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Research article

odd skipped-related 2 as a novel mark for labeling the proximal convoluted tubule within the zebrafish kidney

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A B S T R A C T

The proximal convoluted tubule (PCT) of the kidney is a crucial functional segment responsible for reabsorption, secretion, and the maintenance of electrolyte and water balance within the renal tubule. However, there is a lack of a well-defined endogenous transgenic line for studying PCT morphogenesis. By analyzing single-cell transcriptome data from the adult zebrafish kidney, we have identified the expression of odd-skipped-related 2 (*osr2*, which encodes an odd-skipped zinc-finger transcription factor) in the PCT. To gain insight into the role of *osr2* in PCT morphogenesis, we have generated a transgenic zebrafish line *Tg(osr2:EGFP)*, expressing enhanced green fluorescent protein (EGFP). The EGFP expression pattern closely mirrors that of endogenous Osr2, faithfully recapitulating its native expression profile. During kidney development, we can use EGFP to track PCT development, which is also preserved in adult zebrafish. Additionally, *osr2:EGFP*-labeled zebrafish PCT fragments displayed short lengths with infrequent overlap, rendering them conducive for nephrons counting. The generation of *Tg(osr2:EGFP)* transgenic line is accompanied by simultaneous disruption of *osr2* activity. Importantly, our findings demonstrate that *osr2* inactivation had no discernible impact on the development and regeneration of *Tg(osr2:EGFP)* zebrafish nephrons. Overall, the establishment of this transgenic zebrafish line offers a valuable tool for both genetic and chemical analysis of PCT.

1. Introduction

The PT (proximal tubule) is a critical component of the nephron and performs the majority of reabsorption and processing of the glomerular filtrate. It consists of two distinct segments: PCT and the proximal straight tubule (PST). In mammals, the PCT is characterized by its convoluted structure, while the PST is straight and lacks convolutions. Functionally, both segments share similar roles, including the reabsorption of water, glucose, amino acids, and ions, as well as the excretion of waste products [1–3]. However, these functions are mainly contributed by PCT, while the PST's contribution is relatively limited [4]. Injured PCT cells play a pivotal role in the pathogenesis of acute kidney injury (AKI), chronic kidney disease, and diabetic kidney disease [5,6]. Considering the high conservation of genes between zebrafish and mammalian PCT [7], zebrafish models hold great value for the study of PCT development and diseases.

Tissue-specific transgenic technology has emerged as an invaluable tool for studying tissue development. In zebrafish, several transgenic lines with fluorescent protein have been constructed to visualize kidney organ morphogenesis. For instance, the transgenic line *Tg(cdh17:DsRed)* (the line ID: ZDB-FIG-230223-3) labels renal tubules [8]. The *Tg(dβh:GFP)* zebrafish line utilizes the *dopamine-β-hydroxylase* (*dβh*) promoter to drive GFP expression, marking PT development [9]. Another transgenic line, *Tg(ret1:GFP)*, exhibits GFP expression driven by the *ret* loci, marking the most distal pronephric duct [10]. The *ET33-D10* zebrafish line with GFP expression marks the PT [11]. Additionally, the *Tg(gtshβ:GFP)* zebrafish line, featuring green fluorescent protein under the control of

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the grouper *gtshβ* promoter, provides the details of PT development within the transgenic zebrafish line [12]. Despite these advances, specific endogenous transgenic lines for visualizing PCT development and morphogenesis are still lacking.

The *osr2* gene, which encodes a zinc-finger protein, is detected in higher vertebrates such as mouse [13]. Studies on mouse *osr2* mutant lines have revealed their essential role in proper patterning of the endoderm, palate, bones, teeth, and synovial joints in the limbs [14–17]. Notably, *osr2* has been identified as a key regulator of palate development and a potential candidate gene associated with human cleft palate formation [18,19]. Furthermore, *osr2* is expressed in the kidney, and its presence has been observed in the genomes of *Xenopus* and zebrafish, where it appears to contribute, to some extent, to kidney formation [20,21]. A previous study demonstrated the requirement of *osr2* for zebrafish pronephros development, as evidenced by the expression of *osr2* in the pronephric kidney and the occurrence of pericardial edema in *osr2* knockdown embryos [20]. However, conflicting reports indicate that *osr2* knockdown embryos do not exhibit abnormalities in kidney development [22]. Therefore, further evidence is needed for the specific effects of *osr2*.

In this study, we successfully generated a transgenic zebrafish line, *Tg(osr2:EGFP)*, utilizing the CRISPR/Cas9 knock-in technology. Our findings demonstrate that *osr2* serves as a reliable marker for PCT. The *EGFP* expression in the *Tg(osr2:EGFP)* kidney enables labeling of PCT. Importantly, the complexity of nephrons poses challenges in their counting. Traditionally, *in situ* hybridization has been employed for nephron counting; however, this method is time-consuming and intricate. In contrast, the *osr2:EGFP*-labeled fragments displayed short lengths and infrequent overlap, providing a practical advantage for efficient nephron counting. Furthermore, our investigations revealed that *osr2* inactivation in *Tg(osr2:EGFP)* transgenic fish did not adversely affect the development and regeneration of the kidneys, showing results comparable to the control group. Overall, our study highlights the utility of the *Tg(osr2:EGFP)* zebrafish line as an innovative resource for studying PCT development, regeneration, and related research in a genetic or chemical context.

2. Materials and methods

2.1. Zebrafish maintenance

Adult and embryonic zebrafish were reared following established protocols outlined in the zebrafish book [23]. The adult zebrafish selected for experiments ranged in age from 3 to 12 months. These zebrafish were maintained under standard conditions, subjected to a 10-h dark cycle and a 14-h light cycle, and were fed twice daily. Before performing experiments, zebrafish individuals were anesthetized using 0.0168% buffered tricaine (MS-222, Sigma). The AB strain (ZFIN ID: ZDB-GENO-960809-7) of zebrafish was employed as the wild-type (WT) for line generation. For this study, we utilized transgenic zebrafish lines expressing the *GFF (Gal4FF, Gal4 transcription activator)* and the *EGFP* [24,25]. This transgenic line was generated through CRISPR/Cas9 knock-in technology, as previously described [24,25]. Additionally, the *cdh17:DsRed* zebrafish line was established in our previous study [8]. The animal care and use protocol employed in this study received approval from the Institutional Animal Care and Use Committee of the Army Medical University, China (SYXK-PLA-2007035).

2.2. Single-cell gene expression pattern analysis

To analyze the expression pattern of *osr2* within zebrafish kidneys, we employed a previously published single-cell RNA-sequencing dataset (GSE100910) [26]. For the purpose of data preprocessing, normalization, dimensionality reduction, and subsequent clustering analysis (resolution = 0.4), we applied the Seurat package (version 4.2.0), a widely utilized computational tool in the field of single-cell genomics. PTEC was further classified into PCT and PST by R packages, including ggplot2 (version 3.3.6), dplyr (version 2.2.1), Seurat (version 4.2.0), and tidyverse (version 1.3.2) for dimensionality reduction, and subsequent clustering analysis (resolution = 0.2). This classification was based on marker genes that are specific and highly expressed in PCT (*slc20a1a*) and PST (*slc13a1* and *slc13a3*) [27].

2.3. Production of sgRNA and zCas9 mRNA

The zCas9 expression plasmid, *pGH-SP6-zCas9* [1], was enzymatically linearized using *Xba*I and subsequently utilized for in vitro synthesis of Cas9 mRNA employing the mMACHINE SP6 Ultra kit (Ambion). The resulting zCas9 mRNA was purified utilizing the same kit. According to previously described criteria [2], the single guide RNA (sgRNA) was designed. To minimize potential off-target effects, the CRISPR/Cas9 design tool (http://chopchop.cbu.uib.no/) was employed to identify specific target sequences. The sequences of the designed sgRNA is 5′-TCACACTTTTACCCCGGGGTTGG-3'. The sgRNA was annealed and subsequently cloned into the PSP6-sgRNA vector downstream of the SP6 promoter. To synthesize the sgRNA, the MAXIscript SP6 Kit (Ambion) was utilized, and purification of the sgRNA was performed using the mirVana™ miRNA Isolation Kit (Ambion).

2.4. Cloning of donor plasmids

The donor plasmid consists of four parts: a right arm, *P2A-GFF*, a left arm, and *cryaa-EGFP*. The right and left arms corresponding to

osr2 were amplified by the KOD-PLUS Neo DNA polymerase from genomic DNA of WT zebrafish (right arm Forward: 5′-ATGCGGTGGGCTCTATGGAAACCCTTCAAGTGTCAGGAGTG-3’; right arm Reverse: 5′-GAATAGGAACTTCGGTACGG CGGCGGCATGTGTTTGTGT-3’; 840 bp; left arm Forward: 5′-GTAAAACGACGGCCAGTGGCAGACTCCCGGCCAAGAGC-3’; left arm Reverse: 5′-ATTAGTAGCTCCGGATCCCTCCTTAGAGTGGATATATCTGAGGAAC-3’; 840 bp). Subsequently, the amplified arms were individually inserted into the 3′ and 5′ regions of the *P2A-GFF* fragment, leading to the generation of the donor plasmid.

2.5. Embryos micro-injection

For the micro-injection procedure, a mixture of sgRNA, zCas9 mRNA, and donor plasmids were co-injected into one-cell stage zebrafish embryos following fertilization. Each egg received an injection of 1 nl of solution, consisting of 600 ng/μl zCas9 mRNA, 100 ng/μl sgRNA, and 15 ng/μl donor plasmid.

2.6. Generation of transgenic zebrafish lines

Both the donor plasmid and the second intron of *osr2* contain the sgRNA target site. Consequently, the simultaneous cleavage mediated by the sgRNA/Cas9 complex would facilitate precise and efficient integration of the donor DNA into the *osr2* locus via the homologous recombination (HR) mechanism. Based on whether they expressed the green fluorescent protein cerulean in their eyes, four founders were identified for each transgenic line. These founders were crossed with WT zebrafish to obtain offspring. Subsequently, the extracted genomic DNA from these offspring served as the template to amplify the 5′ and 3′ junction fragments of targeted genes by the primers (F1: 5′-CGGTGACCGGACAGAGCAGACT-3′, R1:5′-ACTGGGTGCTCAGGTAGTGGTTGT-3’; 3.8 kb; F2:5′-GGAAGGCAAAATGCCGCAAAAAAGG-3′, R2:5′-AGCGGCCGCAGCAGAAAGGCTTGA-3’; 2.2 kb), and to sequence the 5′ junction using primer (F1: 5′-CGGTGACCGGACAGAGCAGACT-3’; R: 5′- ACACGCGCGCGCGCACTGAAC-3′) to identify potential indel mutations. Subsequently, the homozygous *TgKI(osr2:GFF)* lines was identified through sequencing and used in subsequent maintenance and experiments.

2.7. Zebrafish AKI model

The zebrafish AKI model was constructed through the intraperitoneal injection of gentamicin [28,29], following a previously described protocol [30]. In brief, adult *Tg(osr2:EGFP)* zebrafish lines of similar weight were subjected to intraperitoneal injection of gentamicin (2.7 μg/μL, 20 μL per fish). Subsequently, each injected adult zebrafish was individually placed in a separate container. Five to six adult zebrafish that produced similar amounts of visible solid proteinuria at 1 dpi (days post-injury) were used as experimental subjects for subsequent studies.

2.8. Nephron counting

Nephron counting was performed following a previously protocol [31]. Each nephron consists of only a segment of *Tg(osr2:EGFP)* PCTs. Therefore, quantification of nephron number relies on counting *osr2:EGFP*-labeled PCTs fragments. For counting, we carefully extracted kidneys from adult *Tg(osr2:EGFP)* fish and then performed whole-kidney imaging using a Nikon A1 confocal microscope. Subsequently, the total count of *EGFP*-positive segments was determined utilizing ImageJ. For unbiased analysis, all images were relabeled and counted by analysts without knowledge of each sample’s treatment conditions. For evaluating variation in nephron number following Gentamicin-induced AKI, the baseline nephron count in uninjured kidneys was considered 100%. Subsequently, the extent of renal regeneration was characterized using the percentage change in nephrons after AKI compared to the uninjured state.

2.9. WISH (whole-mount in situ hybridization) of zebrafish embryos

The *slc20a1a* probe was synthesized (*slc20a1a* probe F: 5′-CATCGGAGGATCGGCAGAAACCGACC-3’; *slc20a1a* probe R: 5′-TCCGATGTTGGAAGCGACAACCACAG-3′) and WISH was performed following previously protocol [30]. Zebrafish embryos were fixed in 4% paraformaldehyde (PFA) overnight at 4 °C. Subsequently, the samples were washed five times with PBT (PBS with 0.1% Tween-20) and were permeabilized with proteinase K (10 μg/μL, Roche) in PBT for 5 min with rocking. The digoxigenin-labeled *slc20a1a* probe, synthesized from cDNA fragments containing zebrafish-specific sequences, was used for hybridization as previously reported [30]. Zebrafish embryos were subjected to hybridization with the digoxigenin-labeled *slc20a1a* probe. Anti-DIG AP antibody and NBT/BCIP substrate (Roche) were used to detect the probe. Following the color reaction, images were captured using a BX3-CBH microscope (Olympus, Japan).

2.10. Acquisition of EGFP signal

To analyze the expression pattern of *EGFP*, zebrafish embryos were treated with PTU (Sigma) (0.16 mM) from 16 h post-fertilization (hpf) to suppress pigmentation. At the indicated time, images were captured using both confocal microscopy and stereoscopic fluorescence microscopy to visualize the *EGFP* signal.

2.11. RT–PCR and qRT–PCR

RNA was extracted from kidney tissues utilizing TRIzol reagent (Invitrogen, 15596018). Subsequently, cDNA was synthesized from the extracted RNA using a Prime Script II 1st strand cDNA Synthesis Kit (Takara, 9767), followed by PCR using TB Green Premix EX *Taq*II (Takara, RR820A) for quantitative Real-time PCR (qRT–PCR) (*osr1*-qRT: 5′-AGCATCCAGCAAAAGTAAACCC-3’*; osr1*-qRT: 5′-TTATGACAGATATCACAAGTGTACG-3’; 310 bp); *β-actin* [8]) or Taq Master Mix (Vazyme, p112-01) for reverse transcription polymerase chain reaction (RT–PCR) (*osr2*-RT F: 5′- ATGGGCAGTAAGACTCTCCCGGCTCCGG-3’; *osr2*-RT R: 5′-CTA-GACTGTGGCGCCGCAGGTCGCAGTT-3’; 710 bp; *osr2-GFF*-RT F: 5′-AAATCCTACAACCTGCTGATCCAC-3’; *osr2-GFF*-RT R: 5′-CCTGTTAACAATGCTTTTATATCCTGTA-3’; 560 bp). Gene expression data were normalized to the expression of *β-actin* mRNA.

2.12. Statistics

All experiments were performed in at least three independent replicates. Statistical analyses were conducted employing GraphPad Prism (version 10) and Excel (Microsoft Office Home and Student 2016 version). The data were analyzed by two-sided *t*-test and are presented as mean values ± S.

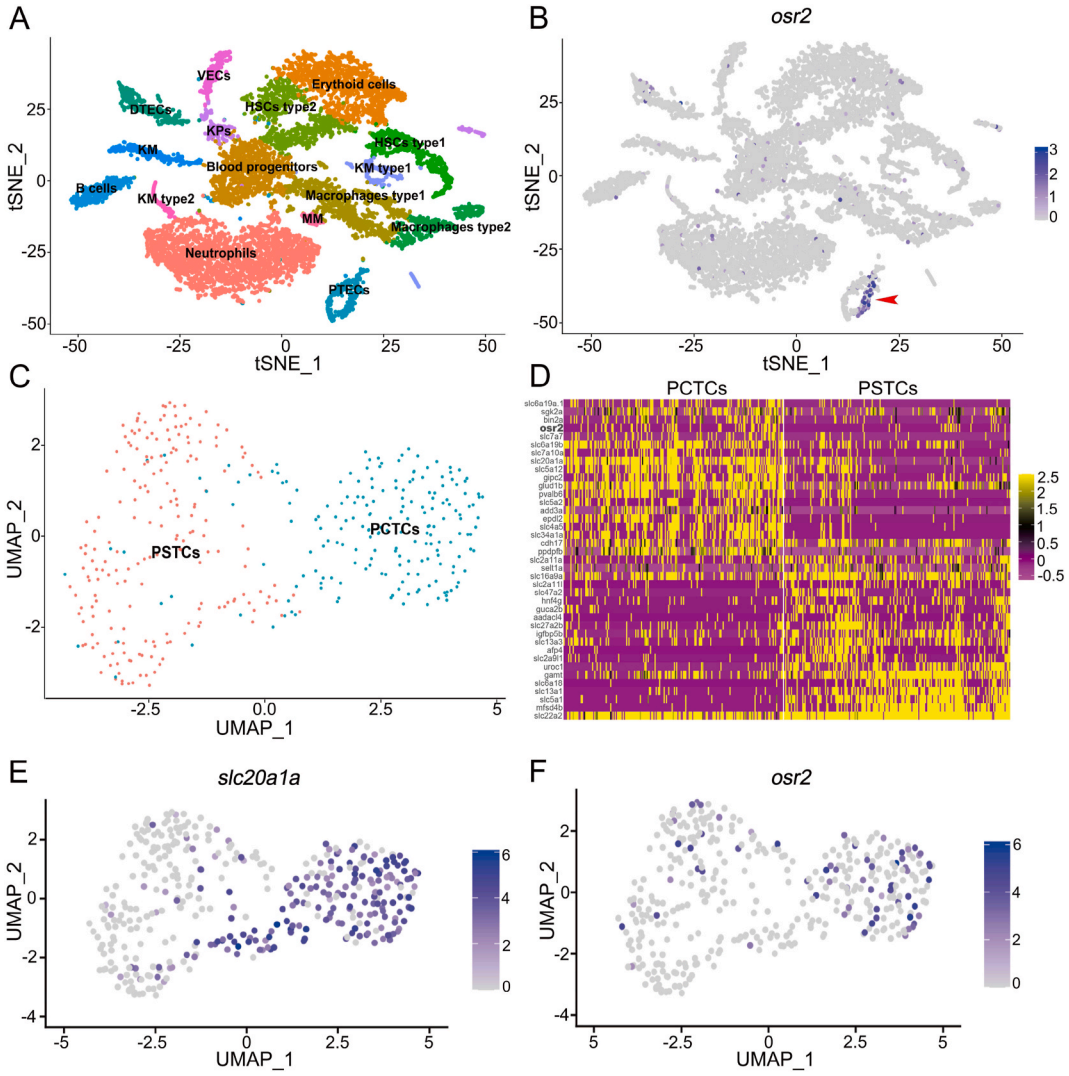

Fig. 1. Expression pattern of *osr2* in zebrafish kidney. **A-B:** scRNA-seq analysis showed that *osr2* was expressed in PTECs. tSNE plots show zebrafish kidney cell clusters and the expression of *osr2*. HSCs, hematopoietic stem cells; KPs, Kidney progenitors; KM, Kidney Multiciliated; MM, Macrophages Myeloid; KM, Kidney mucin; VECs, Vascular endothelium cells; DTECs, distal tubule epithelial cells; PTECs, proximal tubule epithelial cells. **C:** Cluster DTECs was divided into PSTCs and PCTCs subpopulations. **D:** Heatmap shows relative log-expression of some genes in PCT or PST in C. **E-F:** Expression analysis of *slc20a1a* and *osr2* in each subpopulation. PCTCs, proximal convoluted tubule epithelial cells; PSTCs, proximal straight tubule epithelial cells.

3. Results

3.1. Expression of osr2 in zebrafish PTECs

To investigate the expression pattern of *osr2* in the zebrafish kidney, we conducted a reanalysis of previously published single-cell

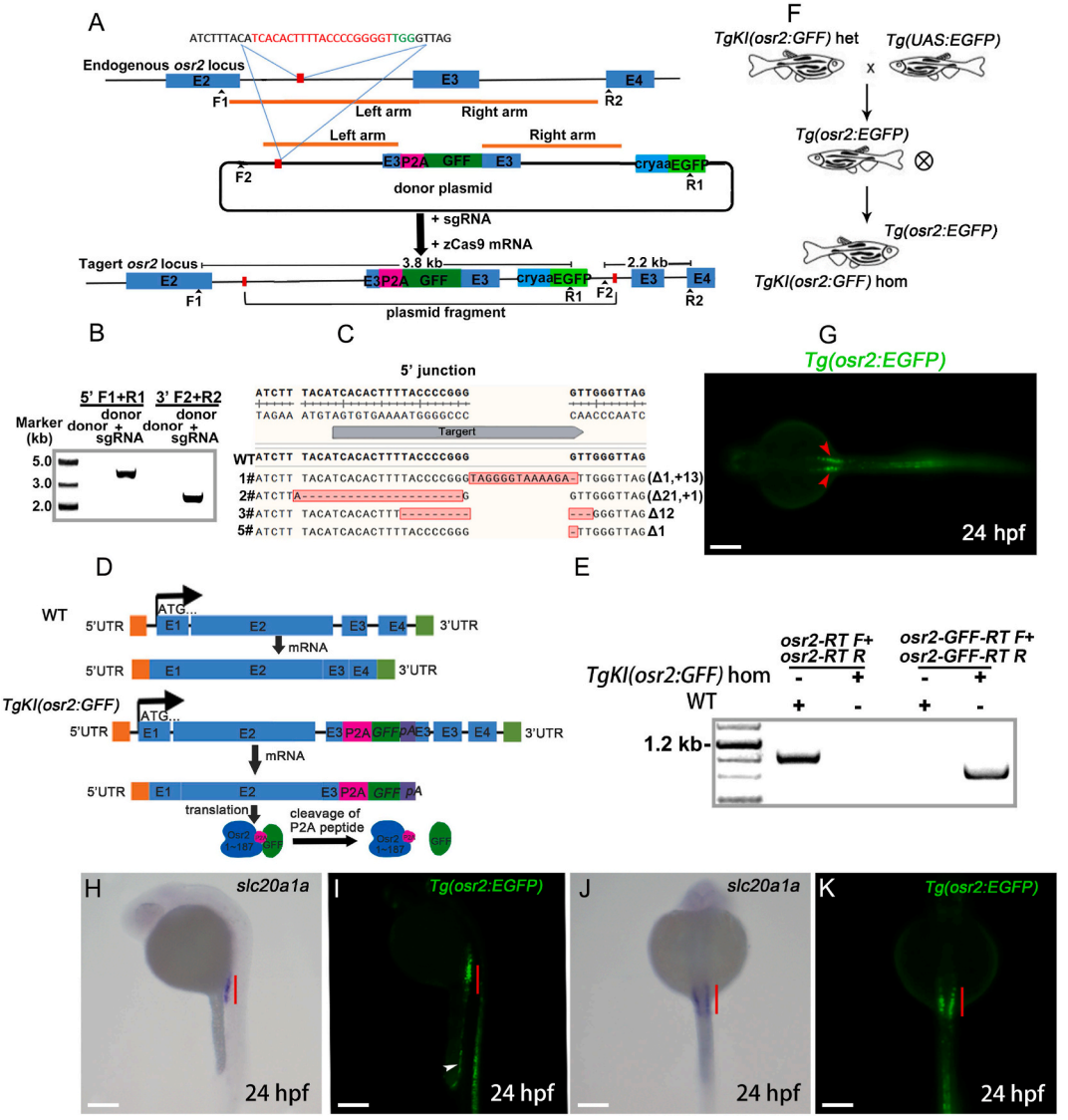

Fig. 2. Intron targeting-mediated donor plasmid knockin at the zebrafish *osr2* locus. **A:** Schematic representation of an intron-targeted mediated strategy for generating *GFF* knockin at zebrafish *osr2* locus using the CRISPR/Cas9 system. The sequence of sgRNA target is shown in red, and the protospacer adjacent motif (PAM) sequence is depicted in green. The right and left arm sequences of the donor plasmid are denoted by the brown lines. The left arm is 840 bp, and the right arm is 840 bp. Following co-injection of the donor plasmid with the zCas9 mRNA and sgRNA, the whole donor plasmid fregment was integrated into the *osr2* locus. The zebrafish *osr2* has 4 exons, with E2, E3, and E4 representing the 2nd, 3rd, and 4th exons, respectively. **B:** PCR analysis of the 3′ and 5′ junctions of F1 progenies from the 3# founder. The F1, R1, F2 and R2 primers are shown in A. **C:** 5′ junction sequences of F1 progenies of four donor plasmid knockin F0 founders. The indel mutations are highlighted in red. **D:** Transcription schematic of *osr2* in *TgKI(osr2:GFF)* and WT zebrafish. **E:** RT-PCR was used to verify the transcription of *osr2* and *GFF* in *TgKI (osr2:GFF)* and WT zebrafish (n = 3 biological replications per group). **F:** Schematic diagram for obtaining *TgKI(osr2:GFF)* homozygotes, *TgKI(osr2:GFF)* heterozygotes, and *Tg(osr2:EGFP)* transgenic fish. **G:** *In vivo* confocal image (dorsal view) of *Tg(osr2:EGFP)*embryos at 24 hpf reveals *EGFP* expression in the pronephros as indicated by the red arrowheads. Scale bar, 500 μm. **H–K:** The expression patterns of *slc20a1a* and *EGFP* were examined in 24 hpf embryos. **H:** WISH analysis of the expression pattern of *slc20a1a* in 24 hpf embryos (side view). **I:** *In vivo* confocal images (side view) of *osr2:EGFP* embryos at 24 hpf. Posterior gut (white arrowhead). **J:** WISH analysis of the expression pattern of *slc20a1a* in 24 hpf embryos (dorsal view). **K:** *in vivo* confocal images (dorsal view) of *Tg(osr2:EGFP)* embryos at 24 hpf. hom, homozygotes; het, heterozygotes; *pA*, *poly(A)*. Scale bar in H to K, 500 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

RNA sequencing (scRNA-seq) data (GSE100910) [26]. Analyzing scRNA-seq data revealed that *osr2* is expressed in proximal tubule epithelial cells (PTECs) (Fig. 1A and B). Since the PT consists of the PCT and PST, we aimed to further validate the expression pattern of *osr2* in the PT of the zebrafish kidney. To accomplish this, Seurat (version 4.2.0) was used to divide the PTECs cell population into subpopulations of PSTCs and PCTCs (Fig. 1C), based on marker genes that are specific and highly expressed in PCT (*slc20a1a*) and PST (*slc13a1* and *slc13a3*) [27]. Notably, the analysis demonstrated that *osr2* expression localized to the PCT of the zebrafish kidney (Fig. 1F). Moreover, the heatmap illustrated the expression of several PCT marker genes, including *osr2*, *slc20a1a*, *slc6a19b*, *epdl2*, *slc7a7*, *slc34a1a*, *etc* (Fig. 1D). Previous investigations have identified *slc20a1a* as a PCT marker [27], and our findings consistent with this observation, indicating that both *slc20a1a* and *osr2* are expressed in the PCT (Fig. 1D–F). Collectively, these findings suggest that *osr2* could serve as a valuable new marker for characterizing and studying the PCT.

3.2. PCT are marked by osr2:EGFP in zebrafish

To further determine whether *osr2* can be used as a PCT marker,we designed a short guide RNA (sgRNA) targeting the second intron of zebrafish *osr2*. We then conducted co-injections of this sgRNA with zCas9 mRNA into one-cell-stage zebrafish embryos, resulting in a cleavage efficiency of ~16%. Next, we constructed a donor plasmid *osr2-P2A-GFF* consisting of four parts: a left arm, a *P2A-GFF* coding sequence, a right arm, and *crayy-EGFP* (Fig. 2A). This approach facilitated knock-in of the whole donor plasmid fragment (Genomic location: GRCz11; Chromosome 16:54404070–54404071; Gene ID: ENSDARG00000038006) to generate *TgKI(osr2:GFF)* zebrafish (Fig. 2B;Fig. S1), which has been submitted the details to the zebrafish database ZFIN (the line ID: ZDB-ALT-231019-1). PCR and sequencing analysis of F1 progenies verified the inheritance of the genomic integration of their respective F0 founders, and *TgKI(osr2:GFF)* zebrafish with $osr2^{-12}$ (a 12-bp deletion in intron) was used for subsequent maintenance and experiments (Fig. 2C;Fig. S2). Subsequently, we examined *osr2* transcription in *TgKI(osr2:GFF)* homozygous and WT using RT-PCR (Fig. 2D and E) and did not detect intact *osr2* transcription in *TgKI (osr2:GFF)* homozygous. Therefore, the full reading frame of *osr2* in *TgKI (osr2:GFF)* homozygous was disrupted. Given that GFF can activate transcription through its recognition sequence (upstream activating sequence, *UAS*), genes of placed downstream of *UAS* can be expressed in the *GFF*-expressing cells [24]. *Tg(UAS:EGFP)* transgenic reporter fish carries the *EGFP* gene downstream of *UAS* and can specifically express *EGFP* in *GFF* positive cells and tissues [24]. We crossed *TgKI(osr2:GFF)* with *Tg (UAS:EGFP)* to generate *TgKI(osr2:GFF) x Tg(UAS:EGFP)*, abbreviated as *Tg(osr2:EGFP)*, and observed *EGFP* expression in the pronephros of embryos (Fig. 2F and G). Based on their expression pattern and morphological characteristics, we postulated that the *EGFP*-expressing cells were localized within the PCTs. To further verify this, we employed WISH of *slc20a1a*, an established marker for PCTs [32], as a reference to validate the expression region of *osr2:EGFP* was the PCT. Our results showed that *slc20a1a* and *osr2:EGFP* were expressed at the same position, suggesting that *osr2:EGFP* marked the PCT (Fig. 2H–K), which was consistent with the results obtained from single-cell data analysis.

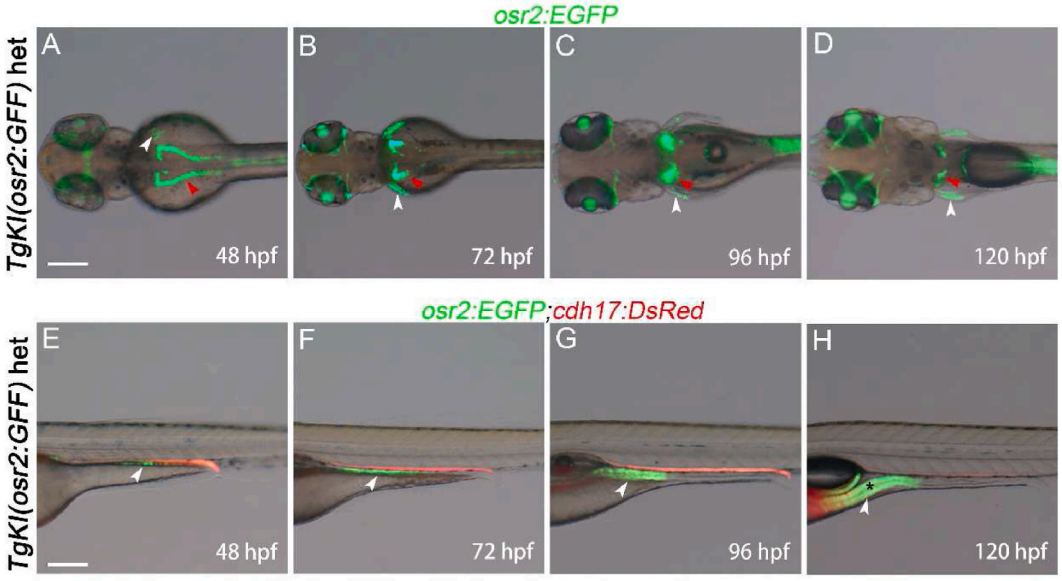

Fig. 3. Dynamic *EGFP* expression pattern throughout embryogenesis in the *Tg(osr2:EGFP)* transgenic line. **A-D:** Showing the PCT dorsal view of *Tg (osr2:EGFP)* embryos at 48 hpf, 72 hpf, 96 hpf, and 120 hpf, respectively. Within these stages, *EGFP* was expressed in the PCT region (indicated by the red arrowhead) as well as in the pectoral fin (indicated by the white arrowhead). **E-F:** Providing lateral view of *Tg(osr2:EGFP;cdh17:DsRed)* embryos at the developmental stages. Posterior gut (white arrowhead) and the gut lumen (*) can be observed. het, heterozygotes. Scale bar in A to H, 500 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.3. Dynamic EGFP expression pattern during embryogenesis in Tg(osr2:EGFP) zebrafish

Expression of *osr2* has previously been documented in various tissues of developing zebrafish embryos, including the pronephros, pectoral fin buds, and gut [33–35]. To assess whether *Tg(osr2:EGFP)* can faithfully reflect the expression of endogenous *osr2*, we performed a dynamic analysis of the *osr2:EGFP* expression pattern using stereoscopic fluorescence microscope. Through observation,

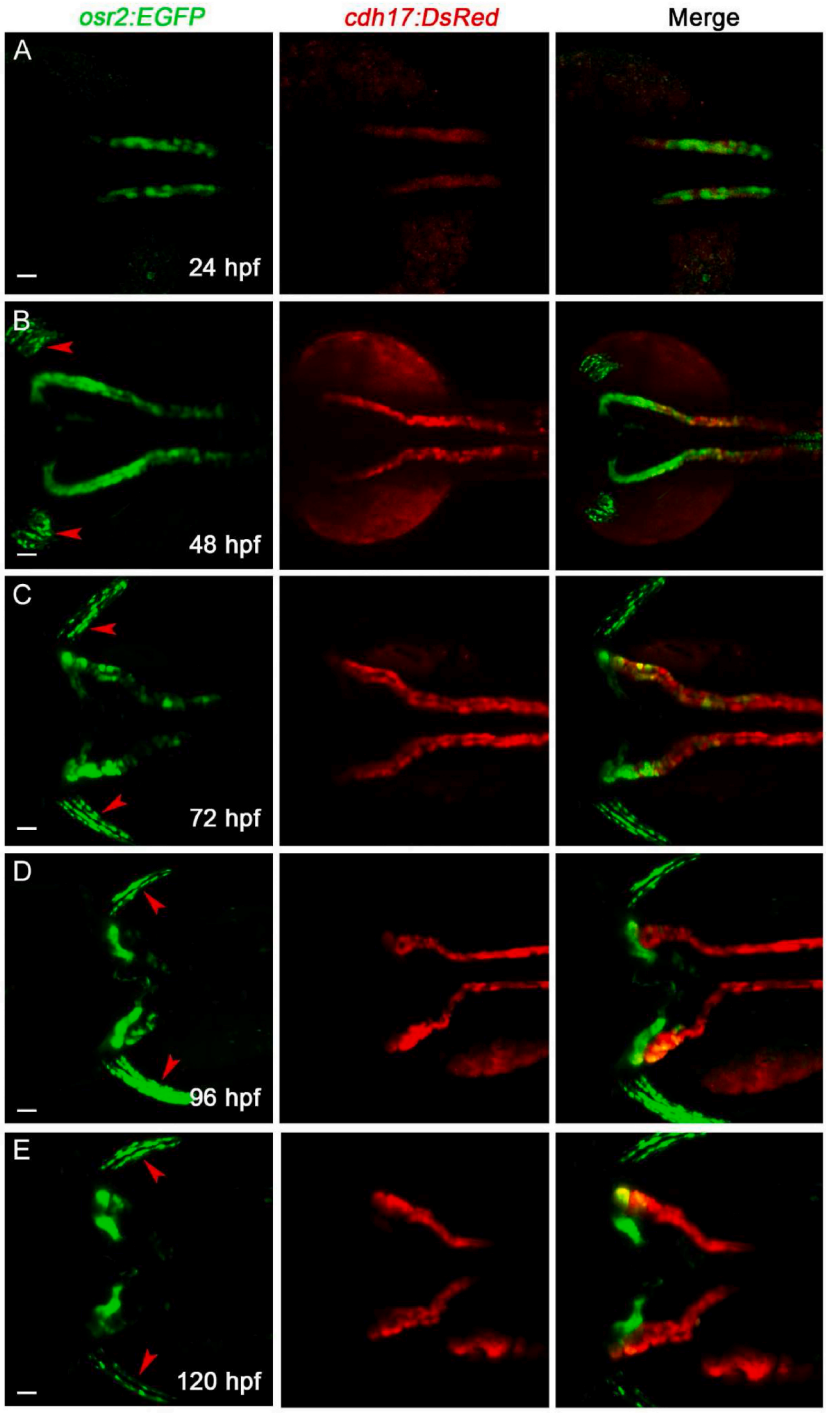

Fig. 4. The PCT morphogenesis is labeled by *EGFP* in *Tg(osr2:EGFP)* embryos. **A-E:** Dorsal view of the PCT cells that are co-labeled by *EGFP* and *DsRed* at the indicated stages are shown (n = 6). *osr2:EGFP* expression in the pectoral fins (red arrowhead) can be observed. Scale bar in A to E, 200 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

we determined that *osr2:EGFP* was expressed in the PCT (Fig. 3A). As embryonic development progressed, the *osr2:EGFP*-labeled PCT showed a progressive aggregation and forward curling phenomenon (Fig. 3B–D). Additionally, *osr2:EGFP* expression was also detected in the pectoral fins, pharygial arch 1, and neuronal cells from 48 hpf to 120 hpf (Fig. 3A–D). Previous study has shown that *osr2* is expressed in posterior gut epithelial cells from as early as 24 hpf, and in posterior gut and gut mesenchyme at 48 hpf [36]. To further validate *osr2:EGFP* is expressed in gut, we crossed *Tg(osr2:EGFP)* with *Tg(cdh17:DsRed)*, which specifically marks the proximal and distal tubules of the pronephron. As a result, we observed that *osr2:EGFP*-expressing cells in the posterior gut were located below the renal tubules at 48 hpf and 72 hpf (Fig. 3E and F). As the embryo developed, posterior gut morphogenesis exhibited a gradual aggregation (Fig. 3G and H). Collectively, these findings demonstrate that *Tg(osr2:EGFP)* transgenic fish faithfully reflect the endogenous *osr2* expression pattern.

3.4. *Tg(osr2:EGFP) marks PCT morphogenesis*

In order to further clarify *Tg(osr2:EGFP)* marks PCT morphogenesis, we employed confocal microscopy to examine the expression pattern of *EGFP* in the PCT. Our observations revealed that *EGFP* fluorescence signal in the developing kidney is initially detectable above the dorsal side at about 20 hpf. By 24 hpf, the *EGFP* fluorescence extends above the yolk sac, exhibiting bilateral tubular structures (Fig. 4A). To provide additional evidence for *osr2* marking PCT morphogenesis, we crossed *Tg(osr2:EGFP)* with *Tg(cdh17:DsRed)*. Subsequently, we tracked the expression patterns of *osr2:EGFP* and *cdh17:DsRed* in the embryos from 24 hpf to 120 hpf (Fig. 4A–E). Our results demonstrated co-expression of *EGFP* and *DsRed* in the PCT throughout embryonic development (Fig. 4) and persisting into adulthood. The temporal progression of *slc20a1a*-expressing PCT morphogenesis, transitioning from a linear tube to a coiled structure, had been previously characterized [27]. Our observed temporal progression of PCT morphogenesis, marked by *osr2:EGFP,* is consistent with these findings. Therefore, based on the results of single-cell analysis, *in situ* hybridization, and transgenic fish, we believe that *osr2* is a new marker gene for PCT.

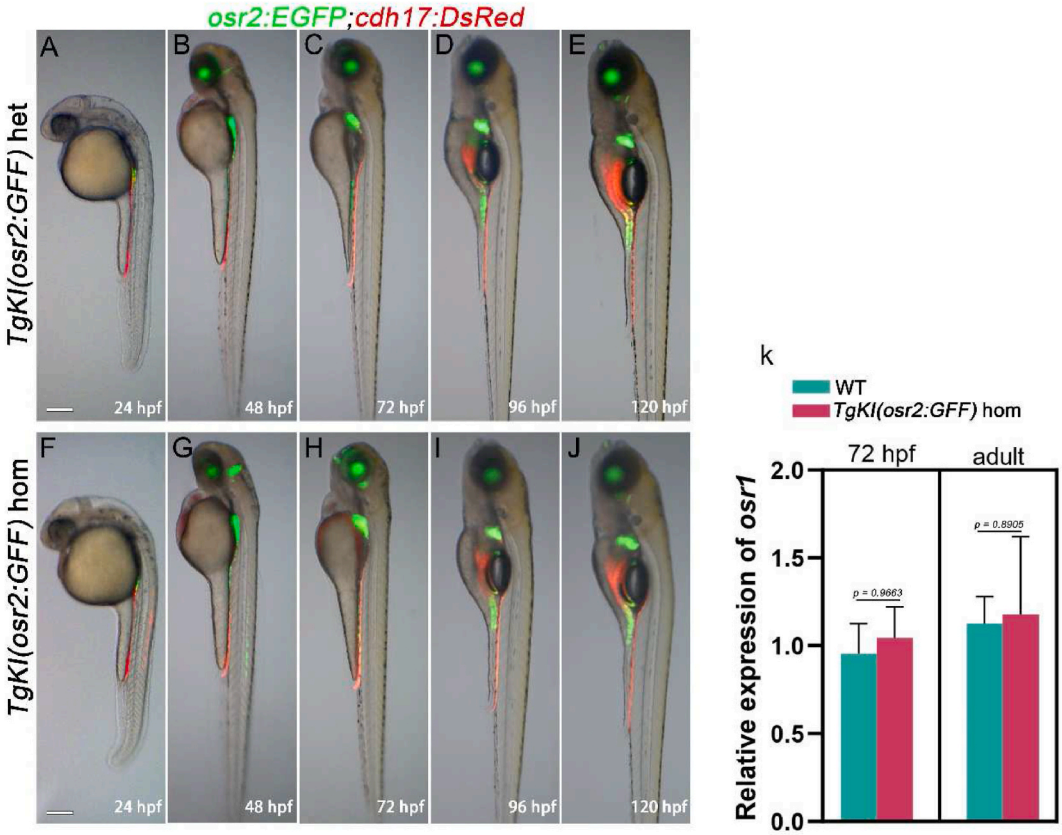

Fig. 5. Embryo morphology of *TgKI(osr2:GFF)* homozygotes and *TgKI(osr2:GFF)* heterozygotes. **A-J:** Use of *Tg(osr2:EGFP;cdh17:DsRed)* background to show *TgKI(osr2:GFF)* heterozygotes (**A-E**) and *TgKI(osr2:GFF)* homozygotes (**F-J**) embryo developmental morphology, development of PCT and renal tubules at the indicated stages are shown (n = 6). **K:** qRT–PCR analyses of *osr1* in embryonic and adult *TgKI(osr2:GFF)* homozygous and WT zebrafish kidneys (n = 3 biological replications per group). hom, homozygotes; het, heterozygotes. Scale bar in A to J, 500 μm. The data in K were analyzed by two-sided *t*-test and are presented as mean values ± SD.

3.5. *osr2* mutants do not affect zebrafish PCT development

Previous studies on the role of *osr2* in zebrafish kidney development have yielded conflicting results. Therefore, it is crucial to gather additional evidence to determine the impact of *osr2*. In our knock-in strategy, the whole donor plasmid was integrated into zebrafish genomic (Fig. 2A and B;Fig. S1), resulting in the full reading frame of *osr2* in *TgKI (osr2:GFF)* homozygous was disrupted (Fig. 2D and E). Consequently, *TgKI(osr2:GFF)* homozygotes are mutants, while the above developmental studies employed *TgKI(osr2:GFF)* heterozygotes that do not exhibit any discernible effects on embryo development. To investigate whether *osr2* mutants affect embryogenesis, we monitored the growth of *Tg(osr2:EGFP;cdh17:DsRed)* zebrafish embryos using a stereomicroscope. As a result, the morphology and development of PCT and renal tubules in *osr2*-inactivated *Tg(osr2:EGFP;cdh17:DsRed)* zebrafish embryos were indistinguishable from the control group (Fig. 5A–J). Additionally, obvious deficiencies were not found in other organizations (pharygial arch 1, pectoral fins, and neuronal cells) in *TgKI(osr2:GFF)* homozygotes zebrafish. We believe that *osr2* inactivation has no impact on the development of nephrons, possibly related to complementation of *osr2* paralogous genes. In addition, we also detected

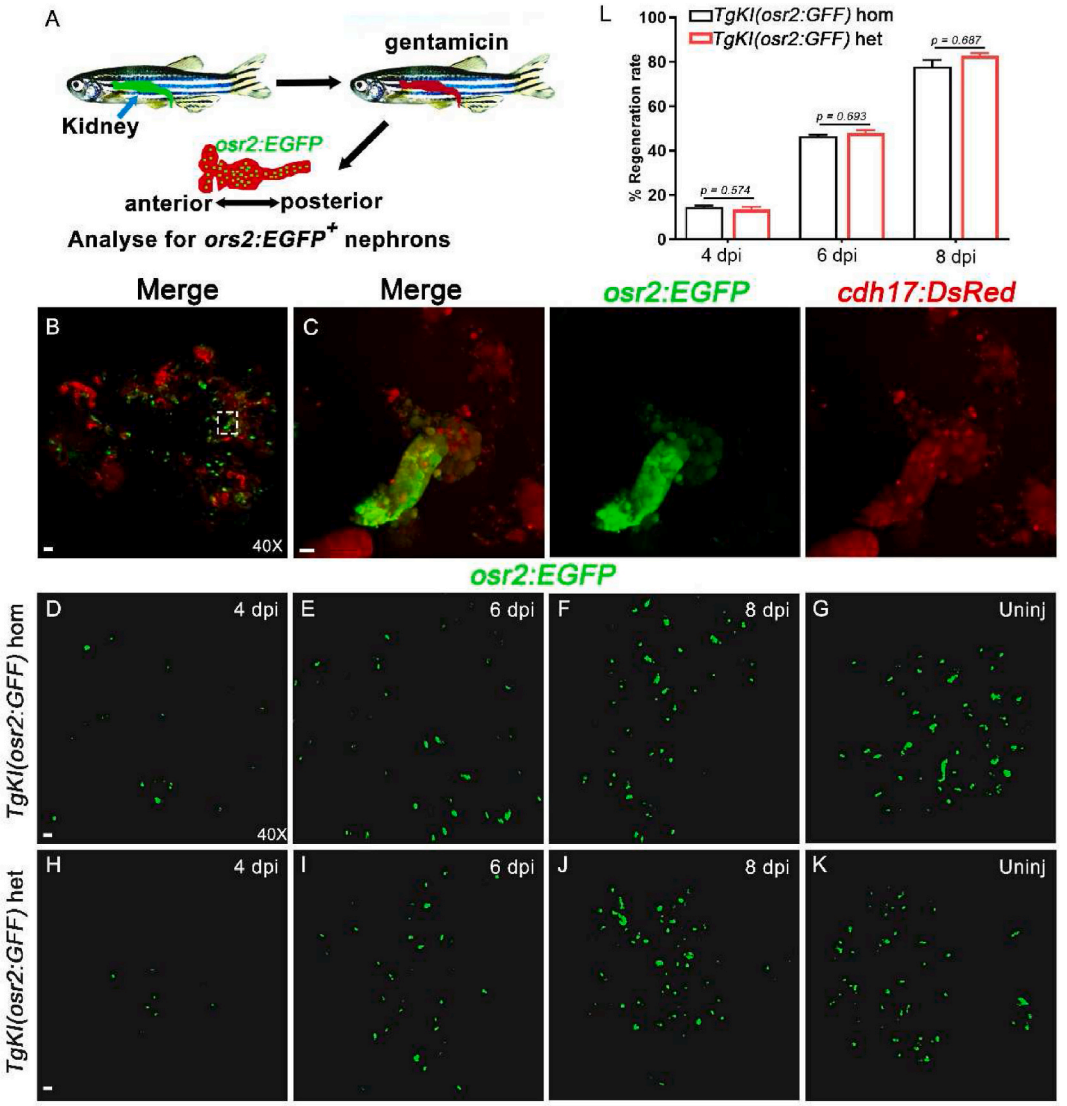

Fig. 6. *EGFP* $^{+}$ cells regeneration in *Tg(osr2:EGFP)* adult zebrafish after kidney injury. **A:** Schematic diagram of the AKI model constructed by intraperitoneal injection of gentamicin in adult zebrafish. **B:** Confocal images of adult zebrafish PCT cells that are co-labeled by *osr2:EGFP* and *cdh17:DsRed* are shown (n = 6). Scale bar in A, 100 μm. **C:** Higher-magnification image of the boxed area showed in B. Scale bar, 200 μm. **D-G:** Use of *osr2:EGFP* background to show of PCTs in *TgKI(osr2:GFF)* homozygotes at the indicated time point after injured (n = 6). Scale bar, 100 μm. **H–K:** Use of *osr2:EGFP* background to show of PCTs in *TgKI(osr2:GFF)* heterozygotes at the indicated time point after injured (n = 6). Scale bar, 100 μm. **L:** After injured, the renal regeneration rate of *TgKI(osr2:GFF)* homozygotes and *TgKI(osr2:GFF)* heterozygotes were counted using ImageJ (n = 5 biological replications per group). hom, homozygotes; het, heterozygotes. Scale bar in A to J, 500 μm.

the transcript level of *osr2* paralogous, *osr1*, in embryonic and adult *TgKI (osr2:GFF)* homozygous and WT zebrafish kidneys by qRT-PCR, however, no obvious upregulation of *osr1 mRNA* was found (Fig. 5K). Collectively, we believe that *osr2* inactivation does not seem to exert any discernible effects on zebrafish kidney development.

3.6. osr2:EGFP adult zebrafish provides a valuable tool for detection nephron regeneration

Fish kidneys have the remarkable ability to regenerate nephrons *de novo* after injury [30,37], designated renal regeneration. In response to injury, renal progenitor cells (RPCs) undergo a process of aggregation, followed by rapid proliferation and subsequent differentiation into new nephrons, providing the basis for new nephron regeneration count [8]. Despite the significance of nephron regeneration, there remains a lack of convenient and effective methods for nephron regeneration counting due to the inherent complexity of nephron structures. Our results indicated that *osr2:EGFP* was expressed in the PCT throughout embryonic development (Fig. 4) and persists into adulthood (Fig. 6B and C). Given that each nephron contains a single PCT segment, evaluating the regeneration of PCT segments enables an approximation of nephron regeneration rate. The labeled fragments observed in *Tg(osr2:EGFP)* transgenic fish are short and exhibit rarely overlap, thereby providing a useful tool for detecting the rate of nephron regeneration. Therefore, the use of *Tg(osr2:EGFP)* zebrafish greatly facilitates nephron regeneration counting. Subsequently, we aimed to determine the potential effects of *osr2* inactivation on nephron regeneration in *Tg(osr2:EGFP)* zebrafish. To achieve this, we induced AKI by intraperitoneally injecting adult zebrafish with a well-established nephrotoxin, gentamicin (Gent; 2.7 μg/μL, 20 μL per fish) [28], according to the schematic diagram outlining the construction of the AKI model (Fig. 6A). Our findings showed that, in comparison to uninjured kidneys, both *TgKI(osr2:GFF)* homozygotes and *TgKI(osr2:GFF)* heterozygotes exhibited similar rates of nephron regeneration at 4, 6, and 8 days post-injury (dpi), approximately 18%, 50%, and 80%, respectively (Fig. 6D–L). Therefore, we conclude that the inactivation of *osr2* may not affect nephron regeneration in *Tg(osr2:EGFP)* zebrafish compared to the control group.

4. Discussion

Transgenic technology offers numerous advantages, such as facilitating the integration of fragments containing fluorescent proteins and target genes, thereby facilitating early screening of experimental animals. In this study, we employed CRISPR/Cas9 knock-in technology to generate *Tg(osr2:EGFP)* zebrafish. Our findings revealed *EGFP* expression in the kidney, pectoral fin, and gut, exhibiting a pattern consistent with the endogenous *osr2* expression profile. Consequently, *Tg(osr2:EGFP)* can faithfully reflect the expression of *osr2 in vivo*. By tracking the expression of *EGFP*, we can investigate the development of PCT. Thus, the establishment of *Tg(osr2:EGFP)* transgenic zebrafish lines laid a crucial foundation for our subsequent investigations on the development of PCT in zebrafish.

The PCT of kidney plays a crucial role in the secretion, reabsorption, and maintenance of electrolyte and water balance within the renal tubule. However, transgenic fish lines that mark the PCT development are still lacking. Two fluorescently labeled transgenes, *Tg(cdh17:EGFP)*^{pt305} and *Tg(enpep:GFP)*, driven by kidney-specific promoters (*cdh17* and *enpep*) can mark the PT, but these transgenes also exhibit fluorescence in other kidney tubule cells [38,39]. Transgenic zebrafish line, *Tg(gtshβ:GFP)*, driven by the grouper *gtshβ* promoter, expresses *GFP* in the pituitary and kidney, and marks the developing details of the PCT and PST segments [12]. However, an endogenous zebrafish line that clearly marks the PCT is still lacking. In this study, through reanalysis of previously published single-cell RNA sequencing (scRNA-seq) data (GSE100910), we found that *osr2* was a novel mark for labeling the PCT (Fig. 1). *osr2* has been reported to have a role in kidney development. Additionally, *osr2* is expressed in the kidney earlier than *slc20a1a* [22,40], which can better track the PCT morphogenesis. Therefore, the establishment of *Tg(osr2:EGFP)* transgenic zebrafish line offers a valuable tool for tracking PCT morphogenesis.

Nephrons are highly complex structures, and their close proximity makes nephron counting challenging. Previous methods, such as *in situ* hybridization, have been utilized for nephron counting [8], but they are complicated and time-consuming. Another approach involves the use of 40 kDa dextran-FITC, which can be taken up by duct cells to count nephrons. However, the labeled fragments are too long, making nephron counting difficult. In contrast, the *Tg(osr2:EGFP)* transgenic fish, which labels the PCT fragment, exhibits shorter and less overlapping fragments, making it highly suitable for nephron counting.

To generate the *TgKI(osr2:GFF)* zebrafish line, the donor plasmid was inserted into zebrafish genome (Genomic location: GRCz11; Chromosome 16:54404070–54404071; Gene ID: ENSDARG00000038006) using CRISPR/Cas9 technology (Fig. 2A–E). This lines were then crossed with *Tg(UAS:EGFP)* to obtain the *Tg(osr2:EGFP)* line (Fig. 2F and G). Notably, the disruption of the full reading frame of *osr2* resulted in the inactivation of *osr2*. Nevertheless, our study revealed no evident abnormalities in *osr2*-inactivated *TgKI(osr2:GFF)* zebrafish embryos development (Fig. 5). To investigate the impact of *osr2* inactivation on nephron regeneration, we induced AKI in adult zebrafish through intraperitoneal injection of gentamicin. Nephron regeneration was subsequently assessed. As a result, the inactivation of *osr2* did not affect nephron regeneration in adult zebrafish (Fig. 6). Based on these findings, we conclude that *osr2* inactivation may not significantly impact zebrafish PCT development and regeneration.

Herein, both *TgKI(osr2:GFF)* homozygotes and *TgKI(osr2:GFF)* heterozygotes could grow normally to adulthood and reproduce normally. No obvious deficiencies were observed in zebrafish with inactivated *osr2*; however, the data from Morpholino experiments present conflicting outcomes, with some studies indicating the presence of defects [33], while others report no such abnormalities [36]. Consequently, we need to exclude the side effects of Morpholino. Additionally, genetic compensation response occurs when a gene mutation results in complete loss of function, prompting the body to increase the expression of other genes to replace its function for normal development and survival [41]. The genetic compensation response triggered by gene-knockout mutations in diverse organisms, such as zebrafish [42,43], mice [44] or *Arabidopsis* [45], were found to be a mechanism contributing to genetic robustness. In

order to determine whether the loss of function of *osr2* did not affect the growth of zebrafish due to genetic compensation, we detected the transcript level of the *osr2* paralogous gene *osr1*, and found that the transcript level of *osr1* did not change (Fig. 5K). Neither the side effects of Morpholino nor the influence of genetic compensation response can be excluded in our results. Therefore, whether *osr2* affects kidney development requires detailed follow-up research.

In this study, we successfully generated *Tg(osr2:EGFP)* zebrafish using CRISPR/Cas9 knock-in technology. The *Tg(osr2:EGFP)* zebrafish line serves as a valuable model to observe the dynamic progression of PCT development throughout the entire lifespan through the tracing of *EGFP* signaling. Moreover, this transgenic fish line enables quantification of nephron regeneration. Overall, the *Tg(osr2:EGFP)* zebrafish line is an excellent tool for screening mutants associated with PCT development, as well as for screening potential drugs targeting PCT development and related diseases.

Data availability

No data was used for the research described in the article.

CRediT authorship contribution statement

Wenmin Yang: Writing – review & editing. **Xiaoliang Liu:** Writing – review & editing, Software. **Zhongwei He:** Software. **Yunfeng Zhang:** Writing – original draft. **Xiaoqin Tan:** Writing – original draft. **Chi Liu:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e27582.

References

[1] B. Lyon-Roberts, K.A. Strait, E. van Peursem, W. Kittikulsuth, J.S. Pollock, D.M. Pollock, et al., Flow regulation of collecting duct endothelin-1 production, Am. J. Physiol. Ren. Physiol. 300 (2011) F650–F656, https://doi.org/10.1152/ajprenal.00530.2010.
[2] L. Yang, G. Frindt, F. Lang, D. Kuhl, V. Vallon, L.G. Palmer, SGK1-dependent ENaC processing and trafficking in mice with high dietary K intake and elevated aldosterone, Am. J. Physiol. Ren. Physiol. 312 (2017) F65–F76, https://doi.org/10.1152/ajprenal.00257.2016.
[3] K.M. Hallow, Y. Gebremichael, G. Helmlinger, V. Vallon, Primary proximal tubule hyperreabsorption and impaired tubular transport counterregulation determine glomerular hyperfiltration in diabetes: a modeling analysis, Am. J. Physiol. Ren. Physiol. 312 (2017) F819–F835, https://doi.org/10.1152/ajprenal.00497.2016.
[4] K.K. McCampbell, K.N. Springer, R.A. Wingert, Analysis of nephron composition and function in the adult zebrafish kidney, J. Vis. Exp. (2014) e51644, https://doi.org/10.3791/51644.
[5] S.C. Thomson, V. Vallon, Effects of SGLT2 inhibitor and dietary NaCl on glomerular hemodynamics assessed by micropuncture in diabetic rats, Am. J. Physiol. Ren. Physiol. 320 (2021) F761–F771, https://doi.org/10.1152/ajprenal.00552.2020.
[6] P. Balazki, S. Schaller, T. Eissing, T. Lehr, A quantitative systems pharmacology kidney model of diabetes associated renal hyperfiltration and the effects of SGLT inhibitors, CPT Pharmacometrics Syst. Pharmacol. 7 (2018) 788–797, https://doi.org/10.1002/psp4.12359.
[7] V. Sander, L. Salleh, R.W. Naylor, W. Schierding, D. Sontam, J.M. O'Sullivan, et al., Transcriptional profiling of the zebrafish proximal tubule, Am. J. Physiol. Ren. Physiol. 317 (2019) F478–F488, https://doi.org/10.1152/ajprenal.00174.2019.
[8] X. Liu, T. Yu, X. Tan, D. Jin, W. Yang, J. Zhang, et al., Renal interstitial cells promote nephron regeneration by secreting prostaglandin E2, Elife 12 (2023), https://doi.org/10.7554/eLife.81438.
[9] S. Zhu, J.S. Lee, F. Guo, J. Shin, A.R. Perez-Atayde, J.L. Kutok, et al., Activated ALK collaborates with MYCN in neuroblastoma pathogenesis, Cancer Cell 21 (2012) 362–373, https://doi.org/10.1016/j.ccr.2012.02.010.
[10] S. Fisher, E.A. Grice, R.M. Vinton, S.L. Bessling, A.S. McCallion, Conservation of RET regulatory function from human to zebrafish without sequence similarity, Science (New York, N.Y.) 312 (2006) 276–279, https://doi.org/10.1126/science.1124070.
[11] A. Vasilyev, Y. Liu, S. Mudumana, S. Mangos, P.Y. Lam, A. Majumdar, et al., Collective cell migration drives morphogenesis of the kidney nephron, PLoS Biol. 7 (2009), https://doi.org/10.1371/journal.pbio.1000009 e9.
[12] Y. Wang, Z.H. Sun, L. Zhou, Z. Li, J.F. Gui, Grouper tshβ promoter-driven transgenic zebrafish marks proximal kidney tubule development, PLoS One 9 (2014) e97806, https://doi.org/10.1371/journal.pone.0097806.
[13] Y. Lan, P.D. Kingsley, E.S. Cho, R. Jiang, Osr2, a new mouse gene related to Drosophila odd-skipped, exhibits dynamic expression patterns during craniofacial, limb, and kidney development, Mech. Dev. 107 (2001) 175–179, https://doi.org/10.1016/s0925-4773(01)00457-9.
[14] Y. Gao, Y. Lan, H. Liu, R. Jiang, The zinc finger transcription factors Osr1 and Osr2 control synovial joint formation, Dev. Biol. 352 (2011) 83–91, https://doi.org/10.1016/j.ydbio.2011.01.018.

[15] S. Kawai, M. Yamauchi, S. Wakisaka, T. Ooshima, A. Amano, Zinc-finger transcription factor odd-skipped related 2 is one of the regulators in osteoblast proliferation and bone formation, J. Bone Miner. Res. : the official journal of the American Society for Bone and Mineral Research 22 (2007) 1362–1372, https://doi.org/10.1359/jbmr.070602.
[16] Y. Lan, C.E. Ovitt, E.S. Cho, K.M. Maltby, Q. Wang, R. Jiang, Odd-skipped related 2 (Osr2) encodes a key intrinsic regulator of secondary palate growth and morphogenesis, Development 131 (2004) 3207–3216, https://doi.org/10.1242/dev.01175.
[17] Z. Zhang, Y. Lan, Y. Chai, R. Jiang, Antagonistic actions of Msx1 and Osr2 pattern mammalian teeth into a single row, Science (New York, N.Y.) 323 (2009) 1232–1234, https://doi.org/10.1126/science.1167418.
[18] N.J. Prescott, M.M. Lees, R.M. Winter, S. Malcolm, Identification of susceptibility loci for nonsyndromic cleft lip with or without cleft palate in a two stage genome scan of affected sib-pairs, Hum. Genet. 106 (2000) 345–350, https://doi.org/10.1007/s004390051048.
[19] Y. Lan, C.E. Ovitt, E.-S. Cho, K.M. Maltby, Q. Wang, R. Jiang, Odd-skipped related 2 (Osr2) encodes a key intrinsic regulator of secondary palate growth and morphogenesis, Development 131 (2004) 3207–3216, https://doi.org/10.1242/dev.01175%JDevelopment.
[20] J.J. Tena, A. Neto, E. de la Calle-Mustienes, C. Bras-Pereira, F. Casares, J.L. Gómez-Skarmeta, Odd-skipped genes encode repressors that control kidney development, Dev. Biol. 301 (2007) 518–531, https://doi.org/10.1016/j.ydbio.2006.08.063.
[21] A. Neto, N. Mercader, J.L. Gómez-Skarmeta, The osr1 and osr2 genes act in the pronephric anlage downstream of retinoic acid signaling and upstream of wnt2b to maintain pectoral fin development, Development 139 (2012) 301–311, https://doi.org/10.1242/dev.074856%JDevelopment.
[22] P.Y. Lam, C.N. Kamei, S. Mangos, S. Mudumana, Y. Liu, I.A. Drummond, odd-skipped related 2 is required for fin chondrogenesis in zebrafish, Dev. Dynam. 242 (2013) 1284–1292, https://doi.org/10.1002/dvdy.24026.
[23] M. Westerfield, The Zebrafish Book. A Guide for the Laboratory Use of Zebrafish (Danio rerio), fourth ed., Univ. of Oregon Press, Eugene, 2000.
[24] K. Asakawa, M.L. Suster, K. Mizusawa, S. Nagayoshi, T. Kotani, A. Urasaki, et al., Genetic dissection of neural circuits by Tol2 transposon-mediated Gal4 gene and enhancer trapping in zebrafish, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 1255–1260, https://doi.org/10.1073/pnas.0704963105.
[25] J. Li, B.B. Zhang, Y.G. Ren, S.Y. Gu, Y.H. Xiang, J.L. Du, Intron targeting-mediated and endogenous gene integrity-maintaining knockin in zebrafish using the CRISPR/Cas9 system, Cell Res. 25 (2015) 634–637, https://doi.org/10.1038/cr.2015.43.
[26] Q. Tang, S. Iyer, R. Lobbardi, J.C. Moore, H. Chen, C. Lareau, et al., Dissecting hematopoietic and renal cell heterogeneity in adult zebrafish at single-cell resolution using RNA sequencing, J. Exp. Med. 214 (2017) 2875–2887, https://doi.org/10.1084/jem.20170976.
[27] R.A. Wingert, R. Selleck, J. Yu, H.D. Song, Z. Chen, A. Song, et al., The cdx genes and retinoic acid control the positioning and segmentation of the zebrafish pronephros, PLoS Genet. 3 (2007) 1922–1938, https://doi.org/10.1371/journal.pgen.0030189.
[28] C.Q. Diep, D. Ma, R.C. Deo, T.M. Holm, R.W. Naylor, N. Arora, et al., Identification of adult nephron progenitors capable of kidney regeneration in zebrafish, Nature 470 (2011) 95–100, https://doi.org/10.1038/nature09669.
[29] J. Augusto, B. Smith, S. Smith, J. Robertson, R. Reimschuessel, Gentamicin-induced nephrotoxicity and nephroneogenesis in Oreochromis nilotica, a tilapian fish 26 (1996) 49–58.
[30] J. Chen, T. Yu, X. He, Y. Fu, L. Dai, B. Wang, et al., Dual roles of hydrogen peroxide in promoting zebrafish renal repair and regeneration, Biochem. Biophys. Res. Commun. 516 (2019) 680–685, https://doi.org/10.1016/j.bbrc.2019.06.052.
[31] C. Liu, X. Liu, Z. He, J. Zhang, X. Tan, W. Yang, et al., Proenkephalin-A secreted by renal proximal tubules functions as a brake in kidney regeneration, Nat. Commun. 14 (2023) 7167, https://doi.org/10.1038/s41467-023-42929-5.
[32] R.A. Wingert, R. Selleck, J. Yu, H.-D. Song, Z. Chen, A. Song, et al., The cdx genes and retinoic acid control the positioning and segmentation of the zebrafish pronephros, PLoS Genet. 3 (2007) 1922–1938, https://doi.org/10.1371/journal.pgen.0030189.
[33] J.J. Tena, A. Neto, E. de la Calle-Mustienes, C. Bras-Pereira, F. Casares, J.L. Gomez-Skarmeta, Odd-skipped genes encode repressors that control kidney development, Dev. Biol. 301 (2007) 518–531, https://doi.org/10.1016/j.ydbio.2006.08.063.
[34] A. Neto, N. Mercader, J.L. Gomez-Skarmeta, The Osr1 and Osr2 genes act in the pronephric anlage downstream of retinoic acid signaling and upstream of Wnt2b to maintain pectoral fin development, Development 139 (2012) 301–311, https://doi.org/10.1242/dev.074856.
[35] M.E. Swartz, K. Sheehan-Rooney, M.J. Dixon, J.K. Eberhart, Examination of a palatogenic gene program in zebrafish, Dev. Dynam. 240 (2011) 2204–2220, https://doi.org/10.1002/dvdy.22713.
[36] P.Y. Lam, C.N. Kamei, S. Mangos, S. Mudumana, Y. Liu, I.A. Drummond, Odd-skipped related 2 is required for fin chondrogenesis in zebrafish, Dev. Dynam. 242 (2013) 1284–1292, https://doi.org/10.1002/dvdy.24026.
[37] W. Zhou, R.C. Boucher, F. Bollig, C. Englert, F. Hildebrandt, Characterization of mesonephric development and regeneration using transgenic zebrafish, Am. J. Physiol. Ren. Physiol. 299 (2010) F1040–F1047, https://doi.org/10.1152/ajprenal.00394.2010.
[38] S. Sanker, M.C. Cirio, L.L. Vollmer, N.D. Goldberg, L.A. McDermott, N.A. Hukriede, et al., Development of high-content assays for kidney progenitor cell expansion in transgenic zebrafish, J. Biomol. Screen 18 (2013) 1193–1202, https://doi.org/10.1177/1087057113495296.
[39] C. Seiler, M. Pack, Transgenic labeling of the zebrafish pronephric duct and tubules using a promoter from the enpep gene, Gene Expr. Patterns 11 (2011) 118–121, https://doi.org/10.1016/j.gep.2010.10.002.
[40] M. Nichane, C. Van Campenhout, H. Pendeville, M.L. Voz, E.J. Bellefroid, The Na+/PO4 cotransporter SLC20A1 gene labels distinct restricted subdomains of the developing pronephros in Xenopus and zebrafish embryos, Gene Expr. Patterns 6 (2006) 667–672, https://doi.org/10.1016/j.modgep.2006.01.005.
[41] Z. Ma, P. Zhu, H. Shi, L. Guo, Q. Zhang, Y. Chen, et al., PTC-bearing mRNA elicits a genetic compensation response via Upf3a and COMPASS components, Nature 568 (2019) 259–263, https://doi.org/10.1038/s41586-019-1057-y.
[42] A. Rossi, Z. Kontarakis, C. Gerri, H. Nolte, S. Holper, M. Kruger, et al., Genetic compensation induced by deleterious mutations but not gene knockdowns, Nature 524 (2015) 230–233, https://doi.org/10.1038/nature14580.
[43] P. Zhu, Z. Ma, L. Guo, W. Zhang, Q. Zhang, T. Zhao, et al., Short body length phenotype is compensated by the upregulation of nidogen family members in a deleterious nid1a mutation of zebrafish, J Genet Genomics 44 (2017) 553–556, https://doi.org/10.1016/j.jgg.2017.09.011.
[44] A.T. De Souza, X. Dai, A.G. Spencer, T. Reppen, A. Menzie, P.L. Roesch, et al., Transcriptional and phenotypic comparisons of Ppara knockout and siRNA knockdown mice, Nucleic Acids Res. 34 (2006) 4486–4494, https://doi.org/10.1093/nar/gkl609.
[45] Y.B. Gao, Y. Zhang, D. Zhang, X.H. Dai, M. Estelle, Y.D. Zhao, Auxin binding protein 1 (ABP1) is not required for either auxin signaling or development, P Natl Acad Sci USA 112 (2015) 2275–2280, https://doi.org/10.1073/pnas.1500365112.