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Pleiotropy of tumor necrosis factor-α in C2C12 myotubes: *in vitro* studies on genes, networks and pathways involved in TNF-α induced skeletal muscle atrophy

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Background

Skeletal muscle atrophy is one of the most common phenomenon occurring in a variety of conditions that range from muscular inactivity to disease states such as diabetes, and cancer. Activation of inflammatory pathways by various cytokines is one of the critical events leading to skeletal muscle atrophy. Though there is significant literature on the role that the cytokine, tumor necrosis factor-α (TNF-α) plays in the induction of nuclear factor-kappa B (NF-κB) and ubiquitin-proteasome pathways, the molecular mechanisms involved in TNF-α induced skeletal muscle wasting remain poorly understood. Understanding how TNF-α regulates the expression/activation of various gene networks and pathways is essential not only to understand the disease prognosis but also to obtain a better molecular insight in skeletal muscle development and wasting.

Results

Oligo-nucleotide microarray analyses of C2C12 myotubes after 18h of treatment with TNF-α (10 ng/ml) showed differential expression of 27,663 genes out of 38,467 spotted on the chip (MEEBO, Invitrogen). Out of these, 1821 genes were differentially expressed with p-values of ≤0.05 and ≥1.2 fold change. Further classifying of these genes based on their p-values, we found about 50 genes with p-values < 0.0001, 179 genes with p-values <0.001, 750 genes with <0.01 p-values and 842 genes with p-values <0.05. The Ingenuity Pathway Analysis showed that TNF-α affects the activation of both classical and alternative NF-κB signaling pathways, 26s proteasome pathway, and chemokine network in C2C12 myotubes. Notch1 signaling and genes involved in oxidative phosphorylation were found to be significantly down regulated. The quantitative real-time-PCR analysis of NF-κB1, NF-κB2, IκBα, IL-6, VCAM-1, CCL5, CxCL5, CCL2, MyoD, muscle creatine kinase, Notch1, and TIMP-2 showed good correlation with the microarray data. Expressions of some of these genes studied were also reflected at their protein levels.

Conclusions

The present study showed that TNF-α induces both classical and alternative NF-κB pathway in C2C12 myotubes. TNF-α also upregulates the expression of genes involved in 26s proteasome pathway, which is one of the major pathway for protein degradation in skeletal muscle atrophy. This observation was further supported by the down-regulation of Notch1, which is a target for one of the ubiquitin ligase Nedd4. Our data also showed a significant induction of other markers of inflammation such as chemokines and their ligands.

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