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The Use of Zebrafish's Flexibility in the Regeneration of Their Hearts After Injury in Human

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Abstract

Myocardial infarction had been an ongoing concern to the medical world as the mortality rates are increasing day by day. An animal having more than 70% of their genes in common with humans as well as the same major organs and tissues caught the attention of scientists because of its extraordinary ability to regenerate their heart after damage, the study revolved around the detailed mechanism of the zebrafish's heart regeneration by a set of experiments. The results were promising as the zebrafish's heart managed to fully recover in terms of their anatomical, histological and physiological makeup when they had been injured. This was further on studied to come up with therapeutic agents that could lead to the same regenerative process in human hearts after cardiac failure.

Introduction

The heart, like any other organ in the body is in need of constant supply of oxygen and nutrients to keep up with the body's needs, and this is mediated via the coronary arteries, they're two in number and only function to deliver oxygenated blood to the heart. One of the leading causes of death in the world, constituting 30% is myocardial infarction (MI), it happens due to blockage to one of the coronary arteries that results in cardiac ischemia and death of about 25% of cardiomyocytes.[1] The heart is considered a non-regenerative organ for its low capacity to regenerate after injury, although at the beginning of the 20th century, it was believed that the adult myocardium had some ability to regenerate. Scientists thought that cardiac hypertrophy, a pathological growth of the heart was due to the production of new cardiomyocytes, and that the injury is what triggered a regenerative response, this hypothesis soon changed when a series of studies were further made to reveal that in fact the cardiac hypertrophy was due to increased cardiomyocyte size without cell division. [1] Other studies through genetic techniques concluded that a small number of cardiomyocytes are renewed during adult life in mammals but the rate of renewal was insufficient to compensate for the loss of myocardium after a myocardial infarction, however these results were promising to scientists because if the human heart has some endogenous regenerative potential then further researches can be done to booster the regenerative process of the heart and overcome the infarction. [2]

The heart muscle is irreversibly lost when exposed to injury. It is replaced by a non-contractile fibrotic scar that works to maintain ventricular wall integrity but impairs the muscle's pumping ability therefore it cannot replace the lost cardiomyocytes and most often result in congestive heart failure. [2] This had been a concern to many scientists as the mortality rates are increasing on a daily basis therefore many researches have been made to come up with therapies to enhance the heart's ability to regenerate not just structurally but also functionally when injured. This inspired scientists to study animals with hearts of high regenerative capacities and the focus was on the zebrafish. [2]

The attention was drawn to the zebrafish for sharing multiple characteristics with humans as it has over 70% of its genes, it is cheaper to experiment than mice and its embryos are transparent however researches developed a new strain of zebrafish named Casper which is an adult zebrafish with a transparent body skin, this allows experimenters to visualize the internal structures, cellular activities and how diseases develop more closely e.g Leukaemia and cancers. [2]

Materials and Methods

The zebrafish heart was examined to test its efficiency and ability to regenerate the damaged area, this was achieved by ventricular resection, amputating part of the ventricle to observe the regenerating process (apex amputation). An alternative method to the resection known as the cryoinjury was introduced, which mimics the sequence of events of myocardial infarction that happen in human. The following method included electrocardiograms to examine the electrical activity of the heart after both resection and cryoinjury.

Results

The results were encouraging as the zebrafish heart regenerated pretty well however ventricular regeneration after cryoinjury is significantly slower than of the apex amputation. Another examination by electrocardiograms have shown that injury causes prolongation of the QT segment, however as regeneration proceeded, the QT interval returned to normal, proving that regeneration corrects the electrical abnormalities induced by injury. [1]

Discussion

The zebrafish adult heart has one atrium and one ventricle, it is smaller and simpler than the human heart with similar histological and structural composition. Despite it's incredible ability to regenerate its own heart, various organs are exposed to regeneration as well for instance all fins, the spinal cord, the retina, the heart, the telencephalon and the kidney. It is worth to mention that the regeneration is organ specific meaning that each organ activate specific cell type for example if the zebrafish's fin get injured a mass of cells capable of growth and regeneration known as blastema will give rise to a new normally functioning fin. The main focus of this study was on how zebrafishes were able to recover after various types of harms. [1] When a zebrafish had up to 20% of its ventricle amputated:

1. An initial fibrin clot was formed and lasted for about 7 – 9 days post injury.
2. The fibrin clot was then replaced by new cardiomyocytes.
3. After almost 2 months the size and shape of the ventricle, in addition to the contractile ability of the heart was back to normal.

This is in contrast to the human heart which fails to compensate for the loss of cardiomyocytes. As a result the injury activates fibroblasts that work to protect the heart from rupturing by secreting collagen, this non-contractile ability of the scar leads to systolic dysfunction and serious complications later on. [1]

The zebrafish's capability to regenerate the heart is mainly done by a series of events in order:

1. Early response to injury (that includes inflammation and endocardial activation)
2. Endocardial and epicardial regeneration;
3. Cardiomyocyte proliferation.
4. Integration of regenerated cardiomyocytes into the myocardium and scar removal. [1]

The process starts by damage occurring to the heart of zebrafish, an inflammatory immune response resembling the one occurring in humans is initiated, proinflammatory cytokines and molecules resulting in the recruitment of phagocytes and neutrophils is seen in the first 3

hours post injury. Inflammation is essential for cardiomyocytes proliferation and eventually recovery of the heart therefore anti-inflammatory therapy lead to phagocytes and neutrophils depletion that will impair revascularization and cardiomyocyte proliferation. This will result in fibrotic scar persistence and support the idea that inflammation promotes proliferation and neurogenesis due to pro-regenerative properties of specific immune cells. [2]

The next regenerative step is the endocardial regeneration, in healthy hearts the endocardial cells are normally flat and tightly attached to the myocardium, however this soon changes when the injury occurs, the endocardial cells then become rounded and partially detached from cardiomyocytes. This is followed by the reexpression of specific markers which is in this case the retinoic acid. Retinoic acid signaling from the endocardium is necessary for cardiomyocyte proliferation and myocardial regeneration in addition to both the endocardium and inflammatory cells release cytokines that will instruct cardiomyocytes to proliferate.[1] These secreted factors activate the Jak1/Stat3 axis in cardiomyocytes, which can bind to DNA and allow the transcription of genes involved in cell division which results in cardiomyocyte proliferation, Cardiomyocyte-specific expression of a dominant negative form of *stat3* during regeneration reduces cardiomyocyte proliferation and blocks regeneration. These results also support the essential role of early inflammation on regeneration. The recovery of epicardium and the endocardium occurs in the first days after injury and is before myocardial regeneration. These non-muscular cells provide environment that facilitates myocardial proliferation. When the endocardium is activated, it migrates to the injured area and starts proliferating and regenerating to provide an internal covering for the wound within 3 to 5 days prior to myocardiocytes proliferation that happens at day 7 post injury. The epicardial cells become highly proliferative as well and starts to invade the underlying tissue. Shortly the damaged area becomes completely covered by regenerated epicardium, the regeneration caused by the endocardium and epicardium is known as regenerative scaffold that provides support for the proliferating cardiomyocytes. [2]

Cardiomyocyte proliferation is stimulated by the endocardium, epicardium and circulating cells, NF-κB activity is induced in cardiomyocytes during regeneration, by inflammatory cytokines released from circulating cells. Suppression of NF-κB signaling blocked cardiomyocytes proliferation and impaired epicardial regeneration. Beside the endocardium

and epicardium there are other factors impacting cardiomyocyte proliferation e.g when the wound became hypoxic after ventricular resection, the scientists introduced experimental hyperoxia, this showed that excessive oxygen administration blocked the regenerative process meanwhile the induction of hypoxia proved an opposite result which was cardiomyocytes proliferation. [1]

Lastly is the reintegration of cardiomyocytes into the myocardium and scar removal, it's worth noting that this phase is the least explored phase of the zebrafish heart regeneration. The extracellular matrix that was deposited in response to injury is gradually replaced by regenerated myocardium, myofibroblasts start progressively disappearing, collagen degradation in the wound region at 14 and 30 days post injury was seen, this increase in collagenolytic activity occurred at the same time when the expression of several matrix metalloproteinases increased, the upregulation of *mmp2* and *mmp14a* expression is specifically found 14 and 30 days post injury this was concomitant with the onset of scar clearance. Now that the mechanism of zebrafish heart regeneration is clear Future studies are made to identify the specific molecules that led to heart regeneration. These molecules can be used further on to promote cardiac repair after myocardial infarction in humans, the zebrafish are being used as a preclinical model, useful to identify new therapeutic strategies to reduce the damages caused by myocardial infarction. [3]

Conclusion

15 years of studies helped clarify plenty of aspects, scientists now have a better understanding of the regenerative process of the zebrafish's heart and can next use this information to develop therapeutic agents to limit serious complications of myocardial infarction and mortality rates.

Future work

Many studies are working on therapeutic drugs to reduce the mortality rates occurring due to myocardial infarction by producing a similar cascade that occurs in the zebrafish in the human's heart.

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