Sex differences in autophagy of VSMCs from human male and female neonates

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Sex has largely been neglected in cellular studies. Autophagy is a sophisticatedly regulated homeostatic mechanism, which ensures cell's constituent turnover. Under physiological conditions, autophagy levels are usually low, but it can be induced by numerous cellular stresses such as starvation (Oczypok et al. 2013). Indeed, it has been reported that autophagy has an important physiological role in the cardiovascular system and in the pathogenesis of numerous diseases, including ischaemic heart disease (Salabei and Hill, 2013). The influence of sex on autophagy has been studied either *in vitro* or *in vivo*, and previous our findings demonstrated the existence of sex differences in rat heart and HUVECs (Campesi et al. 2013; Addis et al. 2014). Moreover, some of the differences in autophagy seem to be linked to the oestrogen receptors (ERs) (Straface et al. 2009). Vascular smooth muscle cells (VSMCs) are a good experimental model for studying the physiopathology of the cardiovascular system, in which autophagy plays an important physiological role. Therefore, we investigated the occurrence of sexual dimorphism in constitutive and starvation-induced autophagy between the VSMCs obtained from human umbilical cord arteries (HUASMCs) of male (MHUASMCs) and female (FHUASMCs) neonates. HUASMCs were isolated, within 12 hours from spontaneous delivery, from the umbilical cord of healthy and normal weight male and female neonates. The expression of oestrogen receptor (ER-α and ER-β) and the primary molecules involved in autophagic process (mTOR, beclin-1 and LC3) were analysed by western blotting. Both cell types expressed both isoforms of ERs: ER-β was higher expressed in the MHUASMCs than the FHUASMCs while ER-α was similarly expressed in both sexes. The level of constitutive autophagy, measured as LC3II/I ratio was higher in FHUASMCs than in MHUASMCs, while male cells had an higher expression of Beclin-1, indicating that constitutive autophagy was at least partly beclin-1-independent. mTOR activity, a regulator of autophagy, did not vary between the sexes, indicating that the observed differences could not be attributed to this central pathway. Starvation promoted autophagy in both MHUASMCs and FHUASMCs, but the increase was more pronounced in FHUASMCs. Our results show that sex-differences start *in utero* and are parameter-specific, suggesting that HUASMCs of both sexes are necessary in *in vitro* studies to elevate the quality and translational value of research results. The observed differences in the autophagic process could help to ameliorate our knowledge on sex-differences observed in cardiovascular diseases.

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