A complete renin angiotensin system (RAS) is expressed in human satellite cells, myotubes and skeletal muscle: evidence for a direct role for angiotensin-II in activating signaling involved in satellite cells *growth*

A. Laurino, L. Diolaiuti, V. Spinelli, S. Blescia, A. Mugelli, L. Sartiani, L. Raimondi

Dept. of Neuroscience, Psychology, Drug Research and Child Health (NEUROFARBA), Section of Pharmacology and Toxicology, University of Florence, Viale G. Pieraccini 6, 50139 Florence, Italy

The healthy skeletal muscle is endowed of a high capacity to regenerate in response to injuries or stress conditions. This ability is due to the presence of resident mature stem cells, the satellite sells (SC), which lie quiescent, bound to the basal lamina of myofibers establishing with them paracrine/endocrine connections. Injures or stress conditions activate SC growth, self-renewal and differentiation (SC cycle), processes aimed to provide proliferating myoblasts capable to fuse with resident muscle fibers or other myoblasts, thereby forming new myofibers and eventually repair the damage. Emerging evidence indicate that the presence of an inflammatory milieu may affect negatively SC cycle and skeletal muscle repair (Sousa-Victor et al., 2015).
Increased levels of Angiotensin-II (AT-II), one of the inflammatory, pro-oxidant signals produced by the renin angiotensin system (RAS), have been proposed as detrimental to muscle integrity and regeneration (Yoshida et al., 2013). Accordingly, clinical evidence suggest that the use of drugs inhibiting angiotensin converting enzyme (ACEi) or AT-II receptor type1 blockers, reduces the age-dependent loss of muscle strength and improves the strength and the fibrosis in models of congenital muscular dystrophy (Sumukadas et al., 2006; Burks et al., 2011; Elbaz, et al., 2012). The presence of a RAS system in human skeletal muscle cells is debated (Johnston et al., 2011) and the direct effects of AT-II on human SC (hSC) remain controversial (Yoshida et al., 2013; Johnston et al., 2010). In order to get insights into these unresolved issues, we aimed to assess which proteins of the RAS system are expressed in hSC and if exposure of hSC to AT-II was able to activate signaling cascades indicative of induction of cell growth and muscle regeneration.
SC were isolated from human skeletal muscle specimens obtained from pediatric patients undergoing surgery for pectus excavatum. The investigation conforms with the approval by the local ethical committee. Specimens were digested with 0.2% (wt/vol) collagenase type I/DMEM for 1-2 h at 37°C. Isolated SC were plated and grown in Promocell Skeletal Muscle Cell Growth Medium and then differentiated using Promocell Skeletal Muscle Cell Differentiation Medium. Sub-confluent cultures were exposed to AT-II (1, 10 and 100 nM) for 10 min to evaluate activation of ERK1/2, p38 and Akt. The expression of differentiation markers (Pax7, MyoD, MHC) was evaluated by immunofluorescence microscopy. All the determinations were performed in cells from passage 2 to 6. RAS protein expression was investigated by western-blot analysis in SC before and after differentiation and in the whole skeletal muscle extract.
Results indicate that undifferentiated hSC express Pax7 and Myf5, indicative that cell phenotype is quiescent or activated. At variance, differentiating conditions allowed the expression of Caveolin3 and MHC, both markers of the myogenic phenotype. Moreover, sub-confluent hSC express 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. A similar pattern of protein expression was observed in differentiated hSC as well as in muscle specimens with the exception of AT2. Interestingly enough, the exposure of hSC to AT-II induced activation of Akt, ERK1/2, p38, which was maximum at 1nM concentration.
Our results indicate the presence of a complete RAS system in hSC, differentiated myoblasts and human skeletal muscle. Moreover, RAS functional phenotype changes with AT-II concentrations and provide a basis to suggest AT-II signaling as modulator of fundamental biological function in hSC such us proliferation and myogenic differentiation.