ANGIOTENSIN-II DRIVES HUMAN SATELLITE CELLS TOWARD HYPERTROPHY AND MYOFIBROBLAST TRANS-DIFFERENTIATION BY TWO INDEPENDENT PATHWAYS: A BASIS TO SUPPORT THE USE OF RENIN ANGIOTENSIN SYSTEM BLOCKERS IN SKELETAL MUSCLE WASTING AND FIBROSIS

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Introduction: The healthy skeletal muscle has a robust self-regenerative ability in response to injuries or stress conditions because of the presence of resident stem cells, the satellite cells (SCs). They establish paracrine/endocrine connections with environmental signals in the muscle, which drive their expansion, differentiation and self-renewal. However, incorrect muscle regeneration and fibrosis may occur as a consequence of inflammatory and/or fibrotic factors, which represent potential drug targets to counteract muscle fibrotic deterioration and favor efficient regeneration. Angiotensin-II (Ang), one of the effectors of the renin angiotensin system (RAS), has a controversial role on SCs fate, despite clinical use of converting enzyme inhibitors (ACE) or Ang type-1receptor (AT1R) antagonists, are beneficial to slow-down muscle decline. Based on data on RAS over-activation able to promote cardiac and renal hypertrophy and fibrosis through activation of AT1R and stimulation of the canonical transient receptor potential (TRPC) channels, we hypothesized a similar role of Ang/TRPC channel axis on human SCs (hSCs).

Matherial and methods: hSCS were isolated from specimens of pectoralis muscle obtained during corrective surgery. Cultured hSCs (passages 1-3) were analyzed by immunofluorescence and Western blot and calcium imaging recording

Results: Cultured hSCs (passages 1-3) stably stained positive for markers of quiescence/activation (Pax7) and myogenic lineage (Myf5and MyoD); while myogenin and myosin heavy chain were absent. With this phenotype, hSCs expressed all individual proteins of the conventional and not conventional RAS, including AT1R, AT2R and MasR, ACE1and 2. Densitometric analysis revealed a prominent expression of MasR and AT1R over AT2R, while ACE1and 2displayed similar levels. When stimulated by Ang (1, 10, 100 nM) for 15min, hSCs dose-dependently increased the phosphorylation levels of intracellular p-mTOR, p-AKT, p-ERK1/2and p-P38, which were all blunted by irbesartan, a selective antagonist of AT1R. hSC conditioning with 100nM Ang for 24h increased significantly cell cross-sectional area (5546±737vs. 2536±288AU, $p<0.05$) that was prevented by Irbesartan. Additionally, Ang conditioning promoted hSC fate switch toward myo-fibroblasts, as assessed by increased expression of a-SMA (+47% vs control, $p<0.001$), transgelin-2(+53% vs control, $p<0.05$) and b-catenin (+13% vs control, $p<0.01$). All these effects were insensitive to Irbesartan, thus excluding the involvement of AT1R. Accordingly, myostatin levels were enhanced by Ang only in the presence of Irbesartan (+42% vs control, $p<0.001$), but not by Ang alone. To provide mechanistic hypothesis on the effects induced by Ang conditioning, we explored the involvement of TRPC channel modification during Ang conditioning. Immunostaining revealed that hSCs expressed all TRPC channel isoforms, which assembled into functional pores. Calcium imaging recordings confirmed the presence of typical properties of TRPC channels, namely activation by depletion of internal calcium stores, inhibition by SKF96365and stimulation by Ang. The latter appeared mediated by both AT1R and AT2R. Interestingly, TRPC channel function in hSCs was enhanced by 24h conditioning with Ang, which increased both basal (+105% vs control, $p<0.001$) and Ang stimulated (+60% vs control, $p<0.001$) calcium entry into cells.

Conclusions: AT1R promotes hypertrophy of hSCs, while a different, Ang sensitive molecular target, independent of AT1R, drives myofibroblast trans-differentiation of hSCs. Both processes are associated to enhanced calcium entry into hSCs, which is mediated by basal and Ang-stimulated TRPC channel activation. These results provide a basis to explain the benefit of RAS blockade in counteracting muscle fibrosis and to hypothesize a potential benefit of TRPC channel blockers in the same setting.