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Original Article

Yin Yang 1 Expression and Localisation in Quadriceps Muscle in COPD☆

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ABSTRACT

Introduction: Yin Yang 1 (YY1) is a transcriptional repressor that inhibits muscle gene expression and myogenesis. YY1 has not previously been investigated in the skeletal muscle of patients with COPD. The aims of this study were to investigate YY1 expression and localisation in the quadriceps muscle of COPD patients compared to healthy age-matched controls, and to examine the relationship between YY1 expression and localisation and quadriceps muscle fibre cross-sectional area (CSA) in COPD patients.
Patients and methods: 15 COPD patients and 8 age-matched controls underwent lung and quadriceps function assessments and a percutaneous quadriceps biopsy. Quadriceps muscle fibre CSA and fibre proportions and YY1 localisation were determined by immunofluorescence. YY1 was immunoprecipitated from muscle and YY1 levels assessed by western blotting.
Results: YY1 levels were inversely correlated with type IIx and type I fibre CSA in patients and controls, though YY1 levels were not significantly different between the groups. Nuclear localisation of YY1 was demonstrated in the patients but not in controls.
Conclusion: YY1 expression is associated with smaller quadriceps fibre CSA in COPD and nuclear localisation of YY1 was found in muscle of patients but not controls. Regulation of YY1 appears altered in COPD and may be implicated in COPD-related muscle atrophy.

Expresión y localización del factor de transcripción Yin Yang 1 en el músculo cuádriceps en la enfermedad pulmonar obstructiva crónica

Palabras clave:
Atrofia
Regeneración muscular
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RESUMEN

Introducción: El Ying Yang 1 (YY1) es un factor de transcripción represor que inhibe la expresión génica muscular y la miogénesis. Este factor no se ha investigado previamente este factor no se ha investigado en el músculo esquelético de pacientes con enfermedad pulmonar obstructiva crónica (EPOC). Los objetivos del presente estudio fueron investigar la expresión de YY1 y su localización en el músculo cuádriceps de pacientes con EPOC, comparado con individuos control sanos, emparejados por edad, y examinar la relación entre la expresión y localización de YY1 en las áreas transversales (AT) de las fibras musculares del cuádriceps en pacientes con EPOC.
Pacientes y métodos: Se sometió a 15 pacientes con EPOC y a 8 individuos de control, emparejados por edad, a valoraciones de la función pulmonar y del cuádriceps y a una biopsia percutánea de este músculo. Mediante inmunofluorescencia se determinó el AT de las fibras musculares del cuádriceps las proporciones de fibras y localización de YY1. YY1 se inmunoprecipitó a partir del músculo y sus niveles se evaluaron mediante inmunotransferencia.

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Resultados: Los niveles de YY1 se correlacionaron inversamente con el AT de las fibras de tipo IIx y de tipo I en pacientes e individuos de control, aunque los niveles de YY1 no fueron significativamente diferentes entre ambos grupos. En los pacientes, pero no en los individuos control, se demostró la localización nuclear de YY1.

Conclusión: La expresión de YY1 se asocia a un AT más pequeña de las fibras del cuádriceps en pacientes con EPOC, en cuyo músculo también se observa una localización nuclear del factor, a diferencia de los individuos control. La regulación de YY1 parece alterada en la EPOC y podría estar implicada en la atrofia muscular relacionada con la enfermedad.

Introduction

The atrophy and weakness of the peripheral muscles are negative prognostic factors in chronic obstructive pulmonary disease (COPD).[1,2] In order to maintain muscle mass, regeneration of the skeletal muscles is necesary.[3] However, based on animal models in COPD, there is evidence that skeletal muscle regeneration may deteriorate as a consequence of systemic inflammation, which contributes to the muscle atrophy.[4] We still do not fully understand the molecular mechanisms that may translate into a deterioration of the myogenesis in COPD.

Ying Yang 1 (YY1) is a transcription factor that represses myogenesis that has not previously been researched either in the muscles of COPD patients or, to our knowledge, in the skeletal muscles of healthy adults. Nevertheless, it is known that its expression increases in the lung tissue of COPD patients when compared with control subjects.[5] YY1 suppresses the muscle differentiation and gene transcription of the skeletal muscles, such as skeletal alpha actin[6] and muscle creatine kinase,[7] binding with the pertinent promoters and blocking the binding of a transcription activator, serum response factor.[8] For instance, the activation of the nuclear factor kappa B (NF-κB) pathway by tumour necrosis factor-alpha (TNF-α) can inhibit muscle regeneration[3] through an increase in YY1 expression.[9] Furthermore, the location of YY1 affects its activity, as does its expression. When YY1 is limited to the cytoplasm of the muscle cells, it is inactive, which allows for the differentiation and synthesis of the contractile proteins. YY1 is activated by the transport to the nucleus, for example, as a response to the presence of depolymerised actin.[8]

There are at least two mechanisms due to which the muscle YYI activity could increase in COPD. First of all, the patients with COPD and muscle wasting may present an increase in the DNA-NF-κB binding in the muscle[10] and, therefore, a greater expression of YY1.[9] The activation of NF-κB in the quadriceps of COPD patients could be a consequence of the stimulation secondary to the rise in TNF-α in blood[11] or in the muscle. However, in patients compared with control subjects, a decrease in TNF-α values in the quadriceps muscle has been reported[12] in comparison with the findings in the intercostal muscles.[13] Given the fact that TNF-α can also stimulate the activation of satellite cells through the activation of the serum response factor,[14] its reduction in the muscles could inhibit muscle regeneration regardless of YY1. In the second place, in the muscle of COPD patients, the activity of YY1 could increase due to the increase in the nuclear transport of YY1 in the presence of a rise in depolymerised actin, a consequence of the accelerated degradation of proteins through the ubiquitin-proteasome pathway.[8,15] Therefore, the hypothesis of this present study was that the deregulation of YY1 signalling is involved in the atrophy of the quadriceps of patients with COPD. We have investigated the expression and location of YY1 in the quadriceps muscle of a small group of patients with COPD and control individuals, paired for age, and we have examined the relationship between the expression and location of YY1 and the cross-sectional area (CSA) of the quadriceps fibres.

Individuals and Methods

Individuals

From the respiratory department, 15 COPD patients were included for study (Global Initiative for Chronic Obstructive Lung Disease [GOLD] stages II [*n*=4], III [*n*=4] and IV [*n*=7]). The exclusion criteria were heart, kidney or liver failure or a systemic inflammatory, metabolic or neuromuscular disease or a moderate-severe exacerbation (meaning, with the need for antibiotics, oral steroids or hospitalisation) in the previous four weeks. Using an advertisement, 8 healthy control individuals were recruited. All individuals gave their written informed consent and the study was approved by the research committee of the Royal Brompton, Harefield NHS Trust and Ealing and West London Mental Health Trust.

Physiological Determinations and Biopsy of the Quadriceps

In accordance with the guidelines of the American Thoracic/European Respiratory Society, we determined postbronchodilator spirometry,[16] lung volumes with plethysmography[17] and carbon monoxide diffusing capacity,[18] while arterial blood gas was examined from an arterialised blood sample obtained from an earlobe. Lean body mass was determined with bioelectric impedance (Bodystat® 1500, Bodystat, United Kingdom),[19] which was corrected for stature to derive the lean body mass index. Physical activity was determined by a triaxial Dynaport® ADL3 accelerometer (McRoberts BV, Netherlands) that the patients used for two days, 12 h each day, during normal activity. The mean locomotion time was calculated as previously described.[20] The strength of the quadriceps (right leg) was evaluated with the maximum voluntary isometric contraction, in supine decubitus, based on the method by Edwards.[21] The percutaneous needle biopsy in the right *vastus lateralis* was done with local anaesthesia using the Bergstrom technique.[22] The samples for the analysis of ribonucleic acid and proteins were immediately frozen in liquid nitrogen, while the histology samples were introduced in pre-cooled isopentane for 15 s before being frozen in liquid nitrogen then stored at −80 °C.

Analysis of the Muscle Biopsy

There was not enough tissue from each and every individual to complete all the analyses, therefore for each analysis a subgroup of samples was used.

Immunofluorescent Detection of Yin Yang 1

The frozen 10 μm slices from 10 patients and 8 control individuals were set in a solution of 10% formaldehyde, washed in Triton X-100 at 0.1% in a neutralised saline solution with phosphate buffered saline (PBS) and were blocked with 5% bovine serum albumin (BSA) in PBS, before incubation with rabbit anti-YY1 antibody (dilution 1:400, sc-281; Santa Cruz Biotechnology, United States) and murine anti-heavy chain myosin fast contraction antibody (MYSN02, dilution 1:200, Abcam, United Kingdom) in 3% BSA