Doutoramento em Biologia

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Title: "Disease mechanisms in *Lama2*-deficiency: a multiorgan approach"

LAMA2-congenital muscular dystrophy (LAMA2-CMD) is caused by mutations in *LAMA2*, encoding the laminin α2 chain, a structural component of the extracellular matrix. LAMA2-CMD is a highly debilitating disease, characterized by severe muscle weakness and hypotonia, that are apparent from birth. Previously, the host laboratory showed that the onset of LAMA2-CMD in skeletal muscle of the dy^W mouse model of LAMA2-CMD occurs *in utero*, with a reduction in the pool of muscle stem cells and myoblasts. This finding suggests that *Lama2*-deficiency may impact muscle cell proliferation, differentiation, and/or death. However, the mechanisms responsible for this phenotype, and if they can be reversed, remains unknown. Thus, the aim of this PhD project was to explore cellular and molecular mechanisms involved in this fetal skeletal muscle phenotype. For that, a *Lama2*-deficient C2C12 myoblast cell line was generated to use in parallel with the dy^W mouse model. Here, we showed that disease onset, previously established to occur between embryonic days (E) 17.5 and E18.5 in dy^W mouse fetuses, is characterized by a profound downregulation of gene expression, defects in myoblast proliferation and a marked effect on muscle cell differentiation and fusion. These changes are further accompanied by increased DNA damage and a compromised oxidative stress response. Moreover, our results demonstrate that p53 inhibition reverts the proliferation defect, but not the differentiation impairment, suggesting that *Lama2*-deficiency triggers proliferation and differentiation defects through distinct pathways. In addition to skeletal muscle, *LAMA2* is also expressed in other less studied organs including the heart, kidney and liver. This PhD project also intended to understand how these organs are affected in LAMA2-CMD, focusing on oxidative stress, mitochondria function and DNA damage. Our results showed that *Lama2*-deficiency leads to alterations in the antioxidant response in all three organs, including altered levels of the master regulator of the oxidative stress response, NRF2, and its target genes. Additionally, absence of *Lama2* was shown to lead to mitochondria dysfunction in the kidney. To better understand if these defects are cell-autonomous, we isolated cells from the heart and kidney, cultured them *in vitro* and validated the most prominent makers identified in the whole organ. While some alterations observed in these organs were shown to be cell-autonomous, most seem to be non-cell autonomous. Together, our findings provide unique insights into the cellular mechanisms that rely on laminin α2 chain in skeletal muscle and revealed a critical importance of laminin α2 to maintain muscle cell homeostasis already at fetal stages. Moreover, we showed the first evidence of how the heart, kidney and liver are affected at the molecular level in LAMA2-CMD, providing a novel and unique analysis of the impact of *Lama2*-deficiency beyond the well-characterized muscle phenotype. Overall, these findings provide an important framework for studies aiming to therapeutically target LAMA2-CMD and possibly other muscular dystrophies.