University of Fribourg / Faculty of Science and Medicine / Department of Endocrinology, Metabolism and Cardiovascular System

Multimodal regulation of the oxygen-binding protein androglobin

Koay Teng Wei

Androglobin (ADGB) is the fifth and latest addition to the globin superfamily alongside with hemoglobin, myoglobin, cytoglobin and neuroglobin. First identified via genomic-based mining with high conservation in metozoa, the presence of ADGB was further validated in animal models showing high selectivity in expression sites, with most abundant expression in the testis. The organ-specific expression of ADGB suggests a thus far unexplored tightly regulated promoter- and enhancer-dependent transcription. In this study, we identified and confirmed the minimal upstream genomic sequences with *ADGB* promoter activity, a region that can also be activated by CRISPR-based system to upregulate *ADGB* expression in transcriptionally silent cellular models. In addition, two distal genomic regions (termed 3'-AE1 and Int35-AE1) that loop to the promoter were found to be enhancing promoter activity, contributing to the complexity of *ADGB* regulation. Furthermore, we identified that the ciliogenesis master regulator, FOXJ1, upregulates *ADGB* expression via direct binding to the promoter, and mutational studies pinpoints a highly conserved RFX-binding domain as the key binding site for FOXJ1. Additionally, a FOXJ1 binding partner and master regulator of spermatogenesis, RFX2, was confirmed to also regulate *ADGB* gene expression and acts in cooperation with FOXJ1, in a manner dependent on the remote 3'-AE1 enhancer. Altogether, these findings contribute to the understanding of the complex regulatory mechanism of *ADGB* gene expression, and convincingly indicate an association of ADGB with ciliogenesis.

ADGB is a large chimeric protein with a calpain-like domain and a permutated globin domain with an internal IQ motif. These domains are interlaced by a 350-residue and 700-residue domains with uncharacterized homologies. Calpains are known to undergo proteolysis as part of their activation and/or function. Here we show that ADGB displays calcium-dependent cleavage that produces two major cleaved products of 60 kDa and 135 kDa, among other detectable products. Although this process can be inhibited by the pan-calpain inhibitor, calpeptin, we found no involvement of CAPN1 and CAPN2. Instead, the small regulatory CAPNS1 is involved in the proteolysis albeit without direct interaction with ADGB. Further investigations indicate that a cleavage hotspot(s) exists between the calpain-domain and the globin domain, and that proteolysis trims and releases the globin from the chimera. Intriguingly, the liberated ADGB globin domain was found to interact with the calcium-binding calmodulin, in contrast to the full-length ADGB where the C-terminal portion of ADGB impedes this interaction. Moreover, the single domain globin displays a preferential shift in localization to the centriole, an indispensable organelle for ciliogenesis. Collectively, this study bridged the post-translational proteolysis of ADGB to its ciliogenesis-associated functions.

Jury:
Prof. Dr. David Hoogewijs (thesis supervisor)
Prof. Dr. Ben Wielockx (external co-examiner)
Prof. Dr. Zhihong Yang (internal co-examiner)
Prof. Dr. Anna Lauber-Biason (president of the jury)