

Developmental research in zebrafish



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Over the past 20 years, the zebrafish is an organism that has proved to be a powerfully adept model for heart development. Zebrafish have the ability to survive in their respective environments without requiring an active circulation. They provide a helpful tool for research in cardiac development as well as genetics. Furthermore, the transparent, externally developing embryos of the Zebrafish provide a means for a much more detailed cellular analysis than regular animal embryos. Studies have indicated that there is a combination of molecular and cellular processes which are involved in zebrafish heart development. These processes comprise of progenitor specifications as well development of the conduction system and valves. There are also imaging studies as well as studies of cardiac development in mutant Zebrafish that have led to breakthroughs in the idea of heart development as a whole.

Before discussing the influence of Zebrafish on cardiac development. It is important to note how the general mechanism of cardiac development takes effect. Heart developments usually occur during gastrulation. The most pronounced movement that is present during zebrafish gastrulation is epiboly, which is the process of spreading of the blastoderm over the yolk cell from the animal to the vegetal pole. This process can occur via radial intercalation, and causes the thinning out and spreading of the tissue. Mediolateral intercalation can follow, or occur during late epiboly and is a type of convergent extension. It also forms the notochord tissue by using migration of mesendoderm cells toward the dorsal midline. The two main differences between radial intercalation and medio-lateral intercalation are that 1) during radial intercalation the surface covered by the cells extends in

all directions while in mediolateral intercalation the surface is ultimately extended in only one direction and 2) during mediolateral intercalation the cells first undergo a change in cell shape through widening and flattening and then are pulled toward the midline through intercellular contact. In the gastrulation phase, the heart is able to develop a mesoderm. The mesoderm is induced by prospective pharyngeal endoderm that presents itself during the neurula and postneurula stages. These stages are present in the early time development which comes directly after gastrulation. This explains that the mesoderm is capable of heart formation. During beginning of the gastrulation phase the superficial pharyngeal endoderm was removed and experimental embryos forms heart.

Heart developments are also present during organogenesis. In this, the heart begins with the formation of endocardial tubes which then merge to form tubular heart. Later, these tubes separate into four chambers and arterial trunks to form adult heart and this happens within the 4th week of development. The heart develops from embryonic mesodermal germ cells which differentiate in to mesothelioma, endothelium and myocardium after gastrulation. The outer lining of the heart is made up of mesothelial pericardium whereas the inner lymphatic and blood vessels lining is made up of endothelium.

There are many factors which make the development of the Zebrafish unique and make them a model organism. Zebrafish and vertebrates share more sequence and genetic similarity than many organism.. Due to the conservation of cell biological and developmental processes all vertebrates, studies in zebrafish can give great insight into human disease processes.

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Due to their small size and the simple nature of their natural environment, it is also easier to keep zebrafish in what appear to be more natural conditions. Zebrafish have a much larger number of offspring in each generation which provide for an extensive expanse of studies. They are easy to introduce changes to their genes using nothing more than chemical mutagens. Another factor is that cleavage pattern in zebrafish embryos have zebrafish egg is telolecithal, cleavage is meroblastic and discoidal. All teleosts show a discoidal meroblastic cleavage pattern. where the large yolk volume restricts cell division to a small area at the animal pole close to the micropyle. The heart of the Zebrafish begins to beat as it forms well-delineated chambers. Blood begins to circulate bilateral pair of aortic arches appear pharyngula period (Period of Early Development) “ pharyngula” to refer to the embryo that has developed to the phylogotypic stage. This leads to an important population of cells called somites becoming vital to heart development.

Somites are transient, segmentally organized structures. In vertebrate embryo, the somites contribute to multiple tissues, this are blocks of mesoderm located on either side of the neural tube in the developing vertebrate embryo. They are a population of cells which contribute to developing many of the important structures in vertebrates. Somite development is called somitogenesis. In the process of somitogenesis, paraxial mesoderm cells are organised into a whorl of cells called somitomeres which are not differentiated. As the cells mature, outer cells differentiate into epithelial cells which results in the formation of distinct boundary between cells. Somites are then separated into cranial and caudal portion, and metameric shifting occurs when the cranial portion of each fuses

with the caudal portion of somites anterior to it, Finally, as the body matures in a gradual process, the , somites gets differentiated into specific tissues of the body. The heart can several somites which help develop the cartilage and tissue of the heart. The somites in Zebrafish work in conjunction with a protein called CYPHER to induce heart development.

CYPHER is a protein that has been associated largely with cardiac and muscular myopathies. It has been found to not only aid in heart development, but in the development of Somites as well. It is a mRNA that was detected in the 3-somite stage in Zebrafish, and seemed to gradually grow to show expression within the whole somite. Working with the mechanism of hedgehog signaling, leads to the expression of CYPHER in zebrafish. An experiment which disrupted the signaling of hedgehog using cyclopamine lead to the diminishing of the mRNA in the somites. Overall, CYPHER is found to have an effect differentiation of muscle fibers and tissues in somites that aid in heart development, using the mechanism of downstream sonic hedgehog signaling.

Another protein factor known as proteoglycan exhibits significant effect in the heart development of Zebrafish. Proteoglycan is a type of heparan sulfate which is necessary for the migration of bilateral heart fields towards the midline. These are proteins that are heavily glycosylated. The basic proteoglycan unit consist of a core protein with one or more covalently attached glycosoaminoglycan chain. They are found in all connective tissue. Intracellular proteoglycan , serglycin serve as biological glue for most of intracellular proteases stored within the granule. It is packaged in the granule of most cells. Serglycin bind and modulate activity of several inflammatory

mediator chemokines, cytokines and growth factor. It is also found in wide variety of non immune cells such as endothelial, chondrocyte and smooth muscle cells. Cell surface seroglycin promote adhesion of myeloma cells to collagen and affect expressiin of MMP. It is a key componant of cell inflammatory response in activated primary human endothelial cells. It act as marker of immune myeloid cells and interact with TNF- alpha and proteases. It can also protect breast cancer cells from complement attack and support cancer cell survival. Heparin sulfate proteoglycan (HSPG), syndecan are prevalently associated with cell surface or the pericellular matrix. It is associated with plasma membrane of the cell either directly or via GPI anchor and function as biological modifier of growth factor FGF, VEGF and PDGF. It is responsible for presentation of growth factor to their coagnate receptor in their biological favorable form. It is also participate in long range maintenance of gradient for morphagen during embryogenesis and regenerative processes. Syndecan act as endocytic receptor and involving uptake of exosomes. Soluble syndecan -1 promote the growth of myeloma. Chondrin and dermatan sulfate containing proteoglycan function as structural constituents of complex matrix such as cartilage, brain , tendons and corneas. They also provide vesoelastic property, retain water, keep osmotic pressure, dictate proper collagen organization. CSPG act as receptor for clostridium difficile toxin B. Largest class of proteoglycan called small leucin rich proteoglycan (SLRP) function as both structural constituents and signaling molecule. SLRP interact with receptor tyrosine kinase that regulate fundamental process such as migration, proliferation, and angiogenesis. These proteins and signal cascades are in turn what lead to heart development in Zebrafish.

With the research conducted in regard to human and Zebrafish genomes, there are many ways to uncover more similar relationships. In order to use animals like Zebrafish for research, it is very important that they should be altered from the pair genes so that the effect that one desires could be determined. In many cases, some genes of the human DNA have been identified, which are incorporated in the animals so that the functions of these genes could be identified in a better manner. This results in altered functions in these genetically altered species and allows the researchers to study the effects of these genetic alterations in the species. This is done when a disease is suspected that might be having a genetic basis. Once this is done, the full spectrum of the action of the gene causing the disease could be identified. There are a number of methods for alteration of the animals on a genetic basis that includes micro injection Where, human DNA segment would be directly injected in the nucleus of a fertilised animal egg, that would be gestated in a surrogate female. When genes would be introduced in the DNA as these are expressed better. Retrovirus mediated transfer is where the virus is inserted the transgene of the cell. Transgenic animal strains are created when the germ cells have the required DNA. Somatic gene therapy is used to integrate genes into somatic cells where modified vectors are integrated into somatic cells so that the normal function of the cell can be restored. For species including frog, mouse, rat and pig, sperm mediated transfer is used where the DNA is introduced in the head of the sperm. For drug research, animals like zebrafish are frequently used, where one of the first demonstrations was done for cystic fibrosis. For expressing human proteins, transfected cell lines are frequently used. For Huntington disease, animal models have been used a s drugs inducing autophagy were

identified. There have also been things like fluorescent proteins found in Zebrafish embryos. Using these proteins we can conduct a fate map of the embryo. In developmental biology, fate map is a method used to determine the embryonic origin of various tissues. They tell us about the origin of particular cell groups, and movements of cells in an embryo. When the same method is carried out at single-cell resolution, this process is called cell lineage tracing. Recent advancement of science made it possible to use fluorescent peptide tracers to determine fate map. In this method, the donor embryo is treated with fluorescent dye. The host embryo continues to develop. To know the fate of the tissue, at any point in the overall developmental process the embryo is sectioned using microtome, prepared and examined under a fluorescent microscope.

Things such as biological clocks may also be used for Zebrafish and uncovering genetic relationships. A circadian clock is a biochemical oscillator in our biological system that is synchronized with solar time. It is our natural system which tells our body to secrete different biochemical products for day and night, it helps our body to differentiate between day and night. The circadian clock in zebrafish is able to regulate the timing of the cell cycle, and demonstrate the profound impact that this type light sensitivity and daily rhythmicity may have in developmental and genetic biology. Generally, circadian clock depends on photoreceptors which are normally the eyes in higher animals like mammals, but in zebrafish, experiments show that peripheral cells show photoreceptors. In zebrafish, the circadian clock is important as the presence of sunlight helps in DNA Transcription and repair mechanism in the organism, the replication of DNA depends on circadian

clock even in embryos. This is as far as research goes this is what is known till now. Zebrafish also display similarities in the CRYPTIC protein of their retina and heart. Both of the organs display the same protein but there has not been much research conducted to see if the protein is the same or a variation. The CRYPTIC gene in heart and retina have a difference of 100 amino acids. This can be from the same mRNA or from different. To find out, we can isolate mRNA and carry out microarray experiment. The probe can be synthesized which will bind to CRYPTIC mRNA of one of the organ. If the probe binds to mRNA of both the organ then it might be possible that they are from the same mRNA. We can use the RNA editing enzyme, which enzyme can alter the U to C change point mutations. This can add a stop codon or remove a stop codon. This would change the mRNA in a cell where the editing is active. The mRNA length would be same but protein will be of different length. There is also post-translational processing in which, the protein can be edited after it is translated and the signal sequences and other leader sequences can be removed. This can change the length of the protein. The mRNA will be of the same length, but the protein in retina will be trimmed to give smaller protein. It is also possible to use mutations in the DNA that will result in a longer protein. This can change the DNA which might add new bases to the protein. The DNA in the heart can contain this mutation which results in longer protein in the heart. The mRNA in heart and retina will have different lengths and so is the protein. While there is a large amount of research present on Zebrafish, experiments like these could help fill in the small gaps that remain.

Zebrafish provide close relationships of development in correlation to humans. They are something that can be used to study a spectrum of diseases. Human genomes share similarities to Zebrafish, further clarifying why some developmental processes may be so similar. All in all, Zebrafish are an important aspect of developmental research. They have the ability to regenerate their fins, skin, heart and, in larval stages, brain. Zebrafish heart muscle regeneration does not make use of stem cells; instead, mature heart muscle cells regress to a stem cell-like state and differentiate. Zebrafish can also regenerate photoreceptor cells and retinal neurons following injury. This has been shown after research to be mediated by the dedifferentiation and proliferation of Müller glia. Researchers frequently amputate the dorsal and ventral tail fins and analyze their regrowth to test for mutations. It has been found that histone demethylation occurs at the site of the amputation, switching the zebrafish cells to an “ active”, regenerative, stem cell-like state. There is another focus of zebrafish research that concentrates on understanding how a gene called Hedgehog which was present in the CYPHER-somite regulation for the heart, is also a biological signal that underlies a number of human cancers, controls cell growth. In probing disorders of the nervous system, including neurodegenerative diseases, movement disorders, psychiatric disorders and deafness, researchers are using the zebrafish to understand how the genetic defects underlying these conditions cause functional abnormalities in the human brain, spinal cord and sensory organs. Researchers are also delving into the complexities of muscle degeneration in genetic models of human musculoskeletal diseases, such as muscular dystrophy. These fish have helped uncover many aspects to the world of development and their similarities to human development is a

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factor we can use to find solutions for the developmental questions that have evolved over time. A heart is one of the most vital and intriguing organs of the body. It is what gives us life. By using organisms like Zebrafish, we can find numerous answers to not only the questions surrounding things like heart development, but also how to remedy cardiovascular diseases, and further technological advances into creating something like an artificial heart.

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