Angiotensin-II activates p-38 and the growth of human satellite cells: a role for type 1 receptor activation in self-renewal?

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The healthy and young skeletal muscle has a high capacity to regenerate in response to injuries or stress conditions because of the presence of resident mature stem cells, the satellite cells (SC). Associated to the basal lamina of myofibers, SC establish paracrine/endocrine connections with many microenvironmental signals in the muscle, including growth factors and cytokines, which drive SC expansion or differentiation and then muscle regeneration. The p38 mitogen-activated protein kinase (MAPK) and the AKT pathways are among the master regulators of extracellular cues to cell nucleus, with p38α activity initiated by inflammatory signals (Sergales et al., 2016). The latter may derive from different sources, including the local or systemic renin angiotensin system (RAS). Consistently, RAS pharmacological blockers showed favorable clinical effects in reducing the age-dependent sarcopenia and in improving the strength and the fibrosis in models of congenital muscular dystrophy (Sumukadas et al., 2006; Burks et al., 2011; Elbaz, et al., 2012). However, whether RAS is expressed in SC and Angiotenisn-II (AT-II) is a bioactive peptide on fundamental SC functions; namely renewal and activation, is unknown.

To this aim, human SC (hSC) were isolated by collagenase type I (0.2% wt/vol DMEM) digestion of human skeletal muscle specimens obtained from pediatric patients undergoing corrective surgery for pectus excavatum. The protocol was approved by the local ethical committee. Isolated hSC were plated and grown in Promocell Skeletal Muscle Cell Growth Medium and splitted when sub-confluent. The effect of AT-II on cell growth and signaling activity was evaluated on cell passage 3.

Cells were exposed to 100 nM AT-II for 24h in the absence or presence of 1□M irbesartan or 1□M PD-123319, angiotensin type 1 (AT1) and type 2 receptor (AT2) antagonist, respectively. The number of viable cells was then evaluated by cell counting and by measuring the amount of oxidized MTT (Mossman et al.,1983). In parallel, AT-II signaling capacity was evaluated exposing cells to 1, 10, and 100 nM AT-II in the absence or in the presence of 1□M irbesartan or 1□M PD-123319 for 10 min. The levels of expression of p-AkT, p-mTOR, p-ERK1/2 and p-38, of conventional and not conventional proteins of the RAS system were investigated by Western-blot analysis. The expression of stemness, myogenic and differentiation markers (Pax7, MyoD, MHC) were tested by immunofluorescence microscopy.

Results indicate that hSC express both Pax7 and Myf5, indicative of a quiescent or activated cell phenotype, and all the proteins of conventional and not conventional RAS system, including ACE1 and ACE2, AT1, AT2 and MAS receptors, with AT1 and ACE1 more expressed than AT2 and ACE2, respectively. Interestingly enough, exposure of hSC to 100 nM AT-II induced activation of p-38, which was prevented by pre-exposure to irbesartan. Activation of Akt, mTOR and ERk1/2 were also observed at the same dose. In addition, 100 nM AT-II induced a significant increase of cell number (+30% vs. control) but not of cell area. The increase of cell number was not followed by a

parallel increase of MTT cell oxidation. The increase of cell number was prevented by irbesartan, but not by PD-123319.

Our results indicate that hSC have a complete RAS system and AT-II, one among the main RAS system effectors, is a bioactive signal for hSC, activating kinases involved in cell growth/regeneration. Furthermore, the stimulatory effect of AT-II on cell number but not on MTT oxidation suggests that AT-II promotes a specific renewal activity of hSC, which generate cells with low mitochondrial metabolic activity. Interestingly enough, both cell growth and activation and of p38 were dependent on AT1 receptor activation, thus suggesting a potential role of this receptor cascade on hSC renewal.

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Burks et al. (2011). Sci Transl Med. 3, 82-37.

Elbaz et al. (2012). Ann Neurol . 71, 699–708

Sousa-Victor et al. (2015). Trends Endocrinol Metabolism

Sumukadas et al. (2006). Gerontology 52, 237–242

Mosmann et al. (1983). J Immunol Methods. 65, 55-63.

Segalés J (2016) Front Cell Dev Biol. 4: 91.