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The p75^{NTR}-mediated effect of nerve growth factor in L6C5 myogenic cells

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Abstract

Objective: During muscle development or regeneration, myocytes produce nerve growth factor (NGF) as well as its tyrosine-kinase and p75-neurotrophin (p75^{NTR}) receptors. It has been published that the p75^{NTR} receptor could represent a key regulator of NGF-mediated myoprotective effect on satellite cells, but the precise function of NGF/p75 signaling pathway on myogenic cell proliferation, survival and differentiation remains fragmented and controversial. Here, we verified the role of NGF in the growth, survival and differentiation of p75^{NTR}-expressing L6C5 myogenic cells, specifically inquiring for the putative involvement of the nuclear factor κB (NFκB) and the small heat shock proteins (sHSPs) αB-crystallin and Hsp27 in these processes.

Results: Although NGF was not effective in modulating myogenic cell growth or survival in both standard or stress conditions, we demonstrated for the first time that, under serum deprivation, NGF sustained the activity of some key enzymes involved in energy metabolism. Moreover, we confirmed that NGF promotes myogenic fusion and expression of the structural protein myosin heavy chain while modulating NFκB activation and the content of sHSPs correlated with the differentiation process. We conclude that p75^{NTR} is sufficient to mediate the modulation of L6C5 myogenic differentiation by NGF in term of structural, metabolic and functional changes.

Keywords: NGF, p75^{NTR}, Myogenic differentiation, Energy metabolism, NFκB, sHSPs

Introduction

Skeletal muscle regeneration depends upon optimal activation, proliferation and differentiation of myogenic precursors, the satellite cells, whose behavior is controlled by components, such as cytokines and growth factors, contained in the satellite cell microenvironment [1]. During myogenic differentiation, muscle cells produce NGF and other neurotrophins as well as their receptors, tyrosine-kinase receptors (TrkA and TrkB) and p75-neurotrophin receptor (p75^{NTR}), able to act in autocrine manner on cell morphology, proliferation and differentiation [2, 3]. Beside some contractictory results [4], several evidences demonstrate that cellular signaling pathways activated by the neurotrophin/p75^{NTR} axis stabilize the cytoskeletal architecture and increase the fusogenic properties of myotubes, thus promoting "in vitro" myogenic differentiation, myotubes survival and muscle repair "in vivo" [3, 5]. Thus, despite the well known pro-apoptotic role of p75^{NTR} in neuron cells, this receptor might represent a key mediator of survival in myoblasts and myotubes and its activity during myogenesis seems important for developing skeletal muscle [6, 7].

We previously demonstrated that the activation of NF-κB and the parallel modulation of αB-crystallin (αB-Cry) and Hsp27 play a major role in the antiapoptotic effect exerted by vascular endothelial growth factor (VEGF) in C2C12 myoblasts exposed to oxidative or hypoxic-like stress "in vitro" [8], as well as in the positive effect exerted by platelet-rich plasma on rat skeletal muscle healing "in vivo" [9]. NF-κB is a redox-sensitive transcription factor known to regulate several cellular processes (i.e. inflammation, cellular survival, proliferation and differentiation) that recently emerged as a key player in the regulation of skeletal muscle homeostasis

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[10]. αB-Cry and Hsp27 are small heat shock proteins (sHSPs) abundantly expressed in muscle tissue where they stabilize cytoskeletal structures, especially under pro-oxidant insult [11–13], modulate myogenic differentiation [14, 15] and interact with several growth factors through the c-Jun N-terminal kinase (JNK)- and/or NFκB- dependent regulatory mechanisms [9, 11, 12].

Considering these observations altogether, the main aim of the present study was to analyze in L6C5 myogenic cells expressing exclusively the $p75^{NTR}$ receptor, whether the effect of NGF on myoblast growth, survival, fusion rate and expression of early and late markers of myogenesis correlates with NFκB activation and/or modulation of αB-Cry and Hsp27 expression.

Main text

Materials and methods

Cell culture, growth and viability

All experiments were carried out on L6C5 rat myogenic cells (ICLC, AL00001). As already described [16], L6C5 myoblast cultures were maintained and subcultured in growing medium (GM) (DMEM with 4.5 g/l glucose and Corning® glutagro™, w/o sodium pyruvate, Corning 10-102-CVR; 100 U/ml penicillin, 100 μg/ml streptomycin, Euroclone ECB3001D; 10% FBS, Gibco 10270). Differentiation medium (DM) containing 2% FBS was utilized in 85%—confluent cells to induce myotubes. The process was monitored through microscopy and expression of myogenic markers [17, 18]. NGF (Promega, 10–100 ng/ml) was added to the GM or DM medium at the indicated time and replenished with media changes every 3 days. For cell growth and viability, 5×10^5 cells/well were seeded in a 96-well culture plate for 6, 12, 24, and 48 h with or without 10% FBS, in presence or absence of NGF added from the seeding. Cell growth was analysed by MTS assay (Promega) following the manufacturer's recommendations. The absorbance was measured at 490 nm (Bio-Rad680). Viability was evaluated by trypan blue exclusion assay performed at the same culture conditions.

Enzymatic activities

L6C5 myoblasts grown in presence or in absence of NGF (10, 100 ng/ml) for 48 h under standard (GM) or serum starvation conditions were lysed (0.05 M Tris–Acetate, 250 mM sucrose, pH 7.5, 1 mM PMSF) with a protease inhibitor cocktail (P8340, Sigma Aldrich, St. Louis, MO). After gentle sonication (twice 10 s in ice with Vibra-Cell CV 18 SONICS VX 11) and centrifugation (at 14,000 rpm for 10 min at 4 °C), the supernatant was tested for protein content (Bradford method, Sigma Aldrich, St Louis MO) and then analysed spectrophotometrically (20–50 μl sample, Perkin Elmer Lambda 25, Fremont, CA, USA) for glyceraldehyde-phosphate-dehydrogenase (GAPDH), lactate dehydrogenase (LDH), citrate synthase (CS), 3-OH acylCoA dehydrogenase (HAD) and alanine transglutaminase (ALT) enzymatic activities as previously described [19, 20].

Immunoblot analysis

Protein extraction and immunoblotting was performed by standard methodology as already described [9, 21]. Briefly, total cellular proteins were extracted by lysis buffer (20 mM Tris pH 7.5, 150 mM NaCl, 2 mM EDTA, 1 mM sodium orthovanadate, 100 mM PMSF, 10 mg/ml leupeptin, 10 mg/ml aprotinin, 5 mg/ml pepstatin, 50 mM NaF, 1 nM okadaic acid, 1% Triton X-100 and 10% glycerol), and quantified using the Bradford assay (Sigma). From each sample, 15–20 μg of proteins have been utilized for immunoblotting with the following antibodies: myogenin (sc-576), Hsp27 (sc-1048), $p75^{NTR}$ (sc-56448) and TrkA (sc-20539) (1:1000, Santa-Cruz Biotechnology), β-actin (A1978) (1:3000, Sigma), anti-embryonic MyHC (F1652) (1:1000, Biovalley) and MyHC IIB (BFF3) (1:1000, Development Studies Hybridoma Bank), αB-crystallin (SPA-222) (1:1000, Enzo Life Sciences), SAPK/JNK (#9252), Phospho-SAPK/JNK (Thr183/Tyr185) (#4668), and Bcl-2 (#2870) (1:1000, Cell Signalling), Caspase 3 (#44976) (1:1000, Abcam). All immunoblots were visualized with the appropriated horseradish peroxidase-conjugated secondary antibody (1:15,000, Millipore) followed by detection with enhanced chemiluminescence (Amersham-Biosciences). Bands were quantified by ImageJ software. The expression of β-actin was used as a normalizing control.

Fusion rate analysis

Cells, grown 2 and 9 days in DM with or without NGF (20 ng/ml), were fixed with ice-cold methanol (7′ at − 20 °C), permeabilized (0.1% triton X-100 for 20′) and then blocked at RT for 60′ (TBS, 10% FBS, 0.1% triton). Myotubes were identified by double staining with Hoechst 33258 (Sigma) and MyHC antibody (sc-12117, 1:50, Santa-Cruz Biotechnology) as MyHC positive cells with at least two nuclei. Since the number of total nuclei was not modulated by NGF (data not shown), the fusion rate was evaluated by the number of total myotubes in each well and the number of nuclei/myotube [3].

NFκB activity

NFκB activity was measured in nuclear protein extracts (15 μg) by the TransAM NF-κB p65 protein assay (Active Motif), according to the manufacturer's protocol [8]. The assay was performed in presence or in absence of NGF

(20 ng/ml) on proliferating myoblasts (24 h from seeding) or cells grown in DM for 2, 5 and 9 days. Experimental samples and controls were run in duplicate.

Statistical analysis

Statistical comparisons between groups were performed by Student's t test. All values are given as the mean ± standard deviation of the mean (SD). $p < 0.05$ was considered significant.

Results and discussion

Impairment of the satellite cell pool has been observed in age-related muscle dysfunction and muscle degenerative pathologies, while the poor rate of survival and proliferation of myoblasts derived from satellite cell transplantation represents an important limit for the cell replacement therapy in muscle diseases [22]. Various components of the microenvironment play a crucial role in the proliferative and differentiation potential of satellite cells, thus ensuring an adequate regenerative response to muscle insult [1]. This study was indeed designed to confirm and extend the positive role of NGF/$p75^{NTR}$ axis in "in vitro" differentiation of L6C5 myogenic cells.

As already described in other murine cell lines and in human muscle cells [3, 5–7], L6C5 cell line expressed only $p75^{NTR}$ mRNA that, differently from primary myogenic cells [3], was not differentially expressed in myoblasts or during myogenesis, at both mRNA and protein levels (Additional file 1: Fig. S1a–c).

NGF did not affect significantly neither myoblast growth nor viability under standard culture condition (data not shown). Under serum starvation, the MTS-derived OD values of myoblasts treated with NGF 100 ng/ml were statistically higher when compared to untreated cells ($p < 0.05$) (Fig. 1a), although the Trypan blue assay (Fig. 1b) and the analysis of the apoptotic index (data not shown) excluded a significant NGF modulation of the total number of viable and nonviable cells. Since MTS quantification of viable cells depends upon the cellular metabolic rate [23], we verified the activity of some key enzymes. The results showed that, under serum deprivation, the activity of CS and GAPDH, both involved in carbohydrates metabolism, was increased by NGF supplementation compared to control (CS: 21.7 and 55.2% increase for NGF 10 and 100 ng/ml respectively; GAPDH: 37.7 and 95.7% increase for NGF 10 and 100 ng/ml respectively ($p < 0.05$) (Fig. 1c). No NGF-dependent differences were detected under standard conditions (data not shown). Previous data concerning the correlation between NGF and an increased activity in cycle Krebs enzymes also involved the activation of the JNK pathway [24]. Indeed, we demonstrated a significant enhancement of JNK phosphorylation after 15′–30′ min from NGF (20 ng/ml) supplementation in L6C5 myoblasts grown under serum starvation ($p < 0.05$) (Fig. 1d). At present, the biological significance of this result is unknown, but it agrees with published data in L6 myogenic cells showing that modification of mitochondrial homeostasis correlates with JNK phosphorylation and insulin resistance [25], opening to speculations on the role of NGF in the metabolic homeostasis of myogenic cells and possible ergogenic effect in muscle tissue [26, 27].

In 2011, Colombo et al. [7] proved that $p75^{NTR}$ modulates myogenesis and dystrophin expression, proposing this receptor as a novel marker of human differentiation-prone muscle precursor cells. In agreement with Deponti et al. [3], we confirmed that NGF at the concentration of 20 ng/ml, the most effective to promote L6 myoblast proliferation and differentiation [4], did not modulate the expression of myogenin, an early myogenic marker [28], both at protein (Fig. 2a) and transcriptional level (data not shown). However, later during differentiation (9 days in DM), NGF increased both the Embrional (HC Emb) and type IIB (HC IIB) MyHC isoform contents ($p < 0.05$) (Fig. 2b). Thus, we showed for the first time that NGF, similar to growth hormone [29, 30], exerted a positive effect on the terminal markers of myogenesis [31, 32]. We also found that, at the same stage, NGF induced a significant increase in the number of fibers ($p < 0.05$) (Fig. 2c), with an excess of fibers containing more than 20 nuclei ($p < 0.05$) (Fig. 2d, e). Thus, our in vitro model confirmed a hypertrophic effect of the NGF/$p75^{NTR}$ axis due to both increased fusion rate and increased number of fibers [6], but we also demonstrated an effect on the expression of MyHC isoforms, claiming for a putative role of NGF in the contractile properties of skeletal muscle fibers in vivo [7].

It has been recently reported that the chronic up-regulation of NFκB relates to impairment of the myogenic process in skeletal muscle or to muscle atrophy [10, 33]. Nonetheless, we and others demonstrated that the transient NFκB activation plays a role during L6C5 and C2C12 "in vitro" differentiation [17, 34], or during growth factors-promoted survival and/or differentiation of myogenic cells [9, 35]. Actually, L6C5 myogenic cultures supplemented with NGF showed a significant increase in NFκB activity compared to the control at 2 days in DM ($p < 0.05$), while no effects were detected in proliferating myoblasts (data not shown), sub-confluent myoblasts or at a later differentiation stage (Fig. 3a). This result demonstrated that, as in other cell types, also in myogenic cell line neurotrophins transiently regulate the activity of NFκB via $p75^{NTR}$ receptor [36, 37]. We then verified in L6C5 cellular model the possible correlation between NFκB activity, modulation of sHSPs and resistance to

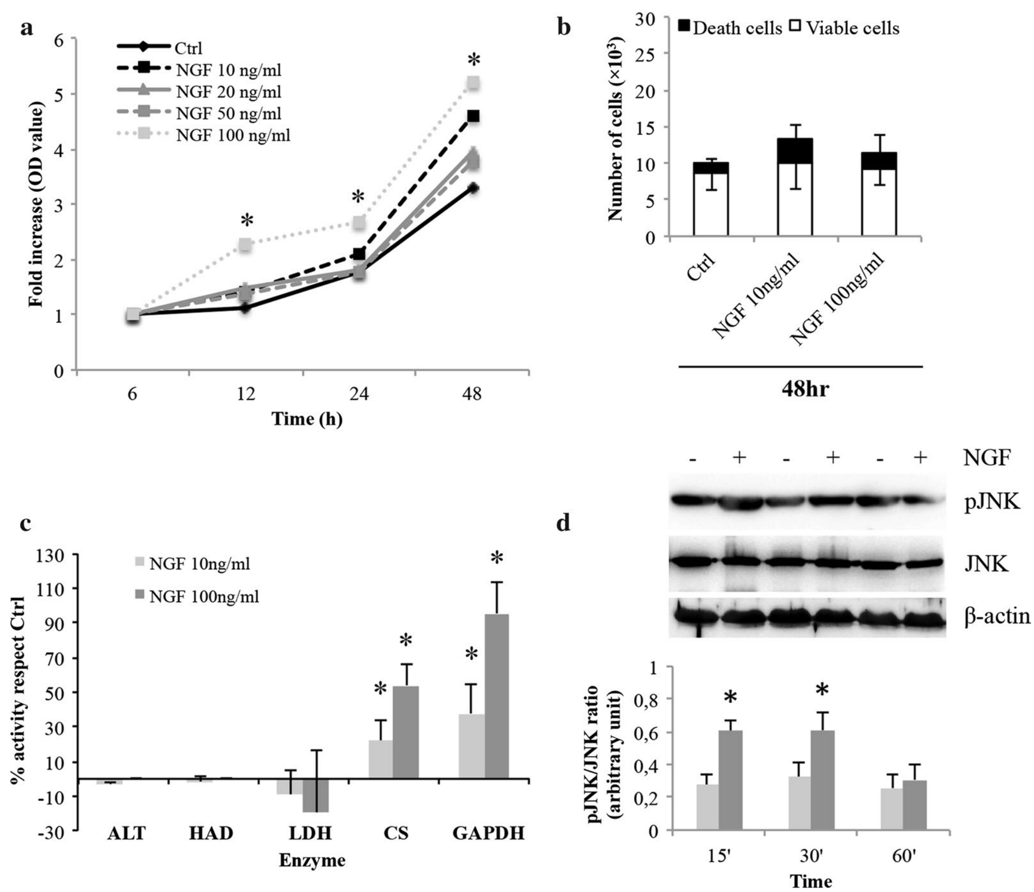


Fig. 1 Cell growth, viability, enzymatic activities and JNK phosphorylation in L6C5 myoblasts under serum starvation condition. **a** MTS viability assay. Cell growth values are expressed as fold increase in absorbance (OD) values after normalization according to values obtained at 6 h from the seeding, when cells usually become adherent to the plate and start growing (NGF 100 ng/ml: 2.2- to 5.21-fold increase vs Ctrl: 1.1- to 3.27-fold increase, $p < 0.05$). **b** Trypan blue exclusion assay. Analysis of viable, trypan blue negative (white) and nonviable, trypan blue positive (black) cells was performed after 48 h with or without NGF (10 and 100 ng/ml) and it is expressed as total number of counted cells (Viable cells: Ctrl, $8.8 \pm 2.5 \times 10^3$; NGF 10 ng/ml, $10.1 \pm 3.6 \times 10^3$; NGF 100 ng/ml, $9.2 \pm 2.2 \times 10^3$; $p > 0.05$; Nonviable cells: Ctrl: $1.3 \pm 0.6 \times 10^3$; NGF 10 ng/ml: $3.3 \pm 1.9 \times 10^3$; NGF 100 ng/ml: $2.1 \pm 2.6 \times 10^3$; $p > 0.05$). Three independent experiments were performed in triplicate. **c** ALT, HAD, LDH, CS and GAPDH enzyme activities performed in L6C5 myoblasts grown in serum-free medium and treated with NGF for 48 h. Data are shown as fold increase with respect to their control and expressed in percent values. One unit of enzymatic activity was defined as the amount of enzyme that forms 1 µmol of product per minute per mg of tested protein. The values have been collected from three independent experiments, each performed in triplicate. *$p < 0.05$. **d** Analysis of JNK activation on L6C5 myoblasts treated with or without NGF under serum starvation conditions. Immunoblot analysis of phosphorylated and total JNK in L6C5 cells at different times (15, 30 and 60 min) since NGF (20 ng/ml) supplementation. The histogram represents phospho JNK/total JNK ratio as mean ± SD of experiments repeated at least three times. *$p < 0.05$

apoptosis. Hsp27 and αB-Cry are key components of the myofibril structure, with a prominent role in skeletal muscle physio-pathology [38, 39] and in exercise-related adaptation to damaging contraction [13, 40]. We found that NGF induced a significant reduction of αB-Cry expression at 5 days in DM and an increase of Hsp27 levels after 2 days in DM ($p < 0.05$) (Fig. 3b–d). This result mirror our previous data on rat muscle regeneration in vivo [9] suggesting an anticipatory effect of NGF in the progression throughout the differentiation process,

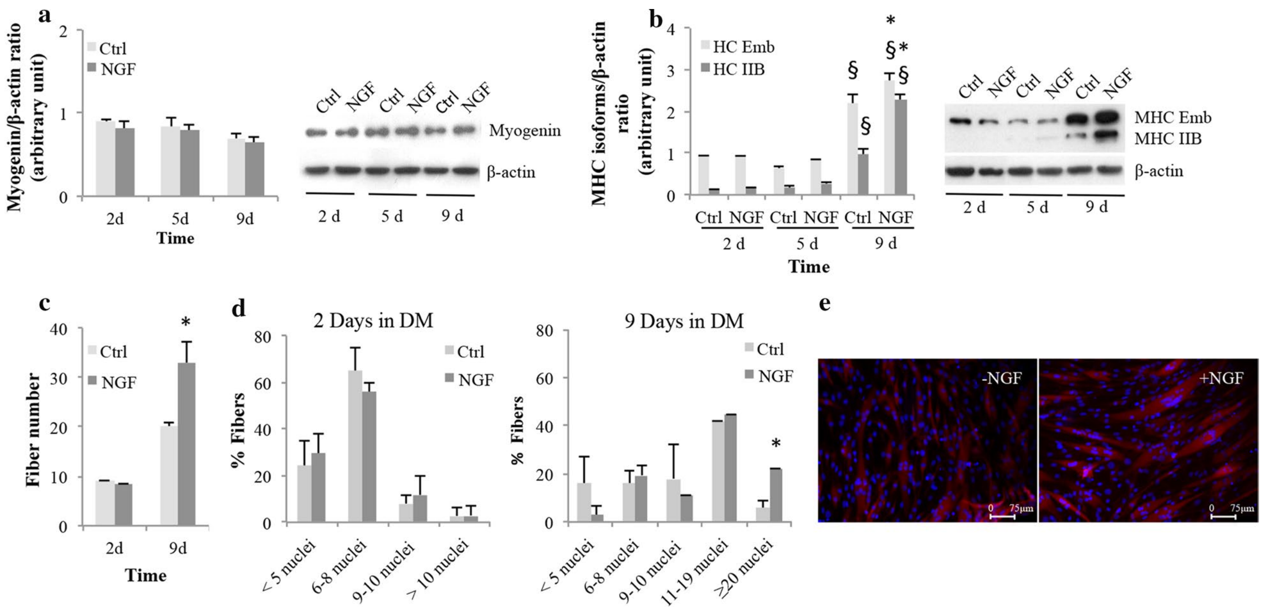
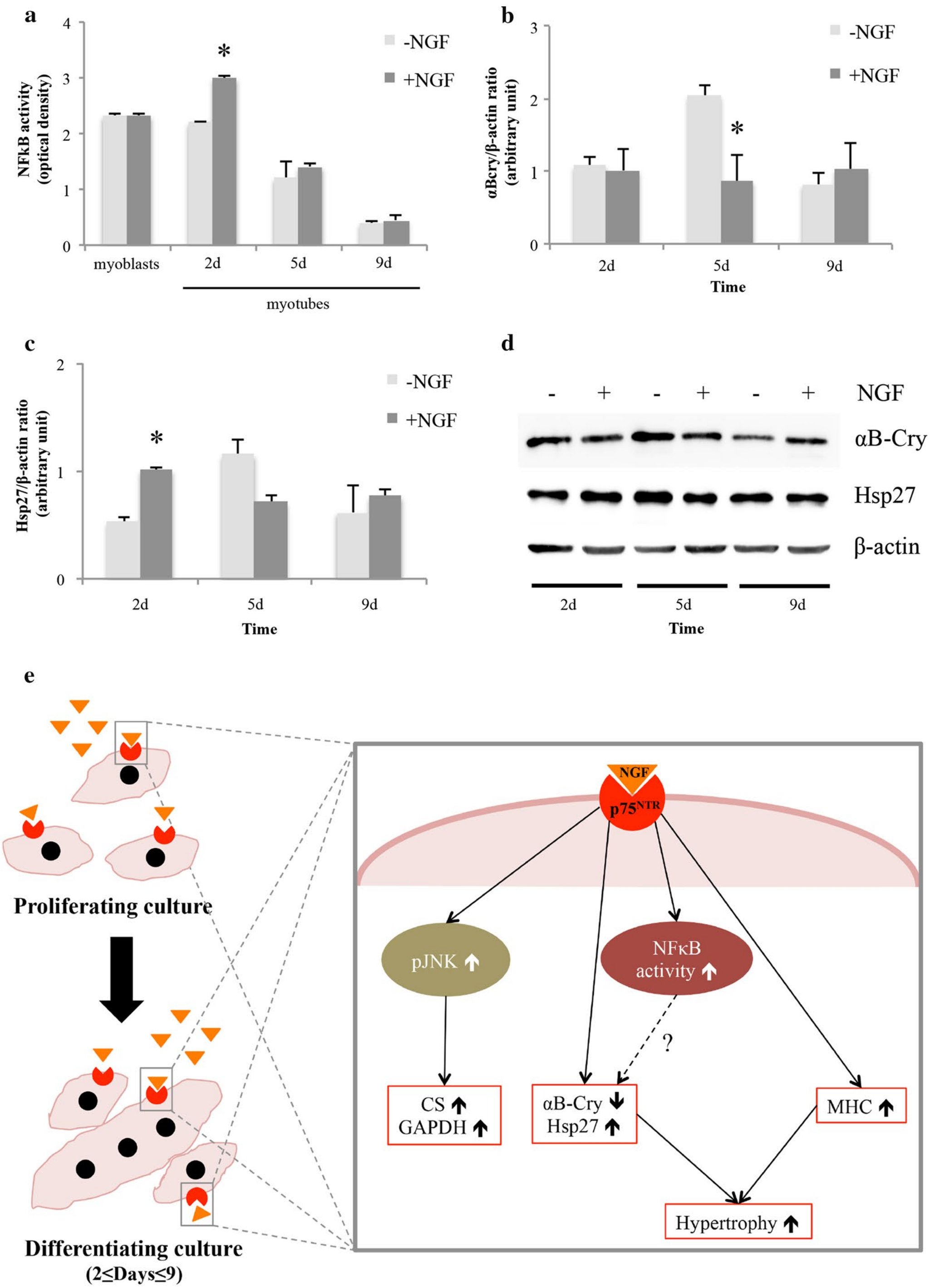


Fig. 2 Effects of NGF supplementation on L6C5 in vitro differentiation. **a** Myogenin and **b** MyHC isoforms expression at different times of differentiation (2, 5, and 9 days post DM) (9 days: NGF MHC Emb: 2.74 ± 0.17 vs Ctrl MHC Emb: 2.19 ± 0.23; NGF MHC IIB: 2.29 ± 0.11 vs Ctrl MHC IIB: 0.98 ± 0.11; $^{§}p < 0.05$ vs 2 and 5 days; $^{*}p < 0.05$ vs Ctrl 9d. The β-actin was used as housekeeping for both markers. **c** Analysis of fiber number during L6C5 differentiation in presence or not of NGF (NGF vs CTRL: 33 ± 4.2 vs 20 ± 1; $p < 0.05$). **d** Each histogram represents the fusion rate of L6C5 myoblasts after 2 and 9 days in DM (9 days: NGF vs CTRL: 22.2% vs 6%, $p < 0.05$). To facilitate cell analyses, the bottom of each well was divided into ten square fields and in each field the number of myotubes and nuclei/myotube were counted manually at a final magnification of ×40 (Olympus BX41 microscope, Olympus) using an ocular grid. **e** Representative images (×40) of L6C5 cells grown in DM medium for 9 days with and without NGF (20 ng/ml). Scale bar, 75 µm. The histograms represent the mean ± SD of experiments repeated at least three times. $^{*}p < 0.05$

consistent with the inhibitory and promoting effects exerted during myogenic differentiation by αB-Cry and Hsp27, respectively [14, 15, 38]. However, in contrast with previous results by our group [8, 11, 17] and by others [41, 42] on the relevance of the neurotrophin/p75^{NTR} axis in the promotion of myofibres survival, in the present study we did not find any protective effects of NGF towards spontaneous or H_2O_2-induced apoptosis, neither in proliferating nor in differentiating L6C5 cells. Indeed, as already demonstrated in a fibroblast derived cell line constitutively expressing rat p75 [43], in our "in vitro" model NGF did not modulate neither the total number of TUNEL-positive apoptotic cells, nor the expression and/or cleavage of Bcl-2 and Caspase-3 in differentiating cells (Additional file 2: Fig. S2) and in myoblasts (data not shown).

As summarized in Fig. 3e, our results demonstrate that p75^{NTR} is sufficient to mediate the NGF modulation of L6C5 cells differentiation inducing structural, metabolic and functional changes as well as NGF direct, or indirect effect on αB-Cry and Hsp27, essential for the myogenic program and myofibrillar stabilization. Moreover, we show that the NGF-mediated hypertrophic at the late stage of differentiation correlates to an increased expression of MyHC isoforms.


a
NFkB activity (optical density)
-NGF
+NGF
*
myoblasts
2d
5d
9d
myotubes
b
αBcry/β-actin ratio (arbitrary unit)
-NGF
+NGF
*
2d
5d
9d
Time
c
Hsp27/β-actin ratio (arbitrary unit)
-NGF
+NGF
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Hsp27
MHC
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Fig. 3 Effects of NGF supplementation on NFκB, αB-crystallin and Hsp27 during L6C5 in vitro differentiation. **a** NFκB activity measurement in myoblasts and differentiating L6C5 cells (2, 5 and 9 days) grown with or without NGF supplementation. Nuclear protein extract from Jurkat cells stimulated by TPA and calcium ionophore was used as positive control (2 days: − NGF vs + NGF, 2.2 vs 3.0, $p < 0.05$). **b, c** Quantitative analysis of αB-Cry and Hsp27 expression during differentiation process (2, 5 and 9 days) of myogenic cells supplemented with or without NGF (αB-Cry: NGF 1.3 ± 0.08 vs Ctrl 2.0 ± 0.11, $p < 0.05$; Hsp27: NGF 1.0 ± 0.03 vs Ctrl 0.53 ± 0.04, $p < 0.05$). The histograms represent the mean ± SD of experiments repeated at least three times. *$p < 0.05$. **d** Representative western blot of αB-crystallin and Hsp27 expression. The β-actin was used as housekeeping for both markers. **e** Proposed mechanism for NGF-$p75^{NTR}$ signaling pathways in L6C5 myogenic cells: during myoblast proliferation and serum-deprivation condition, the supplementation with NGF can sustain the activity of key enzymes in carbohydrate metabolism, such as citrate synthase and glyceraldehyde-phosphate-dehydrogenase, through the activation of the JNK pathway. During the early stage of myoblast fusion, NGF transiently up-regulates NFκB activity and, directly or indirectly trough a NFκB-mediated mechanism, anticipates the myogenic progression by modulating αBcry and Hsp27 expression and promoting, at a late differentiation stage, myonuclear fusion and the accumulation and stabilization of the MyHC myofibrillar component. *CS* citrate synthase, *GAPDH* Glyceraldehyde-phosphate-dehydrogenase, *NGF* nerve growth factor, *αBcry* αB-crystallin, *JNK* c-Jun N-terminal kinases, *NFκB* nuclear factor kappa-light-chain-enhancer of activated B cells, *$p75^{NTR}$* neurotrophin receptor p75

Limitations

Although the results on the NGF-mediated effects on the enzymatic activities, NFκB activation and sHSPs expression are solid, the study did not reveal the causal relationship among these factors.

Additional files

Additional file 1: Figure S1. TrkA and $p75^{NTR}$ expression in L6C5 myoblasts and myotubes. **a**, **b** Relative mRNA levels of TrkA and $p75^{NTR}$ in L6C5 cells at different times since the seeding (proliferating = 24, 48 h; 2, 5, 9, and 12 days of differentiation). Proliferating (Pr) and differentiated (Dif) PC12 cells were used as positive control for the expression of TrkA and $p75^{NTR}$ receptors. **c** Western blot analysis of TrkA and $p75^{NTR}$ in proliferating and differentiated L6C5 cells. RNA extraction and quantitative RT-PCR was performed as already described [44]. Primers for PCR amplification were as follows: housekeeping gene glyceraldehyde-3-phosphatedehydrogenase (GAPDH): 5′-ACCACAGTCCATGCCATCAC-3′ and 5′-TCCACCACCCTGTTGCTGTA-3′; Neurotrophic tyrosine kinase receptor type 1 (TrkA): 5′-CCTGATGCCTTCCATTTCAC -3′ and 5′-TGACATTGACCAGAGTTAGCC-3′; Nerve growth factor receptor ($p75^{NTR}$): 5′-CAAGGAGACATGTTCCACAG-3′ and 5′GGATCTCTTCGCATTCAGCA-3′. *L6C5-P* proliferating myoblasts in GM, *L6C5-D* differentiating cultures in DM.

Additional file 2: Figure S2. Effect of NGF supplementation on spontaneous or H_2O_2-induced apoptosis during L6C5 in vitro differentiation. **a** TUNEL assay (Roche applied sciences) and **b** Bcl-2 and Caspase-3 protein expression in L6C5 cells growing in DM NGF-supplemented under standard and oxidative stress condition (100 μM H_2O_2). For the analysis of H_2O_2-induced apoptosis, cells under differentiation (48 h before, or 2, 5 or 9 days from DM addiction) in presence or in absence of NGF (20 ng/ml) were treated with H_2O_2 100 μM for the last 1-h of culture. The histogram represents the mean ± SD of experiments repeated at least three times. *$p < 0.05$ compared with control (Ctrl). $^{\S}p < 0.05$ compared with control NGF-supplemented (Ctrl + NGF).

Abbreviations

ALT: alanine transglutaminase; αB-Cry: αB-crystallin; CS: citrate synthase; DMEM: Dulbecco modified eagle medium; DM: differentiation medium; GAPDH: glyceraldehyde-phosphate-dehydrogenase; GH: growth hormone; GM: growing medium; HAD: 3-OH acylCoA dehydrogenase; JNK: c-Jun N-terminal kinase; LDH: lactate dehydrogenase; MTS: 3-(4,5-dimethylthiazol-1)-5-(3-carboxymeth-oxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium), inner salt; MyHC: myosin heavy chain; NFκB: nuclear factor κB; NGF: nerve growth factor; $p75^{NTR}$: p75 neurotrophin receptor; sHSPs: small heat shock proteins; TrkA: tyrosin kinase receptor A; TPA: 12-otetradecanoylphorbol-13-acetate.

Authors' contributions

ADP, ID, GD, CF, NM and DC have conceived the work, designed methodology, interpreted data and written the manuscript; RC, LDL and SS participated in data interpretation and manuscript writing. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

Some of the data has been included as additional supplementary material. We will however readily share our datasets and spreadsheets per individual request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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References

1. Almada AE, Wagers AJ. Molecular circuitry of stem cell fate in skeletal muscle regeneration, ageing, and disease. Nat Rev Mol Cell Biol. 2016;17:267–79.
2. Chevrel G, Hohlfeld R, Sendtner M. The role of neurotrophins in muscle under physiological and pathological conditions. Muscle Nerve. 2006;33:462–76.

3. Deponti D, Buono R, Catanzaro G, De Palma C, Longhi R, Meneveri R, Bresolin N, Bassi MT, Cossu G, Clementi E, Brunelli S. The low-affinity receptor for neurotrophins p75NTR plays a key role for satellite cell function in muscle repair acting via RhoA. Mol Biol Cell. 2009;20:3620–7.
4. Rende M, Brizi E, Conner J, Treves S, Censier K, Provenzano C, Taglialatela G, Sanna PP, Donato R. Nerve growth factor (NGF) influences differentiation and proliferation of myogenic cells in vitro via TrKA. Int J Dev Neurosci. 2000;18:869–85.
5. Ettinger K, Lecht S, Arien-Zakay H, Cohen G, Aga-Mizrachi S, Yanay N, Saragovi HU, Nedev H, Marcinkiewicz C, Nevo Y, Lazarovici P. Nerve growth factor stimulation of ERK1/2 phosphorylation requires both p75NTR and α9β1 integrin and confers myoprotection towards ischemia in C2C12 skeletal muscle cell model. Cell Signal. 2012;24:2378–88.
6. Reddypalli S, Roll K, Lee HK, Lundell M, Barea-Rodriguez E, Wheeler EF. p75NTR-mediated signaling promotes the survival of myoblasts and influences muscle strength. J Cell Physiol. 2005;204:819–29.
7. Colombo E, Romaggi S, Medico E, Menon R, Mora M, Falcone C, et al. Human neurotrophin receptor p75NTR defines differentiation-oriented skeletal muscle precursor cells: implications for muscle regeneration. J Neuropathol Exp Neurol. 2011;70:133–42.
8. Mercatelli N, Dimauro I, Ciafré SA, Farace MG, Caporossi D. AlphaB-crystallin is involved in oxidative stress protection determined by VEGF in skeletal myoblasts. Free Radic Biol Med. 2010;49:374–82.
9. Dimauro I, Grasso L, Fittipaldi S, Fantini C, Mercatelli N, Racca S, Geuna S, Di Gianfrancesco A, Caporossi D, Pigozzi F, Borrione P. Platelet-rich plasma and skeletal muscle healing: a molecular analysis of the early phases of the regeneration process in an experimental animal model. PLoS ONE. 2014;9:e102993.
10. Peterson JM, Bakkar N, Guttridge DC. NF-κB signaling in skeletal muscle health and disease. Curr Top Dev Biol. 2011;96:85–119.
11. Fittipaldi S, Mercatelli N, Dimauro I, Jackson MJ, Paronetto MP, Caporossi D. Alpha B-crystallin induction in skeletal muscle cells under redox imbalance is mediated by a JNK-dependent regulatory mechanism. Free Radic Biol Med. 2015;86:331–42.
12. Lavoie JN, Lambert H, Hickey E, Weber LA, Landry J. Modulation of cellular thermoresistance and actin filament stability accompanies phosphorylation-induced changes in the oligomeric structure of heat shock protein 27. Mol Cell Biol. 1995;15:505–16.
13. Dimauro I, Mercatelli N, Caporossi D. Exercise-induced ROS in heat shock proteins response. Free Radic Biol Med. 2016;98:46–55.
14. Kamradt MC, Chen F, Sam S, Cryns VL. The small heat shock protein alpha B-crystallin negatively regulates apoptosis during myogenic differentiation by inhibiting caspase-3 activation. J Biol Chem. 2002;277:38731–6.
15. Brown DD, Christine KS, Showell C, Conlon FL. Small heat shock protein Hsp27 is required for proper heart tube formation. Genesis. 2007;45:667–78.
16. Caporossi D, Ciafrè SA, Pittaluga M, Savini I, Farace MG. Cellular responses to H(2)O(2) and bleomycin-induced oxidative stress in L6C5 rat myoblasts. Free Radic Biol Med. 2003;35:1355–64.
17. Catani MV, Savini I, Duranti G, Caporossi D, Ceci R, Sabatini S, Avigliano L. Nuclear factor κB and activating protein 1 are involved in differentiation-related resistance to oxidative stress in skeletal muscle cells. Free Radic Biol Med. 2004;37:1024–36.
18. Dimauro I, Pearson T, Caporossi D, Jackson MJ. In vitro susceptibility of thioredoxins and glutathione to redox modification and aging-related changes in skeletal muscle. Free Radic Biol Med. 2012;53:2017–27.
19. Sabatini S, Sgrò P, Duranti G, Ceci R, Di Luigi L. Tadalafil alters energy metabolism in C2C12 skeletal muscle cells. Acta Biochim Pol. 2011;58:237–41.
20. Duranti G, La Rosa P, Dimauro I, Wannenes F, Bonini S, Sabatini S, Parisi P, Caporossi D. Effects of salmeterol on skeletal muscle cells: metabolic and proapoptotic features. Med Sci Sports Exerc. 2011;43:2259–73.
21. Dimauro I, Pearson T, Caporossi D, Jackson MJ. A simple protocol for the subcellular fractionation of skeletal muscle cells and tissue. BMC Res Notes. 2012;5:513.
22. Ten Broek RW, Grefte S, Von den Hoff JW. Regulatory factors and cell populations involved in skeletal muscle regeneration. J Cell Physiol. 2010;224:7–16.
23. Goodwin CJ, Holt SJ, Downes S, Marshall NJ. Microculture tetrazolium assays: a comparison between two new tetrazolium salts, XTT and MTS. J Immunol Methods. 1995;179:95–103.
24. Davis LH, Kauffman FC. Metabolism via the pentose phosphate pathway in rat pheochromocytoma PC12 cells: effects of nerve growth factor and 6-aminonicotinamide. Neurochem Res. 1987;12:521–7.
25. Nie Q, Wang C, Song G, Ma H, Kong D, Zhang X, Gan K, Tang Y. Mitofusin 2 deficiency leads to oxidative stress that contributes to insulin resistance in rat skeletal muscle cells. Mol Biol Rep. 2014;41:6975–83.
26. Di Luigi L. Supplements and endocrine system in athletes. Clin Sports Med. 2008;27:131–51.
27. Seidl K, Erck C, Buchberger A. Evidence for participation of nerve growth factor and its low-affinity receptor (p75NTR) in the regulation of the myogenic program. J Cell Physiol. 1998;176:10–21.
28. Corbi N, Di Padova M, De Angelis R, Bruno T, Libri V, Iezzi S, Floridi A, Fanciulli M, Passananti C, et al. The alpha-like RNA polymerase II core subunit 3 (RPB3) is involved in tissue-specific transcription and muscle differentiation via interaction with the myogenic factor myogenin. FASEB J. 2002;16:1639–41.
29. Lange KH, Andersen JL, Beyer N, Isaksson F, Larsson B, Rasmussen MH, Juul A, Bülow J, Kjaer M. GH administration changes myosin heavy chain isoforms in skeletal muscle but does not augment muscle strength or hypertrophy, either alone or combined with resistance exercise training in healthy elderly men. J Clin Endocrinol Metab. 2002;87:513–23.
30. Bigard X, Sanchez H, Zoll J, Mateo P, Rousseau V, Veksler V, Ventura-Clapier R. Calcineurin Co-regulates contractile and metabolic components of slow muscle phenotype. J Biol Chem. 2000;275:19653–60.
31. Wells L, Edwards KA, Bernstein SI. Myosin heavy chain isoforms regulate muscle function but not myofibril assembly. EMBO J. 1996;15:4454–9.
32. Schiaffino S, Reggiani C. Molecular diversity of myofibrillar proteins: gene regulation and functional significance. Physiol Rev. 1996;76:371–423.
33. Shin J, Tajrishi MM, Ogura Y, Kumar A. Wasting mechanisms in muscular dystrophy. Physiol Rev. 2010;90:495–511.
34. Canicio J, Ruiz-Lozano P, Carrasco M, Palacin M, Chien K, Zorzano A, Kaliman P. Nuclear factor κB-inducing kinase and IκB kinase-alpha signal skeletal muscle cell differentiation. J Biol Chem. 2001;276:20228–33.
35. Conejo R, Valverde AM, Benito M, Lorenzo M. Insulin produces myogenesis in C2C12 myoblasts by induction of NF-κB and downregulation of AP-1 activities. J Cell Physiol. 2001;186:82–94.
36. Chao MV. Neurotrophins and their receptors: a convergence point for many signalling pathways. Nat Rev Neurosci. 2003;4:299–309.
37. Ahmad I, Yue WY, Fernando A, Clark JJ, Woodson EA, Hansen MR. p75NTR is highly expressed in vestibular schwannomas and promotes cell survival by activating nuclear transcription factor κB. Glia. 2014;62:1699–712.
38. Ikeda R, Yoshida K, Ushiyama M, Yamaguchi T, Iwashita K, Futagawa T, Shibayama Y, Oiso S, Takeda Y, Kariyazono H, Furukawa T, Nakamura K, Akiyama S, Inoue I, Yamada K. The small heat shock protein αB-crystallin inhibits differentiation-induced caspase 3 activation and myogenic differentiation. Biol Pharm Bull. 2006;29:1815–9.
39. Beltran Valls MR, Wilkinson DJ, Narici MV, Smith K, Phillips BE, Caporossi D, Atherton PJ. Protein carbonylation and heat shock proteins in human skeletal muscle: relationships to age and sarcopenia. J Gerontol A Biol Sci Med Sci. 2015;70:174–81.
40. Beltran Valls MR, Dimauro I, Brunelli A, Tranchita E, Ciminelli E, Caserotti P, Duranti G, Sabatini S, Parisi P, Parisi A, Caporossi D. Explosive type of moderate-resistance training induces functional, cardiovascular, and molecular adaptations in the elderly. Age (Dordr). 2014;36:759–72.
41. Colombo E, Romaggi S, Blasevich F, Mora M, Falcone C, Lochmüller H, Morandi L, Farina C. The neurotrophin receptor p75NTR is induced on mature myofibres in inflammatory myopathies and promotes myotube survival to inflammatory stress. Neuropathol Appl Neurobiol. 2012;38:367–78.
42. Colombo E, Bedogni F, Lorenzetti I, Landsberger N, Previtali SC, Farina C. Autocrine and immune cell-derived BDNF in human skeletal muscle: implications for myogenesis and tissue regeneration. J Pathol. 2013;231:190–8.
43. Cosgaya JM, Shooter EM. Binding of nerve growth factor to its p75 receptor in stressed cells induces selective IκB-beta degradation and NF-κB nuclear translocation. J Neurochem. 2001;79:391–9.
44. Ceci R, Duranti G, Rossi A, Savini I, Sabatini S. Skeletal muscle differentiation: role of dehydroepiandrosterone sulfate. Horm Metab Res. 2011;43:702–7.