

Video Article

Dissection of the Adult Zebrafish Kidney

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Abstract

Researchers working in the burgeoning field of adult stem cell biology seek to understand the signals that regulate the behavior and function of stem cells during normal homeostasis and disease states. The understanding of adult stem cells has broad reaching implications for the future of regenerative medicine¹. For example, better knowledge about adult stem cell biology can facilitate the design of therapeutic strategies in which organs are triggered to heal themselves or even the creation of methods for growing organs *in vitro* that can be transplanted into humans¹. The zebrafish has become a powerful animal model for the study of vertebrate cell biology². There has been extensive documentation and analysis of embryonic development in the zebrafish³. Only recently have scientists sought to document adult anatomy and surgical dissection techniques⁴, as there has been a progressive movement within the zebrafish community to broaden the applications of this research organism to adult studies. For example, there are expanding interests in using zebrafish to investigate the biology of adult stem cell populations and make sophisticated adult models of diseases such as cancer⁵. Historically, isolation of the zebrafish adult kidney has been instrumental for studying hematopoiesis, as the kidney is the anatomical location of blood cell production in fish^{6,7}. The kidney is composed of nephron functional units found in arborized arrangements, surrounded by hematopoietic tissue that is dispersed throughout the intervening spaces. The hematopoietic component consists of hematopoietic stem cells (HSCs) and their progeny that inhabit the kidney until they terminally differentiate⁸. In addition, it is now appreciated that a group of renal stem/progenitor cells (RPCs) also inhabit the zebrafish kidney organ and enable both kidney regeneration and growth, as observed in other fish species⁹⁻¹¹. In light of this new discovery, the zebrafish kidney is one organ that houses the location of two exciting opportunities for adult stem cell biology studies. It is clear that many outstanding questions could be well served with this experimental system. To encourage expansion of this field, it is beneficial to document detailed methods of visualizing and then isolating the adult zebrafish kidney organ. This protocol details our procedure for dissection of the adult kidney from both unfixed and fixed animals. Dissection of the kidney organ can be used to isolate and characterize hematopoietic and renal stem cells and their offspring using established techniques such as histology, fluorescence activated cell sorting (FACS)^{11,12}, expression profiling^{13,14}, and transplantation^{11,15}. We hope that dissemination of this protocol will provide researchers with the knowledge to implement broader use of zebrafish studies that ultimately can be translated for human application.

Video Link

The video component of this article can be found at <http://www.jove.com/video/2839/>

Protocol

1. Dissection of the adult zebrafish kidney from an unfixed animal sample

1. Select an adult zebrafish between 3-6 months in age for dissection.
2. Euthanize the fish by placing it into a dish with 0.2% Tricaine pH 7.0 for approximately 4-5 minutes, watching carefully to see that the gills stop moving and the heart stops beating.
3. Using a spoon, lift the fish from the Tricaine bath in a small amount of solution, decant the solution and gently place the animal on a paper towel.
4. Use a sharp pair of dissection scissors to remove the head of the animal, making a cut just behind the gill operculum (Figure 1, path A). Discard the head in a biohazard waste container.
5. Use the dissection scissors to open the body of the animal, by making a long ventral incision, starting from the head and terminating at the tail at the base of the caudal fin (Figure 1, path B).
6. Remove the internal organs of the animal using a pair of dissection tweezers/forceps, and place them in biohazard waste. Be careful not to brush the dorsal body wall, as this is the location of the kidney (Figure 2). If you have selected a female fish, you will need to scoop developing eggs out of the body cavity, which can be most easily done using an angled pair of forceps or tweezers.
7. Use dissection needles to pin open the body walls to a dissection tray, and angle the pins so that the kidney can be visualized. The kidney will possess a characteristic shape (head, trunk/saddle, tail) and color, being interspersed with black hued pigmentation (Figure 2). You may also see the aorta filled with peripheral blood, which runs down the midline of the organ structure.

8. Use fine forceps to detach the kidney from the dorsal body wall, using one pair of forceps to lift the kidney as you separate the organ from the connective tissues that underlie it.
9. Gently place the kidney into the desired vehicle for further live cell studies, such as a microcentrifuge tube containing 1x PBS for FACS preparation.

2. Preparation and dissection of the adult zebrafish kidney from a fixed animal sample

1. Prepare a solution of 4% paraformaldehyde/1X PBST. Boil the solution to dissolve the PFA into solution, then cool the mixture to 4°C and add 0.1% dimethylsulfoxide. We have had the most success with tissue preservation from using freshly made PFA solutions as opposed to frozen aliquots. Make enough solution to submerge the adult zebrafish during dissection.
2. Fill a dissection tray with enough fixation solution to submerge the adult zebrafish during the dissection. We perform the subsequent steps with this tray positioned under the microscope, which can be placed in a chemical hood to minimize exposure to fixation odors.
3. Select an adult zebrafish between 3-6 months in age for dissection.
4. Euthanize the fish by placing it into a dish with 0.2% Tricaine pH 7.0 for approximately 5 minutes, watching carefully to see that the gills and heart stop beating.
5. Using a spoon, lift the fish from the Tricaine bath in a small amount of solution, decant the solution and gently place the animal on a paper towel.
6. Use a sharp pair of dissection scissors to remove the head of the animal, making a cut just behind the gill operculum (Figure 1, path A). Discard the head in a biohazard waste container, and immediately place the animal into the PFA-containing dissection tray.
7. Use the dissection scissors to open the body of the animal, by making a long ventral incision, starting from the head and terminating at the tail at the base of the caudal fin (Figure 1, path B), and keep the body partially submerged during the procedure.
8. Remove the internal organs of the animal using a pair of dissection tweezers/forceps, and place them in biohazard waste. Be careful not to brush the dorsal body wall, as this is the location of the kidney (Figure 2).
9. Use dissection needles to pin open the body walls, and angle the pins so that the kidney can be visualized. The kidney will possess a characteristic shape (head, saddle, tail) and color, being interspersed with black hued pigmentation (Figure 2). You may also see the aorta filled with peripheral blood, which runs down the midline of the organ structure.
10. Fix the sample overnight, placing the tray at 4°C when you are done with arranging the desired samples.
11. The next day, remove the PFA solution from the tray and replace with 1x PBST.
12. Use fine forceps to carefully detach the kidney from the dorsal body wall, using one pair of forceps to lift the kidney as you separate the organ from the connective tissues that underlie it. The kidney tissue will be soft and pliable from the DMSO in the fixative solution, making it possible to dissect the entire kidney organ in one piece.
13. Use a wide-bore transfer pipet to place the kidney into a microcentrifuge tube.
14. The kidney can now be processed further to conduct histological or whole mount *in situ* gene expression studies based on the desired study.

3. Representative results:

The steps that are diagrammed in Figure 1 indicate the dissection procedure. The kidney can be identified based on its characteristic shape and coloration, and anatomical location on the dorsal wall of the animal's body cavity, as shown in Figure 2. After dissection of a fixed kidney sample, whole mount *in situ* hybridization can be used to localize the expression of a gene of interest, as shown in Figure 3.

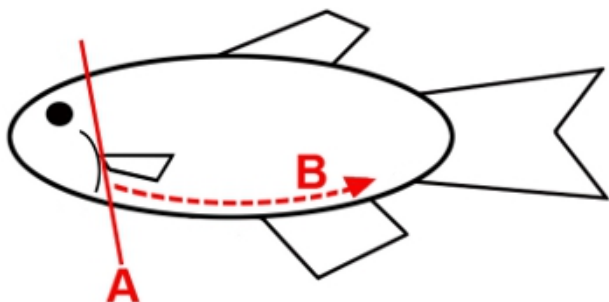


Figure 1: Procedure that enables direct access for dissecting the zebrafish adult kidney. (A) Euthanized zebrafish is dissected in a stepwise fashion (indicated by numbers and arrows) to give the researcher the easiest access to the complete kidney organ. Images of the abdominal cavity before (B) and after (C) removal of the abdominal organs.

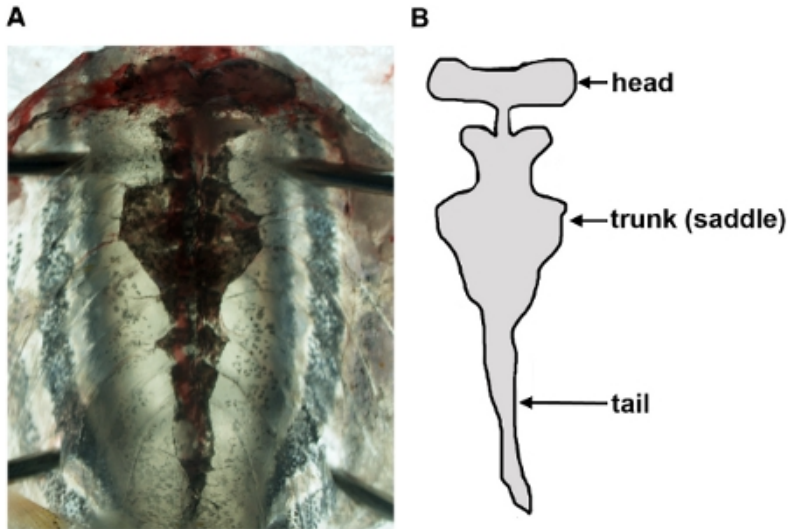


Figure 2: Visualization of the zebrafish kidney in an unfixed sample. Following removal of organs in the body cavity, the kidney appears as a single, flattened organ that is adherent to the dorsal body wall via connective tissues (A), and has been schematized (B) to show its anatomical shape.

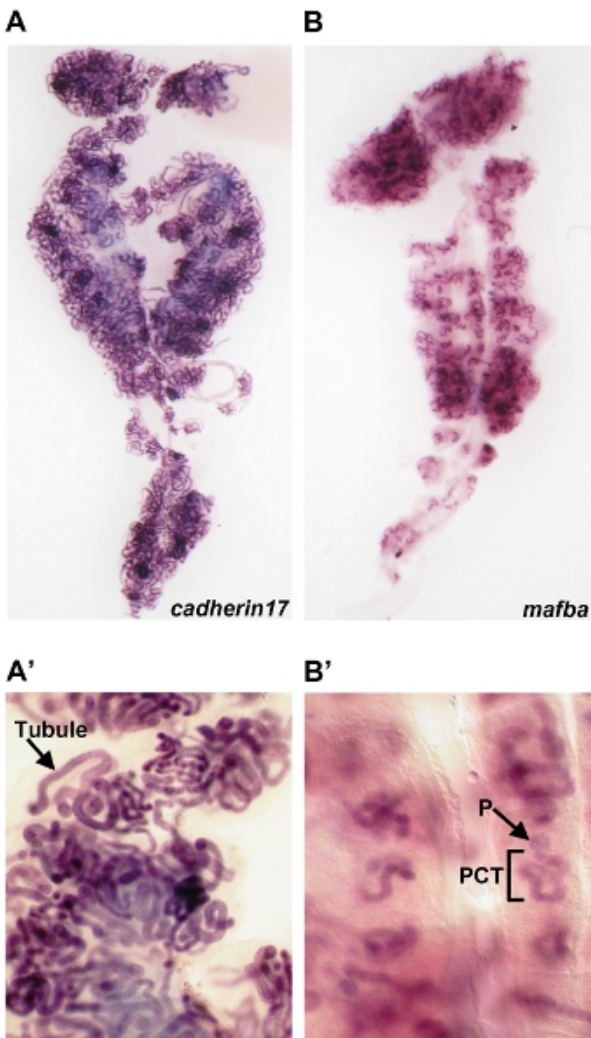


Figure 3: Whole mount *in situ* hybridizations performed on the whole adult kidney organ. (A) *cadherin17* transcripts are detected in the tubule of adult kidney nephrons, enlarged in (A'). (B) *mafba* transcripts are localized proximal nephron cell types in the adult kidney (compare to

entire tubule in panel A). (B') An enlarged view of *mafba* expression reveals that transcripts are specifically localized to the podocyte (P) cells of the blood filter (glomerulus) and proximal convoluted tubule (PCT).

Discussion

Adult stem cells are dynamic and essential components that maintain the adult body form¹. Adult stem cells can also serve to counteract damage that the body incurs during disease states, and the dysfunction or loss of adult stem cells can lead to the decline of organ function and has been implicated to drive cancer malignancies and contribute to aging. Adult stem cells exhibit cellular properties that distinguish them from differentiated cell types. Upon cell division, adult stem cells are capable of self-renewal and the production of progenitor cells that in turn can give rise to distinct differentiated offspring. There is great interest in understanding the pathways that regulate the cell fate decisions and potency of adult stem cells because of their important roles in tissue homeostasis¹. For example, many scientists currently seek to identify the signals that maintain various adult stem cell populations in their niche, or specialized microenvironment, and how this niche is impacted by humoral factors.

Adult hematopoietic stem cells (HSCs) are pluripotent cells that fuel the continual rejuvenation of blood cells in animals⁸. Hematopoiesis is a vibrant process that is constantly ongoing during the life of a vertebrate organism because the mature differentiated blood cell types are short-lived and hence must be replenished regularly. As they divide, HSCs supply this crucial demand by self-renewing and producing a series of progenitors that give rise to the erythroid, megakaryocyte, lymphoid and myeloid lineages. Each of these blood cell types performs unique functions in the circulation, including providing gas exchange to tissue, a way for sealing injuries to blood vessels by means of clot formation, or defense mechanisms against invading pathogens. While HSCs have been recognized for many years, a plethora of fascinating questions remain unanswered about these amazing cells. Recent studies have identified subclasses of HSCs with different long-term self-renewal properties, and the elucidation of niche components and signals that modulate HSC behavior⁸. The zebrafish is now among the cornerstones of hematopoiesis research paradigms, based on the genetic and molecular attributes of this animal model⁸. Research utilizing the zebrafish has led to novel insights about HSC biology that are conserved with higher vertebrates like mammals, emphasizing the biomedical relevance of fish blood investigations⁸.

Historically, knowledge about HSCs has paved the way for the expeditions seeking to discover if other body organs are maintained by adult stem cells. Numerous adult stem cell populations are now appreciated to exist, ranging across diverse structures from the brain to the intestine, skin, and skeletal muscle. Continued efforts in the stem cell field seek to identify how adult stem cells and even other differentiated cell types can display enhanced potency in various settings.

With respect to the kidney, there has been great debate among nephrologists as to whether renal stem cells exist¹⁶. Independent findings have documented populations of kidney cells in mammals that possess varying degrees of regenerative potential, but a cohesive understanding of these reports has yet to be achieved. Interestingly, there is strong evidence to support the notion that many fish species possess cells that can robustly fuel regeneration of damaged nephrons, as well as grow entirely new nephrons during adulthood⁹. Recent work has identified the molecular hallmarks of adult kidney stem cells in the zebrafish^{10,11}, and demonstrated the self-renewal potency of these so-called renal stem/progenitor cells (RPCs)¹¹. Future studies are needed to ascertain whether analogous kidney cells are present in mammals. Nevertheless, continued investigations about the cellular and molecular mechanisms that regulate fish RPCs may provide insight into how regenerative feats may be stimulated in the mammalian kidney. It is evident that much remains to be understood about RPCs, and that the many tools now available to the zebrafish researcher can be implemented to tackle these intriguing questions.

In this protocol, we describe our method for the anatomical identification and dissection of the adult zebrafish kidney. Alternative steps can be used to dissect the kidney, such as opening the abdominal cavity with a ventral incision using dissection scissors; however, we have encountered the most success in accessing the entire kidney when the abdominal wall is pinned open and the head is removed. Blood and kidney researchers alike who wish to isolate and work with these cell types can use either dissection method. It should be noted that the method we describe for isolating and working with the zebrafish adult kidney is certainly not limited to scientists who wish to pursue questions of adult stem cell biology, as the dissection of this organ can facilitate studies for those researchers pursuing investigations on topics ranging from physiology to aging. Further, this method can be coupled with other technologies such as transgenics, so as to molecularly label and then purify and study discrete subpopulations of blood or kidney cells from wildtype or genetically mutant zebrafish¹¹. Looking forward, such detailed purification procedures of stem and progenitor cell types is vital to gaining knowledge about how their behavior is regulated, as operational tests such as transplantation or progenitor culturing constitute the basis by which the potency and identity of these cells is defined. Recent advances in hematopoietic progenitor culture methods open many new avenues for study, and may be adapted to culturing of renal populations¹⁷. Zebrafish chemical genetics have been successful in the identification of pathways that modulate HSCs and renal progenitors in various contexts¹⁸⁻²⁰, and will continue to be a useful avenue to test in combination with the above cell biology techniques. Taken together, there have been a number of groundbreaking contributions to the fields of hematology and nephrology using the zebrafish model, and continued research in these arenas promises to wield further transformative insights in the coming years.

Disclosures

No conflicts of interest declared.

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