Cardiac Neural Crest and Cardiac Regeneration

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Contributor: Shannon Erhardt , Jun Wang

Neural crest cells (NCCs) are a vertebrate-specific, multipotent stem cell population that have the ability to migrate and differentiate into various cell populations throughout the embryo during embryogenesis. Based on the initial axial position and site of contribution, NCCs are divided into specific subpopulations, such as the cardiac neural crest (NC), which mainly contributes to the cardiac valves, interventricular septum, and both the aorta and pulmonary vessel. The heart is a muscular and complex organ whose primary function is to pump blood and nutrients throughout the body. Mammalian hearts, such as those of humans, lose their regenerative ability shortly after birth. However, a few vertebrate species, such as zebrafish, have the ability to self-repair/regenerate after cardiac damage. Recent research has discovered the potential functional ability and contribution of cardiac NCCs to cardiac regeneration through the use of various vertebrate species and pluripotent stem cell-derived NCCs. This potential regenerative capacity to cardiac tissue poses interesting avenues to advance the treatment of various cardiac diseases. Heart disease, a leading cause of death in the United States, results in death or severe damage to the function and/or structural integrity of the heart. Determining the contribution and regenerative capacity of the cardiac NC in mammalian systems is of high clinical significance. Here, the focus is on the NC's regenerative capacity in various tissues and systems, and in particular, the characteristics of cardiac NCCs between species and their roles in cardiac regeneration are summarized. Emerging and future work to determine the potential contributions of NCCs for disease treatment will be further discussed.

neural crest cells (NCCs) regeneration heart structure cardiac regeneration

1. Cardiac Neural Crest Contribution to the Heart Between Species

1.1. Mouse

The cardiac structure of the mouse (Mus musculus) is four-chambered, consisting of two atria and two ventricles, similar to that of the chick. Cardiac neural crest cells (NCCs) in the mouse delaminate to pharyngeal arch arteries 3, 4, and 6, which will then further migrate to the heart[1]. Similar to evidence found in chicks, a growing number of studies also suggested the ability of mouse NCCs to differentiate into cardiomyocytes[2][3][4][5][6][7], yet conclusions may vary using different NCC lineage-tracing mouse models[8][9][10].

Tang et al. examined mice that had NCCs marked using cytoplasmic GFP (Wnt1-Cre;ZsGreen$^{fl/fl}$) and found that at embryonic day (E) 15.5, a large number of cells in the OFT, interventricular septum, and myocardium of the ventricles were NC positive[2]. The authors do note that the number of Wnt1-Cre-positive cells remains stable

postnatally and does not undergo cell division or apoptosis, providing similar information that has been noted in both chicks and zebrafish, indicating an evolutionary role of the NC within these cardiac regions[2][11][12]. The finding of the NC population in both of the mouse ventricles warrants further investigation into the NC-specific function in these regions.

1.2. Zebrafish

Unlike the structure of the amniote heart, zebrafish (Danio rerio) hearts are tubular in structure, consisting of one atrium and one ventricle, and maintain a one-directional flow[12][13][14]. As the developing heart in zebrafish varies compared to amniote models, this suggests that NC contribution varies as well. In contrast to the mouse or chick, the zebrafish heart does not consist of a ventricular septum due to having a single ventricle, clearly indicating varying cardiac NC contribution[12][14]. Sato and Yost found that in zebrafish, cardiac NCCs contribute to the bulbus arteriosus, ventricle, atrioventricular junction, atrium, and muscle formation in the myocardium[11]. The Kirby group also found that by using cell marking, cardiac NCCs migrated to the myocardial wall of the heart tube, and laser ablation of the pre-migratory cardiac NCCs resulted in the loss of NC migration to the heart and failed heart looping, along with reducing the ejection fraction and cardiac output[12]. Although it has further been corroborated that NCCs integrate into the myocardium in the zebrafish heart, the Chen group found that NCCs in the zebrafish can be characterized into two populations: one that gives rise to cardiomyocytes and another that contributes to the endothelium and bulbus arteriosus[15]. Furthermore, the Chen group found that ablation leads to reduced heart rates, defective myocardial maturation, and failure to recruit progenitor cells from the second heart field, indicating the need for further investigation into the cell–cell communication between the various cardiac contributing cell populations[15].

2. Regenerative Capacity of the Neural Crest

2.1. Gastrointestinal Tract and Enteric Nervous System

One field of interest that has sparked an investigation into NC regenerative capacity is the gastrointestinal tract. The coordination of the gastrointestinal tract is regulated by the enteric nervous system (ENS), a network comprised of neurons and glial cells that arise from the NC[16][17]. To understand the contribution of the NC to this highly regenerative area, Kruger and colleagues investigated the gastrointestinal tract in adult rats and found the persistent existence of NCCs. These postnatal NCCs were able to self-renew extensively in culture but were overall not as extensive as NCC regeneration of the fetal gut[18]. Furthermore, the authors were able to determine that the NCCs of the adult gut were still active and able to give rise to neuro-transmitting neurons. However, it was determined that NCCs of the adult gut were unable to give rise to specific neural subtypes that are present in the fetal gut[18]. They suggest that this reduction in producing various neuronal subtypes involves a loss of BMP expression but an increased response to gliogenic factors at postnatal stages[18]. Although the complete functional significance of NCCs in the adult mammalian system is still unknown, these findings of NCCs in the adult gut suggest new possibilities for NCCs in regeneration.

2.2. Cranial Bones, Bone Marrow, and Teeth

One major contribution of the NC is to assist in cranial skeletal formation during embryogenesis, including cartilage and bones of the head[19][20][21][22]. Beyond cranial bones, studies have also indicated that NCCs contribute to bone marrow and tooth formation[23][24]. However, whether the NC can re-activate stem cell-like properties or NCCs maintain multipotency postnatally in such structures is still under investigation.

Although NCCs give rise to the majority of bone, cartilage, and connective tissues of the skull, little is known about the regenerative ability of the NCC lineage in cranial structures after injury or disease. However, regarding the reactivation of stem cell-like characteristics in adult bones, Ransom et al., with the use of a mandibular distraction osteogenesis mouse model, identified that after injury, NC development-related genes, including sox10, sox18, and elk3, were upregulated within newly forming bones of the jaw[25]. Furthermore, it was identified that post-migratory cranial NCCs were not only self-renewing and able to form bone matrix in culture, but that subcutaneous transplanted post-migratory cranial NCCs in mice were able to regain their ability to differentiate into osteocytes and adipocytes, along with assisting in calvaria bone formation[26]. However, the ability and corresponding mechanisms of cranial NCCs to re-active or maintain their multipotency within bones of the skull and to properly contribute to new bone formation have yet to be deciphered.

2.3. Peripheral and Central Nervous System

During development, NCCs from the trunk region give rise to numerous sub-lineages, including glial cells of the peripheral nervous system (PNS). Glial cells contribute to the structure of both the PNC and central nervous system (CNS), assisting in the protection and regulation of neurons, particularly through NCC-derived Schwann cells[27]. The regeneration of nerve fibers and their supporting cells has been a standing field of interest regarding functional recovery to structures such as the spinal cord after injury.

Recent advances regarding PNS regeneration have been made possible due to the use of NC-like stem cells derived from human embryonic stem cells or iPSCs. For example, Huang and colleagues used NC stem cells derived from iPSCs to construct nerve conduits that, when implanted into a rat sciatic nerve transection model, were able to increase functional nerve recovery[28]. Supporting studies using sciatic nerve defect models and NC-derived human iPSCs found that in vivo, grafted cells proliferated and successfully migrated throughout the conduit after transplantation[29]. Furthermore, Kimura et al. discovered that grafted NC-derived iPSCs also contributed to the increased strength of the leg muscle, indicating functional recovery of the sciatic nerve after injury[29]. Similar conclusions have been made by other groups, indicating that NC-derived iPSCs or NC-derived embryonic stem cells are a valuable tool that can contribute to nerve regeneration. However, the mechanisms of this ability have yet to be determined[30][31][32].

3. Cardiac Neural Crest in Cardiac Regeneration

During early mammalian development, the heart maintains its regenerative capacity; however, shortly after birth, this ability is lost. Postnatal cardiac progenitors remain a challenging and controversial issue in the cardiac field. Recent studies have begun to investigate the potential ability of the heart to re-activate regenerative ability, particularly through the NC, to assist in cardiac regeneration after injury.

It has previously been established that zebrafish maintain regenerative abilities throughout their systems, including the fins and retina[33][34][35][36]. Furthermore, it has been identified that adult zebrafish hearts are able to regenerate ventricular myocardium, without scarring, through cardiomyocyte dedifferentiation and proliferation[37][38]. However, until recently, it was unknown whether the NC population assists in this regeneration capacity of the heart. In addition to the established NCC contributions to cardiovascular development, numerous groups recently determined that NCCs in the zebrafish heart also contribute to the cardiomyocyte population[2][39][40]. Based on this, Tang and colleagues further investigated whether the NC population of the zebrafish heart also plays a role in cardiac regeneration. Using the zebrafish transgenic line *Tg(-4.9sox10:eGFP)* to examine NCCs, it was found that although sox10 expression is down-regulated after NCCs reach the heart, the removal of a portion of the adult ventricular apex stimulates sox10-GFP expression, along with the NC marker tfap2a, in cardiomyocytes near the injury site, suggesting the reactivation of a NC-like state for cardiac regeneration[2] (**Figure 1**). Furthermore, Sande-Melón et al. determined that pre-existing sox10-positive NCCs not only contributed to the zebrafish adult heart but that after ventricular cryoinjury, the number of sox10-expressing cells significantly increased in the myocardium near the injured area[39] (**Figure 1**).

Although regeneration in zebrafish has shown promising roles for NCCs in cardiac regeneration, less is known about the contribution of the NC during mammalian cardiac regeneration. Similar to zebrafish, it was identified that NCCs are present in the postnatal mouse heart and can differentiate into cardiomyocytes[3][4][5][6][7]. Tamura and colleagues found that after myocardial infarction in adult mice, GFP-expressed NC-derived cells migrated to the border of the infarcted region and were able to differentiate into cardiomyocytes, contributing to the regeneration of the myocardium[5]. The authors suggest that this migration after myocardial infarction is due to monocyte chemoattractant protein-1 (MCP-1) expression in the infarcted area that provides guidance cues to NCC derivatives[5] (**Figure 1**). In contrast, although Hatzistergos and colleagues found that a population of NCC lineages generate cardiomyocytes postnatally, these cells were not proliferative and had lost their regenerative capacity[3].

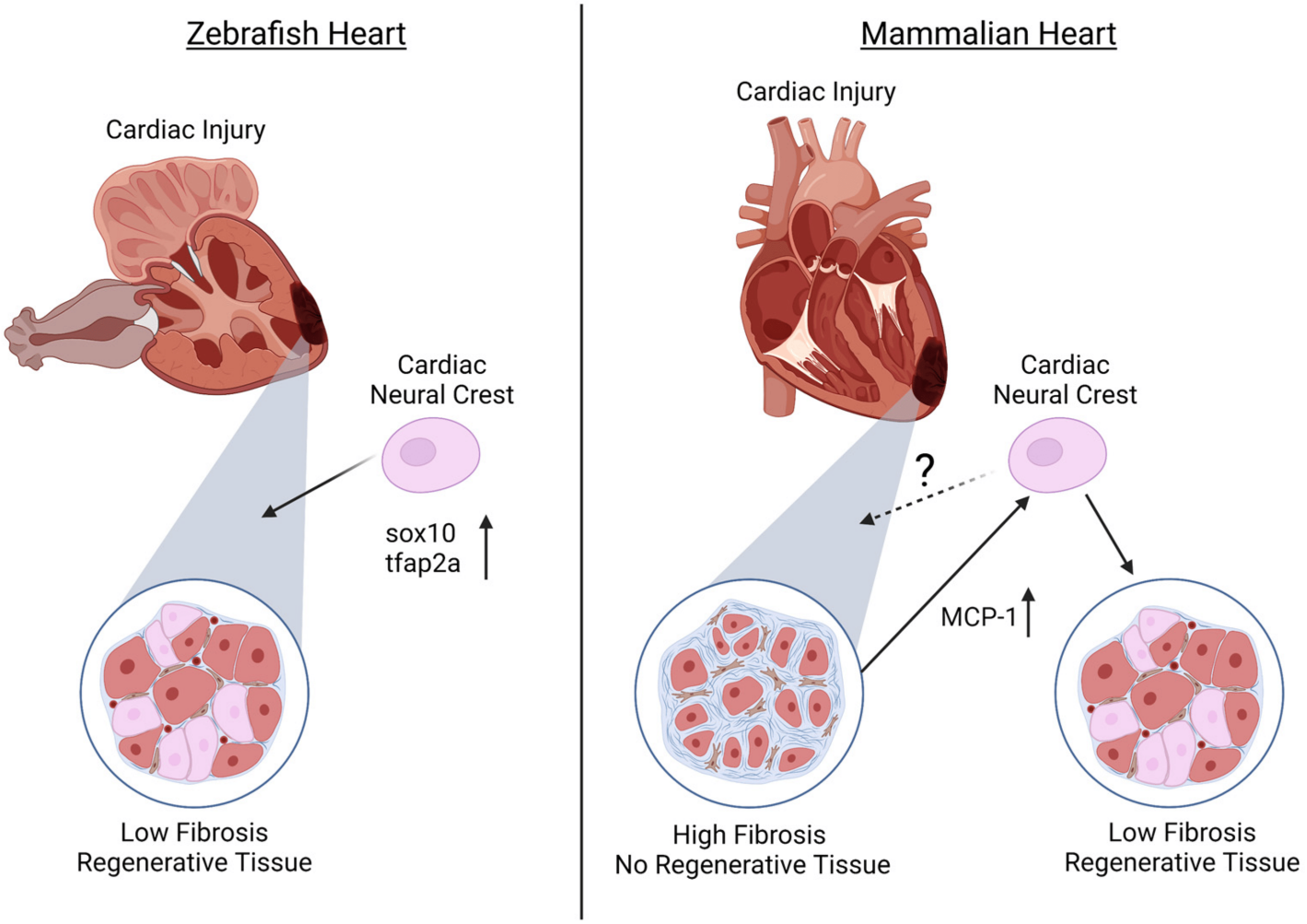

Figure 1. The ability of the cardiac neural crest (NC) to contribute to regeneration after cardiac injury in zebrafish and mammalian hearts. In the zebrafish, injured ventricular tissue has been shown to populate a large number of NC-positive cells that express high levels of *sox10* and *tfap2a* that give rise to cardiomyocytes and myocardium of the regenerated ventricle. In the mammalian heart, the contribution of the NC to the ventricle and their ability to regenerate or maintain a stem cell-like state is still unknown. Although mammalian ventricular cardiac injury results in scarring and fibrosis with little-to-no regenerative ability, there is potential that the injured tissue releases the chemokine MCP-1, signaling to cardiac neural crest cells (NCCs) to migrate to the injury site to assist in tissue regeneration of the ventricle. (Created with Biorender, accessed on 29 November 2022).

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