCGGGTCCCATGACTTT-3′ (nucleotides 461-479), GenBank accession no. NM_183001; and 28S ribosomal RNA (rRNA) sense 5′-TGGGAATGCAGCCCAAAG-3′ (nucleotides 282-299) and 28S rRNA antisense 5′-CCTTACGGTACTTGTTGACTATCG-3′ (nucleotides 342-365), GenBank accession no. M11167. All amplifications were conducted in a final volume of 25 μL of SYBR/Master Mix buffer (Applied Biosystems-Applera) containing the 2 primers (300 nmol/L each), SYBR green, the 4 dNTPs (200 μmol/L each), Taq DNA polymerase, and 1 μL of cDNA. The 28S rRNA, EEF1A1, EEF1A2, and c-MYC were submitted to 40 cycles of amplification with enzyme activation at 50°C for 2 minutes, predenaturation at 95°C for 10 minutes, denaturation at 95°C for 15 seconds, and annealing and extension at 62° for 60 seconds. The $p66^{(ShcA)}$ cDNAs were submitted to 40 cycles of amplification with enzyme activation at 50°C for 2 minutes, predenaturation at 95°C for 10 minutes, denaturation at 95°C for 15 seconds, and annealing and extension at 56° for 60 seconds. Fluorescent levels of amplified products were detected and quantified by ABI Prism 7900 machine; subsequently, they were converted into number of molecules according to an external standard curve by means of the equation $y = A * \mathrm{EXP}(-kx^n)$, where y and x are the number of cycles and the number of molecules, respectively, and A, K, and n are the coefficients of the equation. The standard curve was performed using a dilution series of an external standard. Copy numbers of unknown samples were calculated from the linear regression of that standard curve, with the y-intercept giving the sensitivity and the slope giving the amplification efficiency. Messenger RNA amounts of target genes were normalized by 28S rRNA content.

2.5. Western blotting

Twenty milligrams of muscle biopsies was homogenized on ice in ice-cold lysis buffer (0.25 mol/L sucrose and 0.3% Triton X-100, 1 mmol/L dithiothreitol) in the presence of the protease inhibitor cocktail. After centrifugation at 2000g for 10 minutes at 4°C, the supernatants were collected. Thirty micrograms of protein, assayed by the Bradford method (Biorad Laboratories, Herts, United Kingdom), was mixed with 1 vol of sodium dodecyl sulfate–glycerol/β-mercaptoethanol/bromophenol blue loading buffer, heated to 95°C for 5 minutes, and electrophoresed alongside molecular weight markers (Sigma-Aldrich, St. Louis, MO) on 4% stacking and 10% to 12% resolving denaturing sodium dodecyl sulfate–polyacrylamide electrophoresis gels. Protein samples were electroblotted (20 V and 70 mA for 1 hour and 45 minutes) onto nitrocellulose membrane (Schleicher & Schuell, Keene, NH). The membranes were then incubated with a blocking solution (3%-5% nonfat milk powder/phosphate-buffered saline (PBS)/0.05% Tween 20) for 30 minutes at room temperature, followed by incubation with primary antibodies diluted in PBS or PBS/0.05% Tween 20. The only commercially available anti-eEF1A mouse monoclonal antibody (1:1000 dilution; Upstate, Biotechnology, Lake Placid, NY) did not distinguish eEF1A1 from eEF1A2 forms, and only total eEF1A content can be determined. The other antibodies used were anti-actin rabbit polyclonal antibody (1:1000 dilution; Sigma-Aldrich), anti-PARP mouse monoclonal antibody CD2-10 (1:1000 dilution; purchased from BD Biosciences, San Jose, CA), and anti–glyceraldehyde-3-phosphate dehydrogenase (GAPDH) rabbit polyclonal antibody (1:800 dilution; Santa Cruz Biotechnologies, Santa Cruz, CA). The eEF1A and the cleaved PARP primary antibody incubations were performed overnight at 4°C. The actin and the GAPDH primary antibody incubations were performed at room temperature for 1 hour. Secondary antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnologies and Sigma-Aldrich) were incubated at room temperature for 1 hour. The bands were visualized using the chemiluminescence substrate (Pierce, Rockford, IL), exposing the membrane to autoradiographic film within its linear range; the signal was quantified by scanning laser densitometry.

2.6. Data presentation and statistics

Data were expressed as means ± SEM. Results obtained in patients and controls were compared by the Wilcoxon 2-sample test. Regression analysis was carried out according to standard methods using log-transformed data for parametric analysis. Results were considered to be statistically significant at values of $P < .05$.

3. Results

The trauma patients were in catabolic conditions because the rate of muscle proteolysis exceeded ($P < .05$) that of protein synthesis (57 ± 5 and 30 ± 3 nmol/min per 100 mL leg volume, respectively). Mean values of the mRNA levels of EEF1A1, EEF1A2, $p66^{(ShcA)}$, and c-MYC in the skeletal muscle of patients and controls are shown in Table 1. As expected, the mRNA levels of EEF1A2 were much greater than those of EEF1A1 in the skeletal muscle of healthy controls. The mRNA levels of both genes significantly increased in traumatized patients. Nonetheless, the increase of EEF1A1 was much greater than that of EEF1A2. As evidenced in Table 1, trauma leads to a 29-fold increase in the EEF1A2 mRNA content with respect to healthy conditions, but also to the appearance of a more marked increase of the EEF1A1 transcript (165-fold). The $p66^{(ShcA)}$ mRNA levels were about 3 times greater in patients than in

Table 1
Muscle mRNA levels of EEF1A1, EEF1A2, p66$^{(ShcA)}$, and C-MYC in trauma patients and healthy controls

	Controls	Traumatized patients	Fold increase in mRNA levels (traumatized patients/healthy controls)
EEF1A1	0.6 ± 0.4	81.4 ± 35.0 **	165 ± 87
EEF1A2	$7{\cdot}10^4 \pm 4{\cdot}10^4$	$215{\cdot}10^4 \pm 63{\cdot}10^4$ **	29 ± 15
P66$^{(ShcA)}$	72 ± 20	211 ± 53 *	2.9 ± 2.6
c-MYC	313 ± 166	985 ± 437	3.1 ± 2.6 (NS)

The fold increases in mRNA expression were calculated as the ratio between the mean of mRNA levels in skeletal muscle of individual patients and that of healthy controls. Data are expressed as means ± SEM. NS indicates no statistical significance.

* $P < .05$ patients vs controls.

** $P < .01$ patients vs controls.

controls. Mean c-MYC mRNA levels tended to be greater in patients, but no statistical significance was achieved. In the skeletal muscle of patients, mRNA levels of EEF1A1 and p66$^{(ShcA)}$ directly correlated with the rate of proteolysis (Fig. 1), as previously evaluated [25,26]. Moreover, a direct relationship was found between p66$^{(ShcA)}$ and EEF1A1 mRNA ($R = 0.89$, $P < .01$).

Because a commercial antibody able to distinguish between eEF1A1 and eEF1A2 proteins is not available, Western blotting analysis of total eEF1A content was performed. As illustrated in Fig. 2 (A, Western blots; B-C, data quantification), cytoplasmic total eEF1A protein content tends to increase in trauma patients compared with healthy controls. Moreover, in trauma patients, a decrease in total actin ($P = .008$) and tubulin ($P = .046$) content, both markers of cytoskeletal and sarcomeric disorganization [21-23], was observed. The eEF1A-actin ratio was 4 times greater ($P = .02$) in trauma patients (3.9 ± 1.2) than in the controls (0.8 ± 0.2). Finally, an increased PARP cleavage, a marker of apoptosis [24], was observed in trauma patients.

4. Discussion

The EEF1A1 and EEF1A2 are 2 forms of translation elongation factor EEF1A [8,9]. In skeletal muscle of healthy humans, EEF1A2 is overexpressed and selected over EEF1A1 [15]. In animal models, increases in EEF1A1 expression were associated with muscle injury, denervation, and atrophy [12,29]. In cultured myotubes, the 2 sister genes, EEF1A2 and EEF1A1, regulated survival, with the former exerting antiapoptotic activity and the latter a proapoptotic effect [15].

The level of EEF1A1 mRNA dramatically increased in our patients by 165-fold and directly correlated with the rate of proteolysis, suggesting a production of eEF1A1 as a response to trauma [30]. A more contained increase in EEF1A2 levels (29-fold) was also observed. Moreover, in trauma patients, the eEF1A total protein content tended to be greater than in healthy controls. Based on the mRNA and protein data, we hypothesize that the trauma induced the de novo synthesis of eEF1A1 to exploit its noncanonical functions such as protein degradation and apoptosis triggering [15]. In contrast, eEF1A2 level was less affected, probably because eEF1A2 is just required as translation factor. The modest increase in EEF1A2 mRNA level in critically hypercatabolic patients might be a consequence of the attempt to sustain muscle survival, maintaining or increasing the protein synthesis rate [1].

We observed that the trauma induced a decrease in total actin and tubulin level, suggesting a cytoskeletal and sarcomeric disorganization [21-23]. Moreover, because actin may have a critical role in ensuring translation fidelity [31], its decrease might further contribute to increasing the frequency of translation errors in trauma muscle, thus stimulating eEF1A1-coupled proteosome degradation of damaged proteins [29,32-34]. This observation is consistent with the fact that the eEF1A-actin ratio was 4 times greater in patients than in controls. It is possible that, in muscle cells,

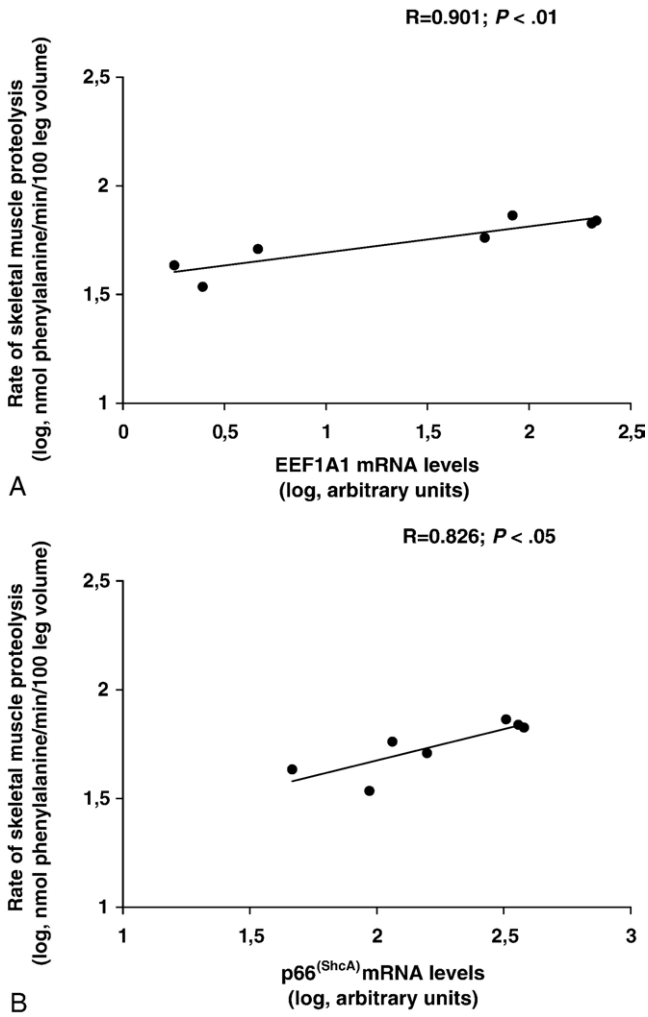

Fig. 1. Relationships between EEF1A1 or p66$^{(ShcA)}$ mRNA levels and the rate of muscle proteolysis. Regression analysis was performed as described in Material and Methods.

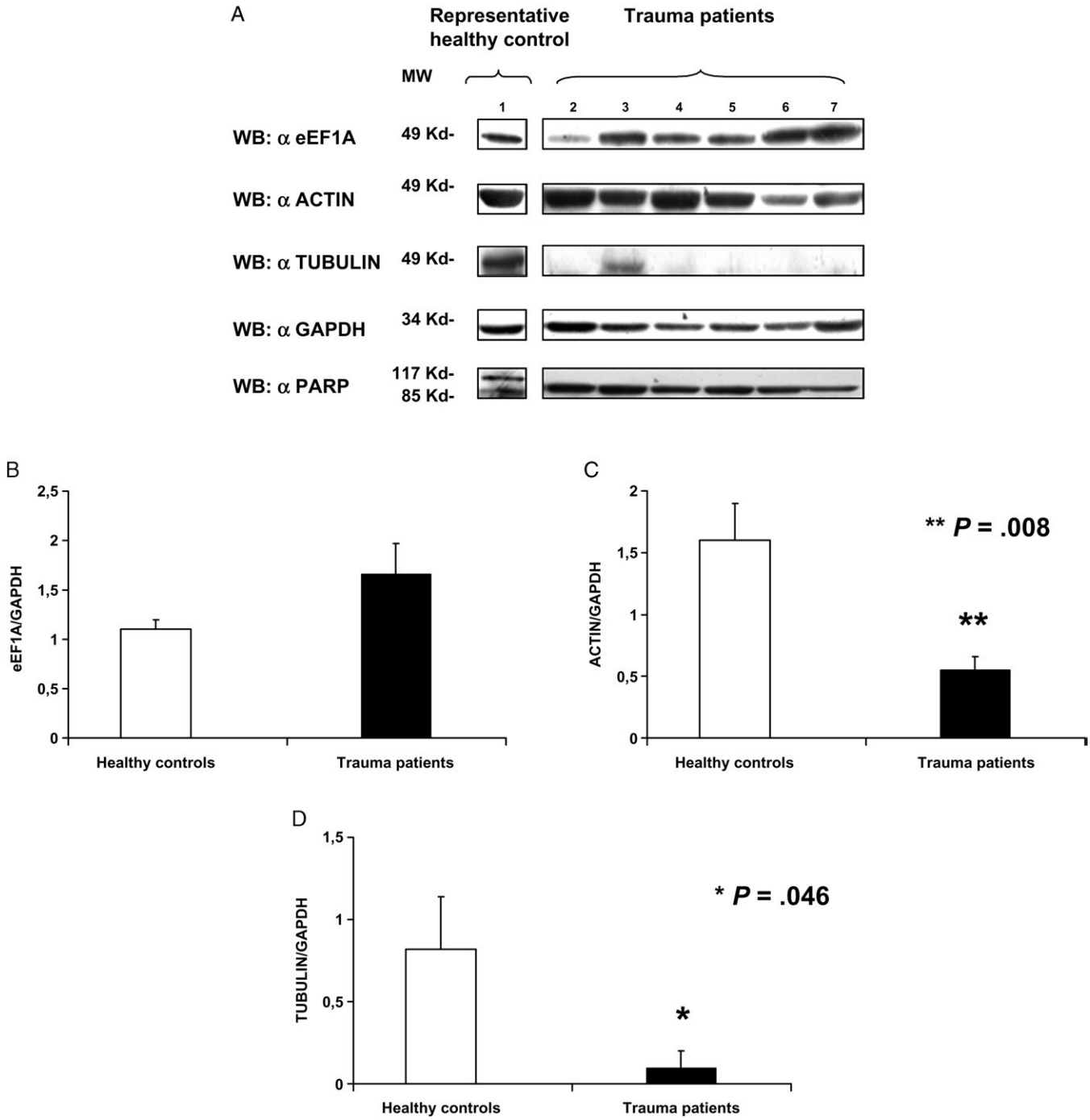

Fig. 2. Protein contents of eEF1A, cleaved PARP, and actin in the skeletal muscle of patients and healthy controls. A, In lane 1, a representative control (with an average band intensity for each protein probed) is reported; in lanes 2 to 7, trauma patients samples are shown. B and C, Reported is the data quantification of eEF1A, actin, and tubulin protein contents expressed as ratio to GAPDH protein levels. Data are reported as means ± SEM.

eEF1A1 may contribute to the ubiquitin-mediated protein degradation by transferring damaged nascent proteins to the proteasome after their release from the ribosome [35,36].

In our patients, muscle $p66^{(ShcA)}$ mRNA levels directly correlated with EEF1A1 expression and proteolysis rate. Both genes are involved in apoptotic programs; and, accordingly, in trauma patients, we found an increase of the cleaved form of PARP. This observation supports previous evidence linking $p66^{(ShcA)}$ overexpression with stress-induced apoptosis [6] and with muscle wasting and atrophy [18]. In contrast to $p66^{(ShcA)}$, c-MYC mRNA levels were not significantly different in patients and controls. This finding is in agreement with the fact that c-MYC was not involved in the regulation of apoptosis in denervated rat skeletal muscle [37]. Therefore, this concept can also be extended to human traumatized muscle.

In conclusion, our results indicate that an increased muscle expression of the eEF1A1 gene in hypercatabolic trauma patients is a molecular marker for injury. The mechanism we described could provide a starting point for further molecular investigations on the role of eEF1A proteins in the stress response of hypercatabolic patients. In particular, studies could be developed with nutritional factors on the expression of EEF1A genes to plan more effective therapeutic approaches for the recovery of muscle-wasting and traumatized patients.

Acknowledgments

This work was supported by grants from MIUR (Ministero dell'Istruzione, dell'Università e della Ricerca Scientifica) and ASI (Agenzia Spaziale Italiana). Dr G Grassi is supported by the program "Rientro cervelli" art. 1 DM n.13, MIUR. We thank Dr Raffaella Antonione for the helpful discussion and suggestion.

References

[1] Biolo G, Fleming RY, Maggi SP, et al. Inverse regulation of protein turnover and amino acid transport in skeletal muscle of hypercatabolic patients. J Clin Endocrinol Metab 2002;87:3378-84.
[2] Du J, Wang X, Miereles C, et al. Activation of caspase-3 is an initial step triggering accelerated muscle proteolysis in catabolic conditions. J Clin Invest 2004;113:115-23.
[3] Du J, Hu Z, Mitch WE. Cellular signals activating muscle proteolysis in chronic kidney disease: a two-stage process. Int J Biochem Cell Biol 2005;37:2147-55.
[4] Mansoor O, Beaufrere B, Boirie Y, et al. Increased mRNA levels for components of the lysosomal, Ca2+-activated, and ATP-ubiquitin–dependent proteolytic pathways in skeletal muscle from head trauma patients. Proc Natl Acad Sci U S A 1996;93:2714-8.
[5] Lamberti A, Caraglia M, Longo O, et al. The translation elongation factor 1A in tumorigenesis, signal transduction and apoptosis: review article. Amino Acids 2004;26:443-8.
[6] Migliaccio E, Giorgio M, Pelicci PG. Apoptosis and aging: role of p66Shc redox protein. Antioxid Redox Signal 2006;8:600-8.
[7] Klefstrom J, Verschuren EW, Evan G. c-Myc augments the apoptotic activity of cytosolic death receptor signalling proteins by engaging the mitochondrial apoptotic pathway. J Biol Chem 2002;277:43224-32.
[8] Browne GJ, Proud CG. Regulation of peptide-chain elongation in mammalian cells. Eur J Biochem 2002;269:5360-8.
[9] Lund A, Knudsen SM, Vissing H, et al. Assignment of human elongation factor 1alpha genes: EEF1A maps to chromosome 6q14 and EEF1A2 to 20q13.3. Genomics 1996;36:359-61.
[10] Lee S, Francoeur AM, Liu S, et al. Tissue-specific expression in mammalian brain, heart, and muscle of S1, a member of the elongation factor–1 alpha gene family. J Biol Chem 1992;267:24064-8.
[11] Lee S, Wolfraim LA, Wang E. Differential expression of S1 and elongation factor–1 alpha during rat development. J Biol Chem 1993; 268:24453-9.
[12] Khalyfa A, Bourbeau D, Chen E, et al. Characterization of elongation factor–1A (eEF1A-1) and eEF1A-2/S1 protein expression in normal and wasted mice. J Biol Chem 2001;276:22915-22.
[13] Carlson BM, Borisov AB, Dedkov EI, et al. Effects of long-term denervation on skeletal muscle in old rats. J Gerontol Biol Sci 2002; 57A:B366-74.
[14] Welle S, Thornton C, Bhatt K, et al. Expression of elongation factor–1 alpha and S1 in young and old human skeletal muscle. J Gerontol A Biol Sci Med Sci 1997;52:B235-9.
[15] Ruest LB, Marcotte R, Wang E. Peptide elongation factor eEF1A-2/S1 expression in cultured differentiated myotubes and its protective effect against caspase-3–mediated apoptosis. J Biol Chem 2002;277:5418-25.
[16] Chen E, Proestou G, Bourbeau D, et al. Rapid up-regulation of peptide elongation factor EF-1α protein levels is an immediate early event during oxidative stress-induced apoptosis. Exp Cell Res 2000;259: 140-8.
[17] Purdom S, Chen QM. Linking oxidative stress and genetics of aging with p66Shc signaling and forkhead transcription factors. Biogerontology 2003;4:181-91.
[18] Jiang X, Edstrom E, Altun M, et al. Differential regulation of Shc adaptor proteins in skeletal muscle, spinal cord and forebrain of aged rats with sensorimotor impairment. Aging Cell 2003;2:47-57.
[19] Dang CV. c-Myc target genes involved in cell growth, apoptosis, and metabolism. Mol Cell Biol 1999;19:1-11.
[20] Di Giovanni S, Molon A, Broccolini A, et al. Constitutive activation of MAPK cascade in acute quadriplegic myopathy. Ann Neurol 2004;55: 195-206.
[21] Percipalle P. Genetic connections of the actin cytoskeleton and beyond. Bioessays 2007;29:407-11.
[22] Korb T, Schluter K, Enns A, et al. Integrity of actin fibers and microtubules influences metastatic tumor cell adhesion. Exp Cell Res 2004;299:236-47.
[23] Heling A, Zimmermann R, Kostin S, et al. Increased expression of cytoskeletal, linkage, and extracellular proteins in failing human myocardium. Circ Res 2000;86:846-53.
[24] Tolosa L, Morla M, Iglesias A, et al. IFN-gamma prevents TNF-alpha–induced apoptosis in C2C12 myotubes through down-regulation of TNF-R2 and increased NF-kappaB activity. Cell Signal 2005;17: 1333-42.
[25] Biolo G, Iscra F, Bosutti A, et al. Growth hormone decreases muscle glutamine production and stimulates protein synthesis in hypercatabolic patients. Am J Physiol Endocrinol Metab 2000;279:E323-32.
[26] Biolo G, Bosutti A, Iscra F, et al. Contribution of the ubiquitin-proteasome pathway to overall muscle proteolysis in hypercatabolic patients. Metabolism 2000;49:689-91.
[27] Bosutti A, Biolo G, Toigo G, et al. Molecular regulation of protein catabolism in trauma patients. Clin Nutr (Edinb) 2002;18:103-5.
[28] Madsen HO, Poulsen K, Dahl O, et al. Retropseudogenes constitute the major part of the human elongation factor 1α gene family. Nucleic Acid Res 1990;18:1513-6.
[29] Duttaroy A, Bourbeau D, Wang XL, et al. Apoptosis rate can be accelerated or decelerated by overexpression or reduction of the level of elongation factor 1-α. Exp Cell Res 1998;238:168-76.
[30] Borradaile NM, Buhman KK, Listenberger LL, et al. A critical role for eukaryotic elongation factor 1A-1 in lipotoxic cell death. Mol Biol Cell 2006;17:770-8.
[31] Kandl KA, Munshi R, Ortiz PA, Andersen GR, Kinzy TG, Adams AE. Identification of a role for actin in translational fidelity in yeast. Mol Genet Genomics 2002;268:10-8.
[32] Attaix D, Ventadour S, Codran A, et al. The ubiquitin-proteasome system and skeletal muscle wasting. Essays Biochem 2005;1:173-86.
[33] Jesenberger V, Jentsch S. Deadly encounter: ubiquitin meets apoptosis. Nat Rev Mol Cell Biol 2002;3:112-21.
[34] Lee SW, Dai G, Hu Z, et al. Regulation of muscle protein degradation: coordinated control of apoptotic and ubiquitin-proteasome systems by phosphatidylinositol 3 kinase. J Am Soc Nephrol 2004;15: 1537-45.
[35] Chuang SM, Chen L, Lambertson D, et al. Proteasome-mediated degradation of cotranslationally damaged proteins involves translation elongation factor 1A. Mol Cell Biol 2005;25:403-13.
[36] Gonen H, Dickman D, Schwartz A, et al. Protein synthesis elongation factor EF-1 alpha is an isopeptidase essential for ubiquitin-dependent degradation of certain proteolytic substrates. Adv Exp Med Biol 1996; 389:209-19.
[37] Siu PM, Always SE. Mitochondria-associated apoptotic signalling in denervated rat skeletal muscle. J Physiol 2005;565:309-23.