Oxidative phosphorylation is required for fish heart regeneration

Oxidative phosphorylation was considered detrimental for heart regeneration, as it produces reactive oxygen species that block cardiomyocyte proliferation by causing DNA damage. However, harnessing natural variation in the regenerative capacity of seven wild-type zebrafish strains has revealed that the activation of oxidative metabolism after proliferation is essential for cardiomyocyte maturation and successful regeneration.

This is a summary of:
Name, A. A. et al. Title. *Nature*
https://doi.org/10.1038/xxxx (202x)
[We will complete this]

Published online: xx xx xxxx

The question

In contrast to humans, zebrafish maintain the impressive capacity to regenerate lost cardiac tissue throughout adulthood. After an injury, the border zone cardiomyocytes, which are located directly adjacent to the wound, dedifferentiate and enter the cell cycle to restore cardiomyocytes lost during injury and to repopulate the wound[1]. Glycolysis has been shown to play a key role in enabling zebrafish cardiomyocytes to proliferate[2,3]. However, oxidative phosphorylation (OXPHOS) has been considered detrimental for cardiac regeneration as it produces DNA-damaging reactive oxygen species that cause cell cycle arrest[4]. Nevertheless, some studies have hinted that OXPHOS is upregulated during heart regeneration. As OXPHOS is the predominant energy pathway in the human heart, deciphering its role in regeneration could have translational applications.

The discovery

Comparing the regenerative capacity within and between species is a useful approach to uncovering novel pathways involved in regeneration. Although zebrafish are a common model in regenerative research, differences between strains have not been investigated in the context of heart regeneration. We compared the regenerative capacity of seven wild-type zebrafish strains (**Fig. 1a**), aiming to identify factors that drive long term regeneration. We found significant differences in wound size at the end-stage of the regenerative period between strains. Surprisingly, the expression of genes involved in OXPHOS — but not cardiomyocyte proliferation — was correlated to a smaller wound size and thus a better regenerative outcome.

The upregulation of OXPHOS seems counterintuitive at first due to its detrimental effect on proliferation. However, our data suggest that OXPHOS and cardiomyocyte proliferation are temporally separated and thus do not interfere with each other. Specifically, we show that OXPHOS gets downregulated after injury to allow cardiomyocyte proliferation to occur. Once the cell cycle is completed, OXPHOS gets activated and drives the redifferentiation of cardiomyocytes. Our analysis suggests that the malate–aspartate shuttle (MAS) is instrumental in activating OXPHOS after cardiomyocyte proliferation, by coupling glycolysis to mitochondrial metabolism to allow for increased ATP production through the electron transport chain. Indeed, pharmacological or genetic ablation of the MAS impaired heart regeneration, whereas cardiomyocyte-specific overexpression of *mdh1ab*, a key gene of the MAS, improved heart regeneration (**Fig. 1b**) through increased cardiomyocyte redifferentiation. Based on these findings, we propose the existence of two phases during heart regeneration: an early glycolysis-dependent proliferative phase and a late MAS- and OXPHOS-mediated redifferentiation phase that has been largely overlooked.

This response appears to be conserved in *Astyanax mexicanus*, a species of tetra that incudes a river-dwelling population that can regenerate its heart and a cavefish population that cannot[5]. In the regenerative surface fish, upregulated OXPHOS drives a dynamic sarcomere gene expression program that allows for successful cardiomyocyte redifferentiation. In contrast, the dampened OXPHOS and redifferentiation response in the cavefish leads to permanent scar formation after cryoinjury.

The implications

Although cardiomyocyte proliferation peaks at seven days post-injury, regeneration is only completed several months later. Our study shows that OXPHOS drives a redifferentiation program that is essential for regeneration after the proliferation stage is complete. This long-term redifferentiation process fills a gap in our knowledge of what happens in the heart after cardiomyocyte proliferation is completed. As OXPHOS is the predominant energy-production pathway in the human heart, its presence during fish heart regeneration suggests that it might be a promising avenue for developing therapeutics that can target pre-existing pathways in the human heart.

Although our work characterises this response, the factors that upregulate or link OXPHOS and redifferentiation remain unclear. The discovery of such factors might inform the development of therapeutics targeting human heart regeneration.

Overall, in order to regenerate human hearts, we not only need to think about how to induce cardiomyocyte proliferation, but also about how to redifferentiate cardiomyocytes to achieve the optimal regenerative outcome. This line of thinking opens up exciting research opportunities that focus on ways to therapeutically harness OXPHOS in the human heart.

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EXPERT OPINION

"The quality of the data in this study is outstanding; I particularly value the detail and accuracy of the methodology and access to materials, including transcriptomics data. Furthermore, the correlation studies of proliferation and regeneration at the different time points will be of great interest to the community, as will the investigation of the regenerative potential of different zebrafish strains and correlation to transcriptomic profiles." **Nadia Mercader, University of Bern, Bern, Switzerland and Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid, Spain.**

FIGURE

Fig.1 | Overexpression of *mdh1ab* improves heart regeneration. (a) Representative images of the seven strains (top; scalebar: 1 cm) and their uninjured hearts (bottom; A: atrium, V: ventricle, BA: bulbus arteriosus; scalebar: 500µm) through which we identified *mdh1ab* as a target for heart regeneration. AB, AB wild-type line; KCL, wild-type from Kings College London; NA, Nadia; SAT, Sanger AB Tübingen; TL, Tupfel long fin; TU, Tübingen; WIK, Wild India Kolkata. (b) The *mdh1ab* gene, which encodes a key enzyme in the malate–aspartate shuttle, was overexpressed in the cardiomyocytes of the KCL strain. Representative images and quantification of *mdh1ab* cOE (cardiomyocyte overexpression) heart wounds, which show improved regeneration compared to *GFP* cOE heart wounds at 21-days post-cryoinjury (dpci). KCL strain; scalebar: 100µm.

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BEHIND THE PAPER

Our laboratory has a long-standing interest in comparative models of regeneration. In 2018 we first described a species of Mexican tetra (*A. mexicanus*) with two populations, the regenerative river-dwelling surface fish and the non-regenerative Páchon cavefish[5]. A natural consequence of this finding was to comparatively investigate regeneration in the more commonly studied zebrafish. This study started amidst a global pandemic as my placement project, and I am lucky for the trust my supervisor showed in allowing me to work in the laboratory during those uncertain times. Progressively, the project grew to include several international collaborators and eventually to form the basis of my Doctor of Philosophy studies. There were many exciting moments during this journey, including the initial observation of variations in the regenerative capacity of wild-type zebrafish strains and the surprising finding that OXPHOS is the hidden force behind those differences! **K.L.**

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FROM THE EDITOR

"This manuscript investigates a very clinically relevant phenomenon with a unique approach, looking for evidence in multiple organisms and reaching conclusions that challenge a common assumption in the field. I appreciated this line of inquiry, and I believe the findings here will have ramifications even beyond this specific field of study." **Andrea Tavosanis, Associate Editor, *Nature Cardiovascular Research***

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