

In contrast to humans, zebrafish have a remarkable ability to regenerate injured heart through a complex and highly orchestrated process involving all cardiac structures. The major source of new myocardial cells are resident cardiomyocytes (CMs), which dedifferentiate and reinitiate proliferation, invading the area of injury to replace the lost myocardium. The response of the myocardium and coronary vasculature is preceded by the activation of epicardium and endocardium, which form active scaffolds to guide regeneration.

This study aimed to identify cardiac structures in which the *ankrd1a* gene is activated during zebrafish heart regeneration.

Zebrafish reporter lines:

***TgBAC(ankrd1a:EGFP)***

- to identify cells expressing *ankrd1a*

***Tg(myf7:nls-dsRedExpress)***

- labeled cardiomyocyte nuclei

***Tg(kdrl:RAS-mCherry)***

- labeled endocardial/endothelial cells

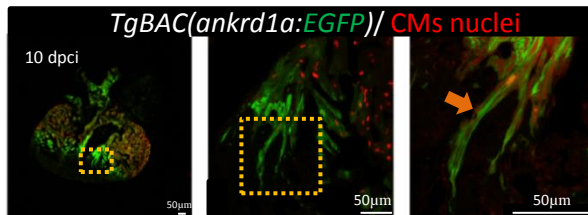
## Methodology

- Cryoinjury
- Fixation/cryopreservation
- Preparation of cryosections
- Immunostaining
- Microscopy

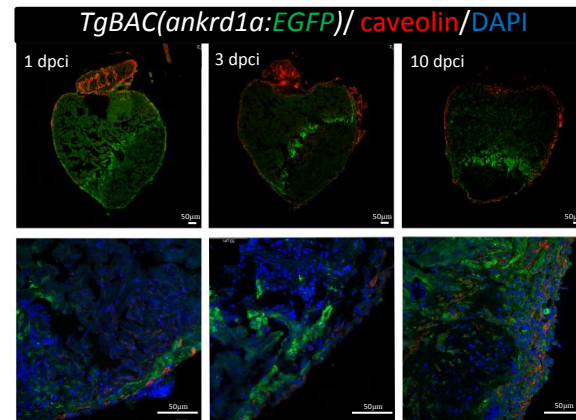


## Myocardium

In the injured zebrafish heart, CMs bordering the injury, dedifferentiate and then proliferate to contribute to regeneration. *ankrd1a* is activated in these dedifferentiating CMs, marked also by embryonic myosin expression (MYH7, white arrows).



CMs migrate into fibrotic scar to replace lost myocardium. CMs protrusions peak in number and length is at 10 dpi (days post cryoinjury). *ankrd1a* is activated in protrusions of CMs that invade scar tissue.



## Epicardium

Transgene expression was not detected in epicardial cells marked by caveolin staining. *ankrd1a* is not activated in regenerating epicardium.

## Conclusion

**Activation of *ankrd1a* during regeneration of zebrafish heart is restricted to border zone cardiomyocytes, implicating this gene in dedifferentiation and proliferation of cardiomyocytes.**

## Endocardium

Transgene expression was not detected in endocardial cells labeled with **mCherry**. *ankrd1a* is not activated in regenerating endocardium.

