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Temporal and spatial expression of tight junction genes during zebrafish pronephros development

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ABSTRACT

The kidney is comprised of nephrons – epithelial tubes with specialized segments that reabsorb and secrete solutes, perform osmoregulation, and produce urine. Different nephron segments exhibit unique combinations of ion channels, transporter proteins, and cell junction proteins that govern permeability between neighboring cells. The zebrafish pronephros is a valuable model to study the mechanisms of vertebrate nephrogenesis, but many basic features of segment gene expression in renal progenitors and mature nephrons have not been characterized. Here, we analyzed the temporal and spatial expression pattern of tight junction components during zebrafish kidney ontogeny. During nephrogenesis, renal progenitors show discrete expression domains of *claudin (cldn) 15a, cldn8, occludin (ocln) a, oclnb, tight junction protein (tjp) 2a, tjp2b*, and *tjp3*. Interestingly, transcripts encoding these genes exhibit dynamic spatiotemporal domains during the time when pronephros segment domains are established. These data provide a useful gene expression map of cell junction components during zebrafish nephrogenesis. As such, this information complements the existing molecular map of nephron segment characteristics, and can be used to characterize kidney development mutants as well as various disease models, in addition to aiding in the elucidation of mechanisms governing epithelial regeneration after acute nephron injury.

The kidney is comprised of specialized epithelial tubules termed nephrons that perform important homeostatic tasks including, but not limited to, the reabsorption of nutrients, water balance, and urine production. Vertebrate nephrons are commonly made up of three different functional parts: a blood filter, tubule, and duct (Reimschuessel, 2001; Saxen, 1987). The filter is specialized to collect plasma from the blood, which is then funneled into the tubule (Kroeger and Wingert, 2014). The nephron tubule is regionalized into a series of proximal and distal segments that accomplish the discrete and complex tasks of modifying the filtrate to recover or secrete macromolecules ranging from metabolites to electrolytes (Cheng and Wingert, 2014). The different tubule segments exhibit unique combinations of ion channels and transporter proteins, allowing them to facilitate the intracellular uptake and secretion of solutes (Reilly et al., 2007). Additionally, the success of the kidney is contingent on the ability of the nephron tubule to control and block the paracellular movement of molecules, enabling the precise regulation and containment of nephron contents (Denker and Sabath, 2011). Nephron cells are thus also characterized by the components of these specialized cell-to-cell junctions. Furthermore, the important functions of these factors in renal physiology are emphasized by the association between various chronic renal disease conditions and abnormal epithelial cell junction formation (Balkovetz, 2009; Hou et al., 2013).

The zebrafish pronephros, or embryonic kidney, is a useful model to study nephron biology because it contains nephrons that have a conserved structure with those of other vertebrates, such as amphibians and mammals (Wingert and Davidson, 2008). Studies of zebrafish nephron segment patterning, morphogenesis, and physiology have emerged as powerful research areas that are applicable to human nephrology (Ebarasi et al., 2011; Gerlach and Wingert, 2013). During zebrafish pronephros formation, bilateral stripes of renal progenitors emerge from the intermediate mesoderm (IM) (Drummond et al., 1998). Over the first day of development, these renal progenitors undergo patterning events that fashion them into parallel nephrons (refer to Fig. 6) (Wingert et al., 2007), and undergo a mesenchymal to epithelial transition (MET) to become

Abbreviations: cldn, *claudin*; CS, corpuscles of Stannius; DE, distal early; DL, distal late; dpf, days post fertilization; EMT, epithelial to mesenchymal transition; G, glomerulus; hpf, hours post fertilization; IM, intermediate mesoderm; *myod1*, *myogenic differentiation 1*; N, neck; *ocln*, *occludin*; P, podocyte; PCT, proximal convoluted tubule; PD, pronephric duct; PM, paraxial mesoderm; PST, proximal straight tubule; *smyhc1*, *slow myosin heavy chain 1*; ss, somite stage; TER, trans-epithelial resistance; *tjp*, *tight junction protein*; WISH, whole mount *in situ* hybridization.

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epithelial tubules (Gerlach and Wingert, 2014). The nephrons join rostrally to form a common blood filter (also known as a glomerulus), comprised of renal podocyte (P) cells and a capillary tuft, that is connected to tubules, comprised of the following segments: neck (N), proximal convoluted and straight tubules (PCT, PST), corpuscles of Stannius (CS), and the distal early and late (DE, DL) tubules (Wingert et al., 2007). The nephrons drain into pronephric ducts (PD) that fuse caudally at the cloaca (C), where waste is finally excreted (Wingert et al., 2007). During pronephric development, the renal progenitors undergo highly dynamic alterations in the spatiotemporal expression domains of transcription factors, leading to the emergence of the aforementioned segments (Li et al., 2014; Serluca and Fishman, 2001; Wingert and Davidson, 2011; Wingert et al., 2007). Comparative analysis of solute gene expression profiles among zebrafish, amphibian, mouse, and human nephrons has demonstrated that segment characteristics are well conserved across these vertebrate species (Wingert and Davidson, 2008).

To date, the characterization of gene expression domains in the zebrafish pronephric kidney segments has been largely limited to transcription factors during nephron patterning and solute transporters at the time points when discrete nephron segments have formed (Li et al., 2014; Marra and Wingert, 2014; Miceli et al., 2014; Naylor et al., 2013; O'Brien et al., 2011; Wingert and Davidson, 2011; Wingert et al., 2007). However, vertebrate nephron functionality is crucially contingent on the aforementioned cell-cell interactions that create tight or leaky attachments between adjacent epithelial cells (Balkovetz, 2006; Denker and Sabath, 2011). This permeability between neighboring nephron cells is governed by interactions between specific proteins that comprise their tight junctions (Balkovetz, 2006; Denker and Sabath, 2011). Annotating the regional differences in tight junction gene expression is integral to understanding the molecular characteristics of the zebrafish kidney, and ultimately its physiology.

Tight junctions are located near the apical surface and consist of a cytoplasmic plaque of proteins as well as intracellular protein fibers (Cereijido et al., 2008; Gonzalez-Mariscal et al., 2007). Across vertebrate tissues, the expression of discrete tight junction components establishes unique properties for various cell types. The cytoplasmic plaque contains the tight junction proteins (TJ; also known as zona occludens (ZO)), which function to link the complex with the cytoskeletal network. Additional proteins found in these junctions include the Occludins (Ocln), a family of tetraspan membrane proteins, and the Claudins (Cldn), a second distinct family of tetraspan membrane proteins. Ocln proteins, which have been found to be non-essential for the formation of tight junctions, influence paracellular permeability by increasing trans-epithelial resistance (TER) between adjacent cells. Cldns, on the other hand, are essential for tight junction formation, and are primarily responsible for regulating the paracelluallar permeability properties of epithelia. Generally, vertebrates possess over 20 Cldn gene family members (Günzel and Fromm, 2012; Hewitt et al., 2006), and these have been greatly increased in the genomes of teleost fishes, where some species possess over 50 Cldn genes (Kolosov et al., 2013). The barrier function that these two protein families contribute to the kidney is essential for its function, and several renal diseases have been associated with mutations in their members (Balkovetz, 2009). Another critical role that tight junctions perform is the maintenance of cell polarity through a so-called fence function, in which they prohibit apical protein complexes from diffusing into the basolateral region and vice versa (Cereijido et al., 2008; Gonzalez-Mariscal et al., 2007). Through their links to the cytoskeleton, junctional complexes help to regulate its organization and functional activities (Fanning et al., 2011). Further, epithelial cell function is modulated by signaling pathways that phosphorylate various tight junction components, situating the tight junction as an assemblage of dynamic elements that significantly influence cell phenotype in both health and disease states (Gonzalez-Mariscal et al., 2008).

Interestingly, genes encoding intercellular junctional proteins, such as Cldns, are known to be regionally expressed in the mammalian kidney, such that TER is increased along the proximodistal length of each nephron (Denker and Sabath, 2011). Whether zebrafish nephrons exhibit analogous regional expression patterns of tight junction genes has not yet been established. Since the simple embryonic kidney of the zebrafish is an advantageous model for nephrology research, it is important to delineate tight junction expression across renal cell types. Previous work by Keiner et al. (2007) has documented the expression pattern of several *tjp* genes throughout tissues of the whole zebrafish embryo during ontogeny. However, sparse information was gathered regarding the expression of these genes within the pronephros, and other tight junction components were not examined. In this study, we performed a detailed analysis of the transcript localization of these *tjp* genes and other junctional components in the developing zebrafish pronephros using whole mount *in situ* hybridization (WISH). We found that zebrafish renal progenitors exhibit dynamic alterations in tight junction gene expression. Furthermore, tight junction genes show an overlapping, nested arrangement in developing nephrons, such that distal nephron regions express the greatest number of factors. With these data, we have thus characterized a spatiotemporal map of zebrafish *tjp, ocln*, and *cldn* gene expression domains during nephrogenesis. Overall, these findings provide a useful addition to the current catalogue of nephron segment characteristics in the zebrafish and can be used to further the understanding of renal physiology.

1. Results and Discussion

1.1. Overview of tight junction genes and pronephros expression analysis

Vertebrate nephrons are characterized by the regional expression of tight junction components which enables relatively leaky proximal tubule segments to reabsorb solutes readily, while distal tubule segments tightly regulate solute movement in order to fine-tune salt and electrolyte levels in the body (Denker and Sabath, 2011). Regional and/or graded expression of Cldn and Occludin genes typifies mammalian nephrons (Denker and Sabath, 2011). Interestingly, previous gene expression analysis has demonstrated that at two *tjp* genes, *tjp2a* and *tjp3*, are expressed in the distal pronephros (Keiner et al., 2007).

To examine whether zebrafish nephrons exhibit a conserved regional distribution of tight junction genes during nephrogenesis, we performed time course studies to examine the expression domains of these genes as well as other tight junction genes annotated in the zebrafish genome. We implemented a modified WISH method which incorporates the use of dextran sulfate to analyze the renal expression of *claudin (cldn) 15a, cldn8, occludin (ocln) a, oclnb*, and *tight junction protein (tjp) 1a, 1b, 2a, 2b*, and *3*, as described in the following sections. To precisely map each gene expression domain in the developing pronephros, we utilized a double WISH approach previously established by our lab that uses a riboprobe to label *slow myosin heavy chain 1* (*smyhc1*) to demarcate the embryonic somites located adjacent to the nephron territory (Li et al., 2014; Wingert and Davidson, 2011; Wingert et al., 2007). This method enables each gene expression domain to be compared to the eventual location of the various pronephric tubule and duct segments. Of note, the renal progenitors emerge from the intermediate mesoderm, and maintain a mesenchymal character until the 20–22 somite stage (ss), at which time they undergo MET, thereby forming tubules (Gerlach and Wingert, 2014). By the 28 ss (approximately 1 day post fertilization (dpf)), discrete segment boundaries are