Time Dependency of Heat-shocking *oep*^{m134} and Its Penetrance

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Neural tube defects are one of the most common birth defects in the United States. (Parker et al., 2010) Approximately 1,500 babies are born with spina bifida and 860 with anencephaly every year in the United States. (Parker et al., 2010) Both anencephaly and spina bifida are abnormalities occurred on the spinal column to the brain. Spina bifida is the most common type of neural tube defects and is caused by the failure to close the posterior region (lumbar region) of the neural tube properly. Anencephaly is the less common type of neural tube defects and is caused by the failure in anterior (brain) neural tube closure. (Wood and Smith, 1984; Copp and Greene, 2013) Pregnancies involving anencephaly usually have miscarriage, or babies die at birth. (Copp and Greene, 2010) The exact causes of these neural tube defects are still unknown. However, recent studies found that environmental factor, nutrition and genetic involved in causing these neural tube defects. In this experiment, genetic level of neural tube defects will be studied.

Nodal signals are secreted proteins in the TGFβ super family, and have many effects on the development of vertebrates including the development of mesoderm and endoderm, the regulation of left-right asymmetry of organs, the patterning of the nervous system and developing anterior-posterior axis (Schier et al., 2000; Schier 2003). Nodal signals are one of many conserved pathways in vertebrate development, and found in mice, chicken, frogs, and zebrafish. Zebrafish has three Nodals: Squint (Sqt), Cyclops (Cyc), and Southpaw (Spaw) (Feldman 1998; Long 2003). All three Nodals work with a receptor connected to a membrane associated protein known as One-eyed pinhead (Oep) (Schier et al., 2000; Schier 2003). It is known that Oep and Sqt are significant players in the anterior neural tube closure (Aquilina-Beck, 2007). When nodal mutations in the *oep* or *sqt* genes occur, mesoderm development is affected indirectly and the result is an open neural tube (Aquilina-Beck, 2007).

Neurulation is the process required for the formation of the neural tube in vertebrates and has two different mechanisms: primary neurulation and secondary neurulation. (Copp et al., 2003; Lowery and Sive, 2004) Primary neurulation starts with folding the epithelial sheet occurring in the anterior area of the future nervous system, while the secondary neurulation begins in the posterior area (Copp et al., 2003; Lowery and Sive, 2004). During the process of the primary neurulation, neural tube precursors (cells) on neural plate start to move upward and toward each other from opposite edges of neural plate. (Copp et al., 2010) During neurulation, notochord acts like a hinge. When neural tube precursors meet in the middle and above notochord, the precursors pinch off from the ectoderm layer, and the whole plate becomes a neural tube (Copp et al., 2010). The precursor cells located laterally on the neural plate was stained in order to trace the formation of neural tube. (Papan and Campos-Ortega, 1994) The stained precursor cells were found dorsally, while the medial cells on the plate ended up on ventral side of the embryos. According to the neurulation process, the neural tube folded upward. Therefore, the NT closure was detected by staining the lateral precursor cells on the neural plate.

In order to understand neural tube defects in humans, a simpler vertebrate species can be studied. Zebrafish are ideal model organisms in general because they are small, reproduce quickly, and are inexpensive to maintain. More specifically however, zebrafish are useful for study of vertebrate development (Dooley and Zon, 2000). This is because not only is the zebrafish genome well known, but there are hundreds of mutant phenotypes that have been identified, some that are similar to human disorders (Dooley and Zon, 2000). For example, neural tube defects in both humans and zebrafish can result from a disruption of nodal proteins (Pei and Feldman, 2009).

In this project, the environmental factor, specifically the effective time range for heat-shocking embryos, is tested. From the previous UROP project, the temperature of the embryo's environment is affecting the severity of the neural defects in *oep* mutants. The hypothesis of this project is that the timing of the heat-shock (placing the embryos at 34°C) is also affecting the penetrance of the complete, or incomplete neurulation in oep^{m134} mutants at different stages of zebrafish (Danio rerio) embryogenesis. Homozygous *sqt* and *cyc* mutants display cyclopia (Pei et al., 2007). Presence of the cyclopic phenotype can therefore be used as a way to identify embryos that are carriers of the *sqt* gene mutation. In this way, mutations of genes that encode the proteins important to the nodal signaling pathway of zebrafish can be used to obtain embryos with neural tube defects. The penetrance of the *sqt* mutant open neural tube phenotype is incomplete however (Pei et al., 2007). When an embryo lacks Sqt due to a *sqt* mutation, cyclopia can be prevented if there is an adequate level of Activin-like signaling. However, since the amount of Activin-like signaling is based on heredity, the penetrance of the *sqt* mutant cyclopic phenotype is variable (Pei et al., 2007). Research on *sqt* mutants has shown that various environmental factors are able to impact the penetrance of the cyclopic phenotype in zebrafish (Pei et al. 2007). One such factor includes the temperature at which embryos are exposed to very soon after fertilization. Ideal temperature conditions for zebrafish embryos are around 28ºC. Pei and his colleagues found that subjecting *sqt* mutants to a 34 ºC heat shock overnight resulted in a marked increase in the cyclopic eye. They found that exposing embryos to the increased temperature between 0-3 hours post fertilization led to increased cyclopia, but also increased lethality. Heat shock between 2.5-5 hours post fertilization also was found to induce cyclopia. After 6 hours post fertilization, no temperature sensitivity could be found (Pei et al., 2007). These findings show that there is key point between the 2.5 and 5 hours post fertilization where applying a heat shock increases the penetrance of the cyclopic phenotype. Ideal time for Nodal effectiveness is up to late mid-blastula stage of 4.3 hour post fertilization. (Gonsar et al., submitted)

To test the hypothesis, multiple heterozygous *oep*$^{m134/+}$ pairs were set up and individual clutches were collected at different time point. Each clutch was separated into half and incubated at the standard temperature (28.5°C) and higher temperature (34°C). By incubating mutant embryos at higher temperature, the higher open neural tubes were expected to find. At 0-2 hours post fertilization, the mutant embryos, incubated at 34°C, had higher percentage of open neural tube than the embryos at 28.5°C. However, there was no significantly difference between two temperatures at the older stages of embryos. Furthermore, the timing at which nodals required was not the same as the previous finding. The effective time range got smaller. The timing at the mid-blastula was not the optimal time at which nodals required for neurulation. Our result varied from the previous experiment. The expected result was higher percentage of open neural tube at the higher temperature. However, there were some opposite result and some did not even show the open neural tube. Could the genetic background be the answer to the vaiation? We refuted the hypothesis because the only correlation was found. There was significant difference between two temperatures at different time points. However, the error bars collapsed at higher time points and the trend only

showed the correlation. Furthermore, the variation in clutches indicated that there were more than timing of heat shock and temperature involved.

Understanding the nodal signals will help better understanding the nature of TGFβ signaling pathway and its roleplays in different time points throughout the lifespan of a vertebrate organisms. Mayo clinc site only claimed that women with pregnancy should not use hot bath tub because the hot tub could create hyperthermia conditions for the babies and would induce NTD. However, there was no data analysis and no primary literature to base on. This research will also further expand more questions to be answered related to the nervous system development in early embryogenesis.

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