A unique angle to discover novel mechanisms in cardiovascular protection

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Project Outline

Background This proposal uses a unique fish model, the Mexican cavefish (*Astyanax Mexicanus*), combined with patient data to uncover key novel mechanisms underlying protection from hypercholesterolemia and atherosclerosis, allowing to identify innovative therapeutic approaches. *Astyanax mexicanus* is a single fish species comprising cave-dwelling and surface populations. Thousands to millions of years ago, surface fish living in rivers became trapped in caves. During their independent evolution in the caves, the fish lost their eyes and pigment, redundant in the absence of light.[1,2] In addition, to be able to survive food scarcity, the fish adapted their metabolism. They developed an increased appetite and increased fat accumulation when food is abundant, such as in the lab.[3,4] This leads to increased body fat, including a fatty liver.[3,5] Alongside these fat-based adaptions, cavefish also display hyperglycaemia and insulin resistance – all phenotypes associated with human obesity and diabetes mellitus. Indeed, cavefish possess a mutation in the insulin receptor (SNP p211L) that has been associated with Rabson-Mendenhall syndrome, a condition of severe insulin resistance.[6] Intriguingly, these same cavefish (lab) populations exhibit robust health and longevity, living much longer than their surface fish counterparts,[4–6] without features of pathologies typically associated with obesity and diabetes such as accumulation of advanced glycation end products (AGEs), chronic tissue inflammation, impaired growth due to insulin dysregulation, and low survivability due to arterial disease.[5,6] From studies using zebrafish, we know that feeding a high cholesterol diet (HCD) without any genetic intervention (fish express *cetp*) results in hypercholesterolemia with atherosclerosis.[7–10] HCD in zebrafish also leads to increased spleen size with higher levels of pro-inflammatory gene expression, in particular strong upregulation of il1b. From published work and our own unpublished scRNAseq data, we know that the immune system of cavefish is altered with a reduced pro-inflammatory response. In particular, il1b upregulation during inflammation is much less strong in cavefish compared to surface fish. Additionally, their fat deposits contain less immune cells.[11] This suggests that cavefish are able to live healthily while obese and diabetic due to adaptations of their immune system and these changes could be crucial for their longevity. While the fish is evolutionary very distinct from human, the specific adaptation seen in cavefish is also unique for fish. Directly comparing surface fish and cavefish will specifically allow to tease out which genomic adaptation and pathways reduce the pathological response to obesity and diabetes. As studies in mice and rabbits also have their drawbacks for studying hypercholesterolemia and atherosclerosis,[12] new inroads into pathway discovery are needed. The identified pathways can directly be compared with mouse and patient data to find conserved mechanisms.

Hypothesis Cavefish are protected from the pathological effects of hypercholesterolemia including atherosclerosis due to evolutionary adaptations to their immune system.

Aims 1. To identify the key regulatory genes underlying the cavefish protective phenotype. 2. To identify similarities/differences in inflammatory gene profile between cavefish and diabetic patients. 3. To identify inhibitors/activators inducing the protective mechanisms.

Description of the work to be undertaken For Aim 1 we will take full advantage of the Astyanax model: As the fish are still one species, this allows for forward genetic screening methods including QTL analysis. We will generate 200 second generation (F2) offspring from a cavefish-surface fish cross which will all receive HCD from birth onwards. 6-month-old fish will be tested for hyperglycaemia and hypercholesterolemia. The spleens will be isolated for bulk RNAseq and the bodies will be embedded for sectioning to analyse atherosclerosis in the aorta and crown-like structures in the fat. DNA will be collected from all F2 fish to perform RADseq to be able to perform QTL analysis on traits such as il1b and other pro-inflammatory gene levels in the spleen and the amount of atherosclerosis in the aorta. This will identify the key regions in the genome regulating the protective response. Aim2: To identify the unique 'protective' inflammatory gene signature and look for conservation, we will also perform scRNAseq on HCD or normal fed cavefish and surface fish spleen and compare this data to existing unpublished diabetic versus control mouse and human scRNAseq data sets. Aim3: We will test inhibitors/activators of the identified 'protective' genes/pathways in fish and mice with the aim to test conservation and find novel ways to induce protection for hypercholesterolemia and atherosclerosis.

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