Zebrafish *ankrd1a* as a common player in heart regeneration and skeletal muscle repair

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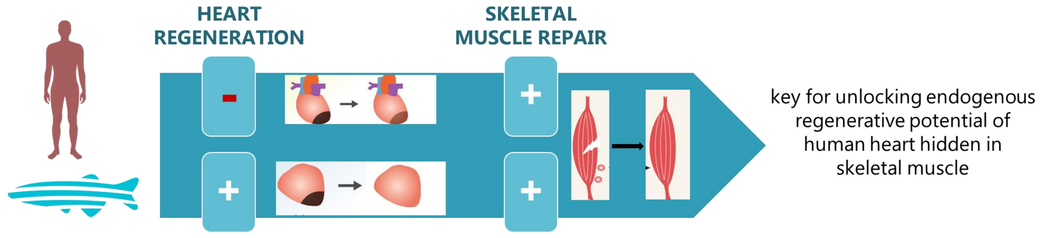
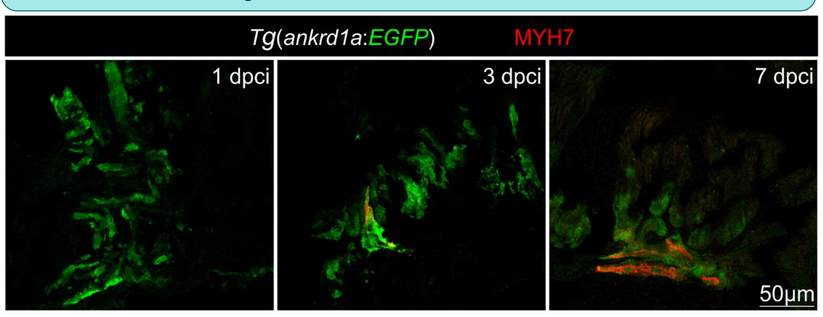
In contrast to humans, zebrafish have a remarkable ability to regenerate their hearts after injury, while both humans and zebrafish efficiently repair the wounded skeletal muscle. Common players in these two processes might represent potential targets for the development of efficient therapies to stimulate the human heart to regenerate after injury.
We identified *ankrd1a* expression to be upregulated in both regenerating zebrafish hearts (1) and in repairing skeletal muscle. Its mammalian homolog *ANKRD1/CARP* encodes a stress-responsive cardiac ankyrin repeat protein involved in transcriptional regulation, sarcomere assembly and mechanosensing (2).



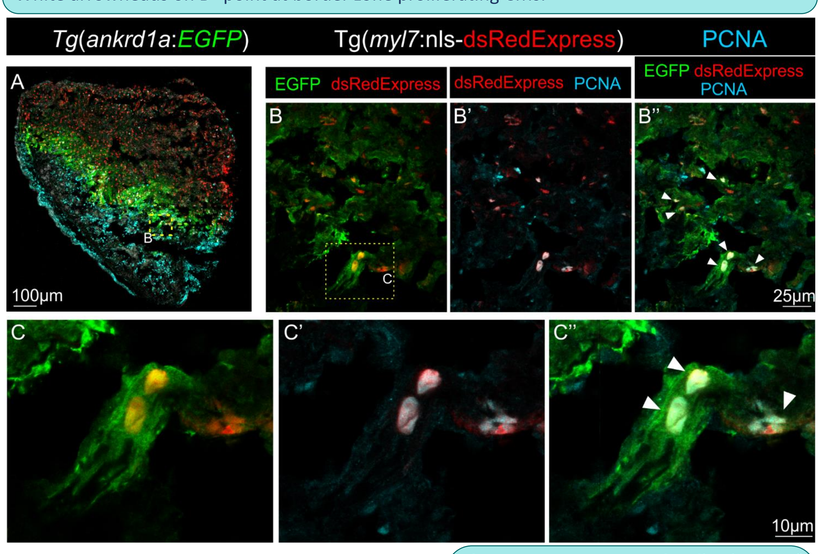
key for unlocking endogenous regenerative potential of human heart hidden in skeletal muscle

Previously we have shown that *ankrd1a* is activated in border-zone cardiomyocytes (CMs) after heart cryoinjury (1).

Colocalization of embryonic myosin MYH7 and EGFP at 3 and 7 dpci suggests that *ankrd1a* is activated in dedifferentiating CMs.

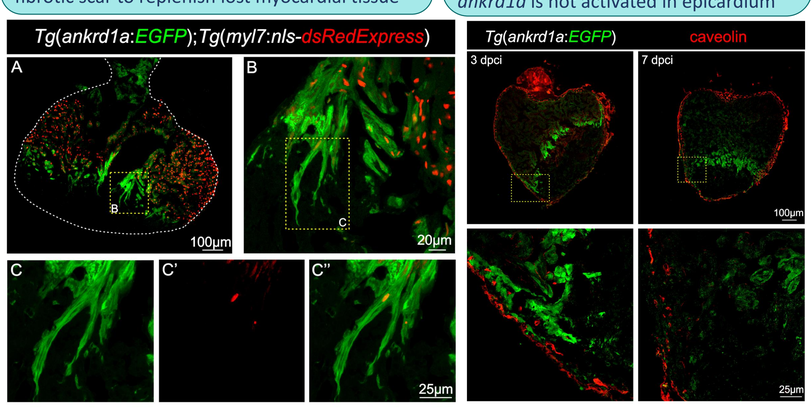


ankrd1a is activated in proliferating CMs identified by PCNA staining.
White arrowheads on B″ point at border zone proliferating CMs.

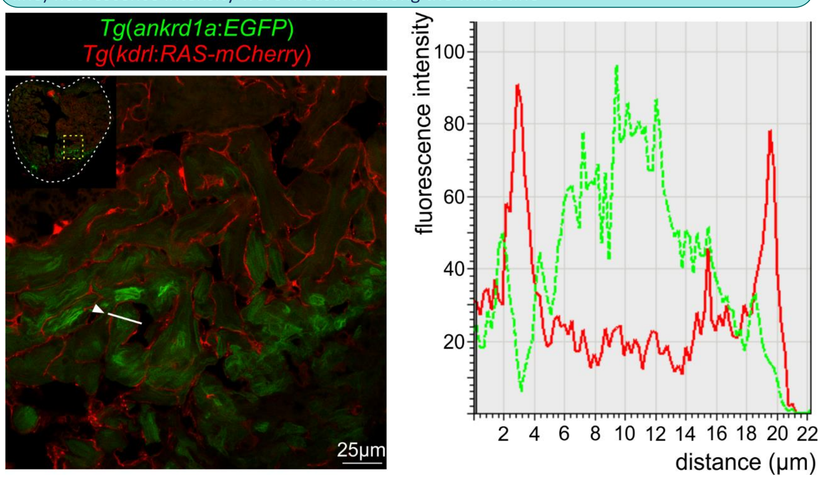


ankrd1a:EGFP transgene was detected in protrusions of CMs (3) that migrate into the fibrotic scar to replenish lost myocardial tissue

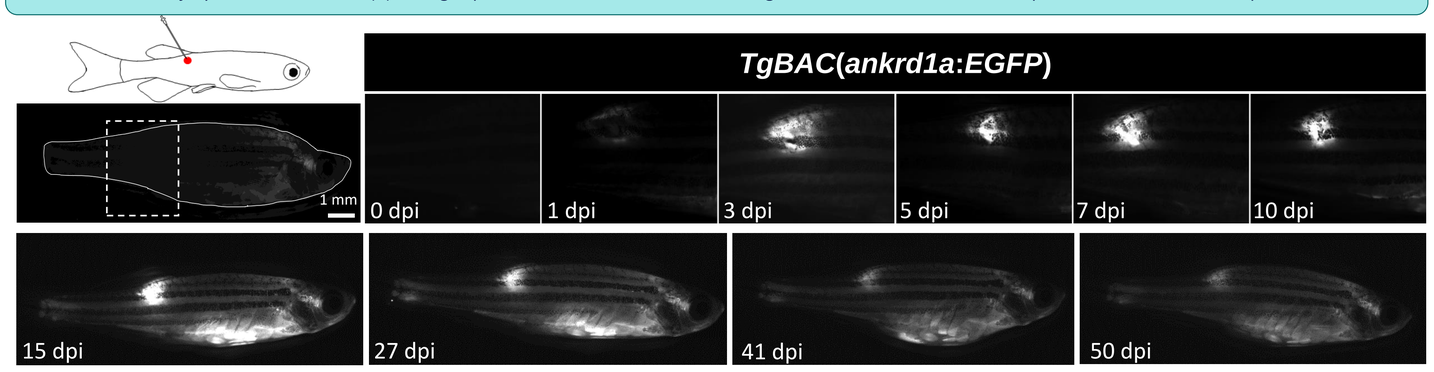
There was no colocalization of *ankrd1a*:EGFP transgene and caveolin in the injured heart suggesting that *ankrd1a* is not activated in epicardium



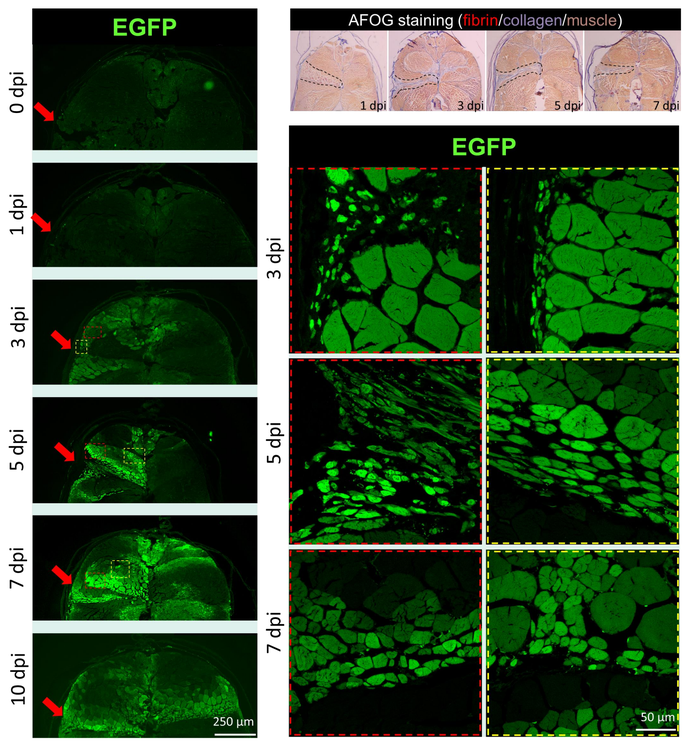
ankrd1a:EGFP transgene was not localized in endocardial cells indicating that *ankrd1a* is not activated in regenerating endocardium. *ankrd1a*:EGFP (green line) and kdrl:RAS-mCherry (red line) fluorescence intensity were measured along the white line



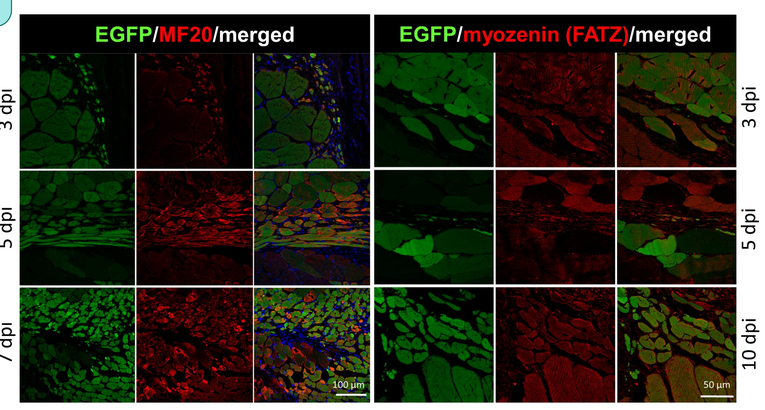
After needle stab injury of skeletal muscle (4) strong expression of the *ankrd1a*:EGFP transgene was observed from the 3 dpi and remained until 50 dpi.



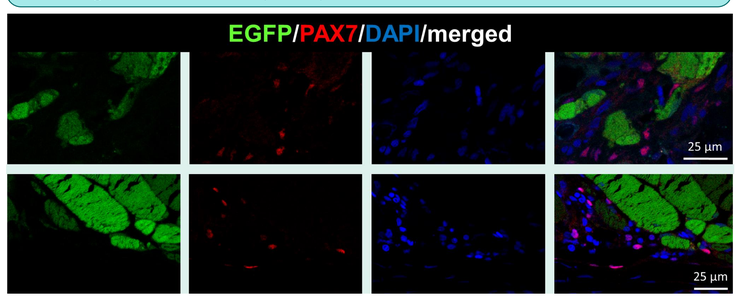
ankrd1a:EGFP transgene is active inside and around the injury. Collagen deposits are visible in the wound by AFOG staining. Expression of the fluorescent reporter was observed from 3 dpi, when new EGFP-positive muscle cells emerged inside the injury zone. At later time points, EGFP-positive myofibers were visible in the deeper tissue layers, concomitant with active repair of the injured tissue.



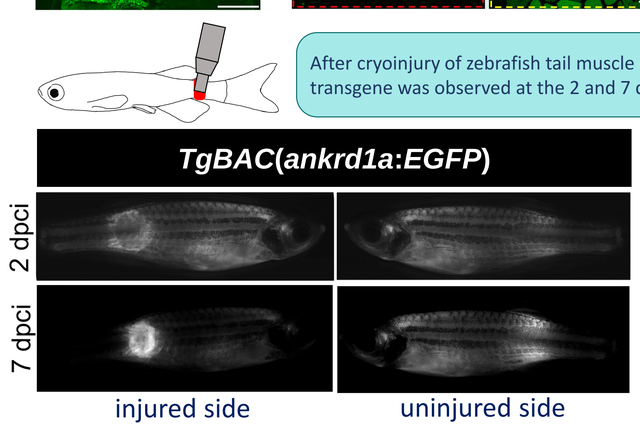
ankrd1a:EGFP transgene is active in muscle fibers after injury as confirmed by staining the sections for markers of differentiated muscle cells (MF20) and mature myofibers (myozenin/FATZ1) (5).

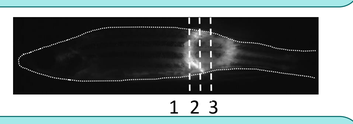


ankrd1a:EGFP transgene is not active in satellite cells. There was no colocalization of the transgene and PAX7 at any time point

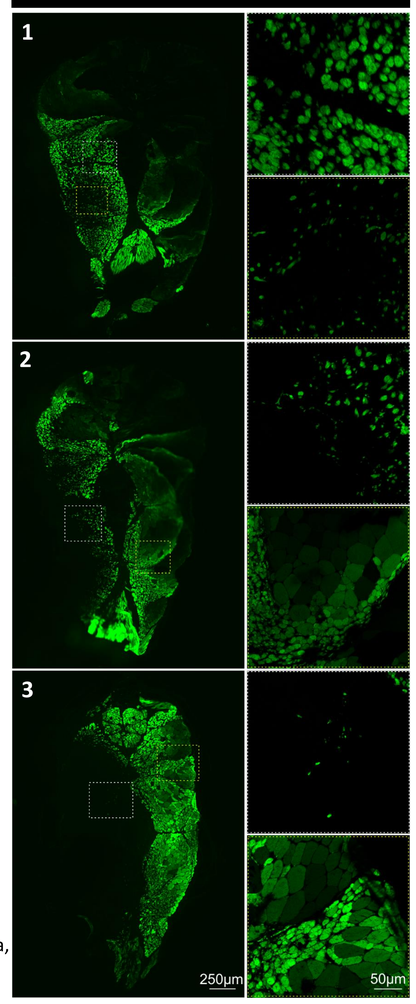


After cryoinjury of zebrafish tail muscle (6) strong expression of the ankrd1a:EGFP transgene was observed at the 2 and 7 dpci





On transversal sections of cryoinjured skeletal muscle, activation of *ankrd1a* was observed in myofibers surrounding the injury, and in newly forming myofibers invading the frozen tissue



CONCLUSIONS

- During heart regeneration after cryoinjury, activation of *ankrd1a* is restricted to the myocardium, specifically to border zone cardiomyocytes, which dedifferentiate, proliferate and invade scar tissue.
- During skeletal muscle repair after a stab wound and cryoinjury, *ankrd1a* is activated in both newly formed myofibers that invade the wound and in the apparently uninjured tissue surrounding the injury, suggesting its role in skeletal muscle tissue repair and adaptive processes in uninjured myofibers surrounding the injury site.
- Our results implicate *ankrd1a* in zebrafish muscle regeneration, repair and remodeling, promoting it as an attractive target for translational studies, as a player in muscle healing and as a sensor of stressed muscle.

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