The proliferation of Duchenne muscular dystrophy fibroblasts cultured under inflammatory conditions is reduced by methylprednisolone through modulation of NFAT5 localization in the cell

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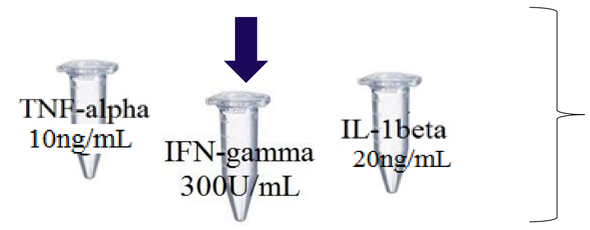
Introduction

Duchenne muscular dystrophy (DMD) is characterized by chronic inflammation and impaired muscle regeneration (Abdel-Salam et al., 2009). The major debilitating factor in DMD is the formation of fibrotic scar tissue (Banker and Engel, 2004). This scar tissue is an excess in extracellular matrix formation (ECM), especially collagen. Two major components induce fibrosis: chronic inflammation and chronic proliferation of fibroblast-like cells (Mann et al., 2001).

The major cell type involved in fibrosis is the fibroblast, producer of collagen. Fibroblasts are sensitive to several cytokines. CD4+ Th1 cells and CD8+ T cells produce IFN-γ, that exerts a pro-fibrotic role by mediating increased TNF-α production in macrophages. Amongst other cytokines, fibroblasts react to IL-1β and produce it in turn (autocrine function), inducing proliferation and ECM production (Kendall and Feghali-Bostwick, 2014).

Material and methods

DMD fibroblasts were cultured *in vivo*



Addition of cytokines for 24 h or 15 d

A part of the cultures received a clinical dose of methylprednisolone every other 3 days during 15 d: 0,75 mg/kg

Immunofluorescence microscopy (IF):
- NFAT5 goat polyclonal (1/100, Santa Cruz, USA).

Western blotting (WB):
- NFAT5 mouse (1/100) (Santa Cruz Biotechnology, Texas, USA).
- Tubuline (1/1000) (Sigma Aldrich, USA).

RT-qPCR: following the protocol of Vandesompele *et al.*, 2002)

IncuCyte ZOOM: (Essen Bioscience, Hertfordshire,UK). The IncuCyte Software acquired and analyzed data at fixed points in each well. (4 images /well every 2 h for 15 d).

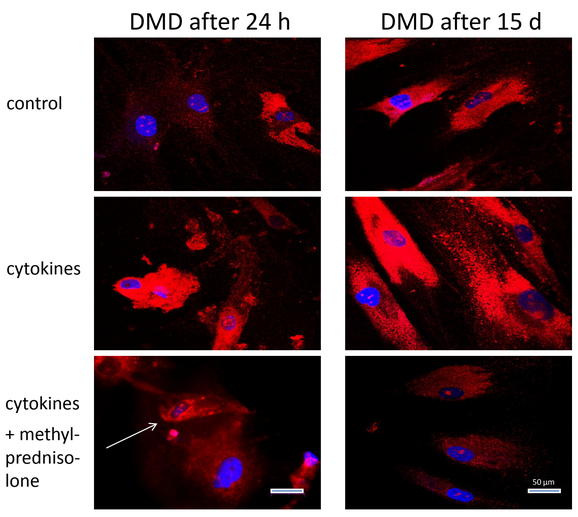
Statistics: SPSS 25.0 (IBM, Armonk, New York, USA).

Take home message

Proliferation of Duchenne muscular dystrophy fibroblasts is reduced by methyl-prednisolone through the modulation of NFAT5 localization in the cell (by trapping in the cytoplasm).

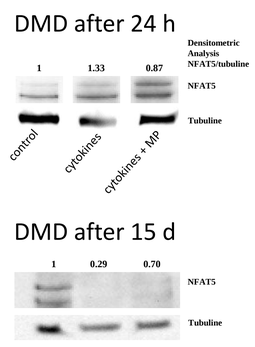
Results

Immunofluorescence microscopy (IF):



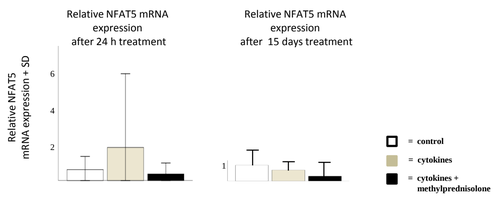
IF: NFAT5 (red) + DAPI in nuclei (blue) merged reveal NFAT5 being localized in the peri-nuclear area after 24 h (white arrows) and shows decreased NFAT5 staining per cell after 15 d in DMD fibroblasts exposed to cytokines + methyl-prednisolone (n=3 passages) (p<0.003).

Western blotting:



WB:Total NFAT5 protein amounts decreased after 15 d exposure to cytokines + methylprednisolone (MP) (n=3 passages).

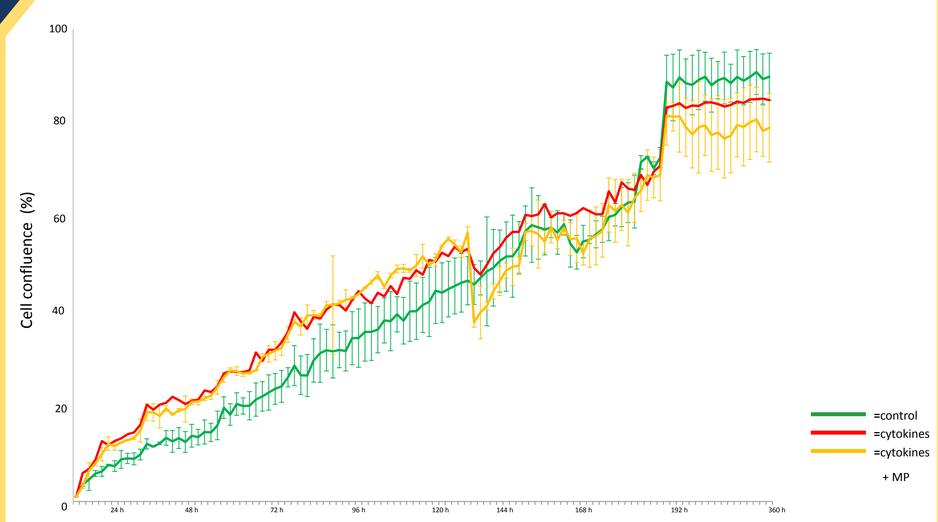
RT-qPCR:

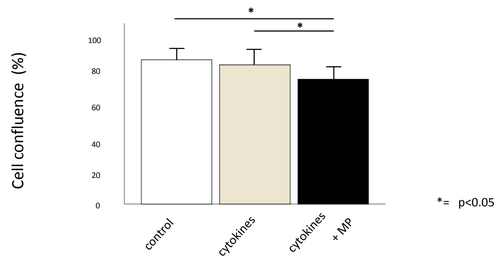


RT-qPCR: Relative mRNA NFAT5 expression is not significantly decreased after 15 d exposure to cytokines + methylprednisolone (n=1 passage).

Results and conclusion

Incucyte:





*= p<0.05

Incucyte: After 15 d, DMD fibroblasts exposed to cytokines + methyl-prednisolone (MP) show less confluence (p <0.05) (n=3 passages).

DMD fibroblasts show reduced NFAT5 staining, decreased NFAT5 protein expression and reduced proliferation after 15 d exposure to cytokines + methylprednisolone (n= 3 passages).

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References
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