# Trehalose prodegradative role on AR aggregates in a muscle model of Spinal and Bulbar Muscular Atrophy.

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Spinal and Bulbar Muscular Atrophy (SBMA) is a motor neuronal disease whose onset and progression have been recently linked also to a muscular defect. SBMA is caused by a polyglutammine tract in the exon 1 of the androgen receptor protein (ARpolyQ). When AR is activated by testosterone a fraction of the protein misfolds and become toxic to cells. Moreover if ARpolyQ is not correctly removed from cellular environment it also forms aggregates that could damage many cellular process. In this work we have studied the protein quality control system (composed of a chaperone network and two main degradative pathways: proteasome and autophagy) in a cellular muscular model of SBMA. We use C2C12 stably transfected with ARwt or ARpolyQ bearing an elongation of 100 glutammine. Initially we performed a filter trap assay (FTA) on both cell line treated with testosterone. We observed that testosterone triggers the aggregation of ARpolyQ but not of ARwt. We also observed that testosterone treatment caused mortality in C2C12 with ARpolyQ. By real time PCR we found that there was not activation of the PQC system in presence of ARpolyQ but that the expression of AchR was significantly lower than in control cell. These data suggest that ARpolyQ led to muscular atrophy. We investigate degradative systems that degrade AR and found that autophagy is highly involved in AR degradation. We facilitate autophagy towards the overexpression of HspB8. We observed that HspB8 counteracted testosterone dependent aggregation of ARpolyQ. We know that trehalose in motorneuronal model of SBMA induce the expression of HspB8. We then enhanced autophagy with trehalose and found that ARpolyQ aggregation was almost completely reverted. We co-treated cells with trehalose and bafilomycin and found that this condition abolished trehalose effect. We demonstrated that trehalose effect depend upon an efficient autophagic flux.
By rtPCR we observed that trehalose enhance the expression of a wide range of genes related to autophagy. Interestingly we also found VCP overexpression in presence of trehalose. The valosin containing protein VCP is a multi-functional protein involved also in the ERAD pathway. So we inhibit VCP with DBEQ a specific inhibitor of the ATPase activity of VCP and found that testosterone dependent aggregation was significantly increased. Interestingly we found that trehalose treatment counteracted this DBEQ associated aggregation.
In conclusion we characterized C2C12 as a reliable muscle model of SBMA. we also found that autophagy is highly involved in ARpolyQ degradation and consequently we demonstrated that autophagy activation rescues ARpolyQ aggregation in muscle cells. We finally observed that also the ERAD pathway plays an important role in ARpolyQ degradation.

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