P24- TARGETING MYOGENESIS MODULATION VIA CRISPR/CAS9-MEDIATION IN FARMED NILE TILAPIA

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SUMMARY

The complex epigenetic regulation underlying teleost fish muscle development and growth remain largely unknown. Just recently, using domesticated Nile tilapia cultured in a RAS system, we evidenced the action of epigenetic marks on several muscle-related genes. In particular, the gene *stub1* was found to be differentially methylated (Podgorniak et al., 2022) and *myo5b* was differentially hydroxymethylated and expressed between wild fish and their progeny reared in captivity (Konstantinidis et al., 2020).

CRISPR/Cas9 is a powerful approach for targeted genome editing that has been proved to be effective in several organisms, including fish. Here, we attempt to induce somatic mutations in domesticated Nile tilapia by microinjecting sgRNA and Cas9mRNA into hormone-induced, fertilized eggs at the single-cell stage. Several guide RNAs were constructed targeting *stub1*, which codes for E3 Ubiquitin ligase CHIP (carboxyl terminus of Hsc70-interacting protein) and is involved in muscle regeneration and negative regulation of cell senescence markers such as p53 and p21. Similarly, guide RNAs were constructed for *myo5b*, whose protein targets and directly activates the phosphatase and tensin homolog (*pten*) that is a natural inhibitor of cell proliferation through the regulation of PI3K. Thus, their genetic modification is expected to result in enhanced fish muscular mass.

In summary, our results intend to demonstrate that CRISPR-Cas9 is an efficient tool for modifying the Nile tilapia genome and open new avenues to facilitate growth selection during the Nile tilapia domestication process by shortening the time to achieve the desired enhanced phenotype benefiting the final aquaculture product.

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