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Mesonephric kidney – a stem cell factory?

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ABSTRACT Mesonephros is a vestige, transient renal organ that functions only during embryonic development. The anatomy, position and even cellular fate of the mesonephric kidney varies drastically among mammalian species. The origin of mesonephros from intermediate mesoderm and the dependence of its differentiation on the nephric or Wolffian duct have been well established. Commonly accepted is also the mesonephric origin of epididymal ducts of the male reproductive tract. Recently, upon the more profound understanding of the molecular mechanisms involved in the development of the permanent mammalian kidney, some light has been shed over the molecular events taking place during the mesonephric development as well. Because of the functional and structural similarities between the mesonephric and metanephric kidneys, it is not surprising that many molecules regulating metanephric development are also activated during mesonephric development. However, the multifunctional nature of mesonephros has been unexpected. First, it serves as an embryonic secretory organ, in some mammalian species more so than in others. It is thereafter removed by programmed cell death. Second, it is a source of multiple stem cells including somatic cells in the male gonad, vascular endothelial cells, and hematopoietic stem cells. Thus, mesonephros is a challenging model for studies on epithelial differentiation and organogenesis, regulation of apoptosis, sex determination and stem cell differentiation. In this review, we focus in the molecular and stem cell aspects in the differentiation of the mammalian mesonephros.

KEY WORDS: *mesonephros, mammalian, stem cells, gonadal differentiation, hematopoiesis*

Morphogenesis of the mammalian mesonephros

All three types of renal organs found in the mammalian species during their embryonic development are derived from the intermediate mesoderm formed after gastrulation. In mammals, however, only the last stage, the metanephros, remains through adulthood, and the others, the pronephros and the mesonephros, disappear during embryogenesis (reviewed by Saxén, 1987). The pronephros consists of few tubule-like structures and has rudimentary, if any, secretory function, but it is believed to be important in the pronephric-mesonephric duct formation, the duct that becomes the nephric duct or Wolffian duct (Toivonen, 1945; Fig. 1). The morphogenesis and molecular mechanisms of the pronephric development are well understood in anamniotes, and an interesting review on this subject has been published recently (Vize *et al.,* 1997). Moreover, the intensive mutation studies on-going in the zebrafish (Drummond *et al.,* 1998) will undoubtedly expand our understanding of the regulation of urogenital differentiation.

Since the mesonephric tubules in the common laboratory rodents, rat and mouse, are relatively few and poorly developed, most anatomical studies on the mammalian mesonephros have been made with other species, such as pig, rabbit, sheep and cat. In these species, as well as in humans, the mesonephros has a similar basic structure that is found in the metanephros: glomeruli with a well-developed vasculature, proximal and distal tubules, and collecting ducts (the Wolffian duct derivatives), in only a somewhat simplified mode (Saxén, 1987). The mesonephros of mouse starts to regress about the same time as the development of metanephros begins. Nevertheless, in mice and rats the most cranial tubules of the mesonephros will remain as the epididymal ducts of the adult male, while the Wolffian duct serves as vas deferens. In females, at least the rete ovarii, a group of anatomising tubules (epididymis-like structure) and epoöphron connecting them (Kardong, 1995), are mesonephric derivatives.

Ten years ago, only little was known about the molecular mechanisms underlying kidney development. Most of the data were collected from cell and tissue culture experiments and tissue transplantation studies (Saxén, 1987). Today several molecules regulating this organ system are known and reviewed in this issue. Since mesonephros is essentially differentiated in a similar

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Fig. 1. Page from Professor Sulo Toivonen's laboratory book from 1940' illustrating the mesonephric structure in the developing rabbit embryo.

fashion to the metanephros, it is not surprising that some key regulatory molecules in the mesonephrogenesis are the same as in the permanent kidney.

The paired box gene *Pax-2* (Dressler *et al.,* 1990; Torres *et al.*, 1995) regulates not only metanephric but also mesonephric differentiation, since *Pax-2* -deficient mice lack all nephrogenic derivatives of the intermediate mesoderm (Torres *et al.*, 1995). Thus, the primary defect in *Pax-2* null mutants is an early event associated with the initial steps in the differentiation of the intermediate mesoderm. Recently, a signalling molecule from the transforming growth factor-β superfamily, bone morphogenetic protein-4 (BMP-4) secreted by the surface ectoderm, has been shown to regulate the expression of *Pax-2* by the nephric duct progenitor cells of the intermediate mesoderm (Obara-Ishihara *et al.,* 1999). As shown by microsurgical tissue experiments (Gruenwald, 1937), the Wolffian duct formation is essential both for meso- and metanephrogenesis. Thus, BMP-4 is a candidate molecule to regulate the first steps in the differentiation of this organ system. The second transcriptional regulator crucial for the urogenital area is Wilms' tumour gene-1, *WT-1* (Pritchard-Jones *et al.,* 1990; Kreidberg *et al.*, 1993). Mice with targeted deletion of this gene lack gonads (and adrenals), most mesonephric tubules, and metanephros (Kreidberg *et al.,* 1993). Nevertheless, a distinct set of mesonephric tubules, the cranial ones, develops almost normally in the *WT-1*$^{-/-}$ null mutants (Sainio *et al.,* 1997). Thus, the development of the cranial mesonephric tubules is regulated differently from the more caudal ones. These cranial tubules will later on form the epididymal ducts in the male mouse.

Mesonephros as a source for gonadal somatic cells

The mesonephros and presumptive gonadal area, the genital ridge, develop next to each other. The long genital ridge faces the inner surface of the equally long mesonephros. Tissue culture experiments of the urogenital ridges from mouse embryos have shown that mesonephros is necessary for a proper differentiation of seminiferous tubules of testis (Buehr *et al.*, 1993; Merchant-Larios *et al.*, 1993). There is extensive cell migration taking place between mesonephros and developing gonad, especially testis (Martineau *et al.,*1997). Three kinds of migratory somatic cells have been identified: endothelial, myoid and fibroblast cells.

The endothelia build up a vascular network in testis, and the angiogenic precursor cells proliferate and differentiate in the mesonephric area before their invasion into the future gonad (Merchant-Larios *et al.,* 1993). These data are supported by the whole-mount staining of rat mesonephros with antibodies against podocyte and endothelial sialoglycoprotein podocalyxin (Schnabel *et al.,* 1989; Miettinen *et al.,* 1990). Podocalyxin is expressed not only in the presumptive podocytes of the mesonephric tubules but also in the mesonephric stroma surrounding the epithelial structures (Sainio *et al.,* 1997). Vascular endothelial growth factor (VEGF) activity, one of the major regulators of angiogenesis, is upregulated by testosterone (Sordello *et al.,* 1998) and VEGF might be a signalling molecule responsible for guiding the migration of the mesonephros-derived endothelial cells into the developing testis.

The other cell types, myoid and fibroblast cells, participate shaping the seminiferous cords (Merchant-Larios *et al.* 1993). The origin of these cells from metanephric stroma has been indicated, because these cells are absent in isolated impaired gonads cultured without mesonephros (Buehr *et al.* 1993; Merchant-Larios *et al.,* 1993). A new study using the mesonephros and bipotent XX gonad recombinant tissue experiments shows that migrating mesonephric cells are essential for testicular cord formation and Sertoli cell differentiation. The writers name myoid cell precursors as candidates to drive the cord formation. The myoid cells form peritubular smooth muscle cells around testicular cords and are in contact with future Sertoli cells synthetising together the seminiferous tubule basement membrane. This close proximity may be essential for Sertoli cell versus follicle cell differentiation (Tilmann and Capel, 1999).

The origin of Sertoli cell precursors has caused some controversy. Several early studies, based on histological criteria, suggested that some or all Sertoli cells are derived from the mesonephros (Upadhyay *et al.*, 1981; Wartenberg *et al.,* 1981; Pelliniemi *et al.*, 1984). On the other hand, expression of Y chromosomal male sex-determining factor *Sry* (Koopman *et al.*, 1990) expressed by pre-Sertoli cells, has not been found in the recombination chimeras of mesonephros and male genital ridge (Buehr *et al.*, 1993; Merchant-Larios and Moreno-Mendoza, 1998). Thus, either the Sertoli cells differentiating in this recombinant culture system are not able to maintain their normal gene expression pattern or the Sertoli cell precursors are derived from other mesodermal tissues than the mesonephros.

Recently, some mesonephric stromal cells have been shown to become testosterone-producing Leydig cells after prolonged culture of the mesonephric/gonadal chimeras between CD-1 mice genital ridges and ROSA26 mesonephroi expressing β-galactosidase marker (Merchant-Larios and Moreno-Mendoza,

1998). Results from another CD-1 and ROSA26 chimeric genital ridge and mesonephros tissue culture experiments suggested that the cell migration from the mesonephros occurs exclusively into testis (Martineau *et al.,*1997). The careful analysis of Wnt-4 -deficient female embryos has shown that the Leydig cell precursors derived from the mesonephros invade the genital ridge of both sexes (Vainio *et al.,* 1999). Wnt-4, a member of Wnt signalling molecule family that is expressed in the stromal compartment of the mesonephros but not in the mesonephric epithelium, suppresses the differentiation of ovarian Leydig cell precursors and is required to inhibit their testosterone production. The fetal ovary in *wnt-4* -deficient female mice embryos has Leydig cells that produce testosterone. Thus, the female embryos are masculinised (Vainio *et al.*, 1999). The further cellular fate of Leydig cell precursors that invade the female ovary is not known. The mesonephric stromal cells are also suggested to differentiate into granulosa cells of the ovary (reviewed by Wenzel and Odend'hal, 1985).

Mesonephric origin of hematopoietic stem cells

Hematopoiesis is a well-known model for stem cell differentiation. Until recently, the initial hematopoietic activity has been assigned to the yolk sac, then it was supposed to shift via fetal liver and spleen to the bone marrow, where it remains in adults (Moore and Metcalf, 1970; Johnson and Moore, 1975). However, the limited differentiation repertoire of yolk sac-derived stem cells (reviewed by Dzierzak and Medvinsky, 1995) suggested that yolk sac hematopoiesis is transitory and has no derivatives in the adult animal, as had been suggested earlier in chicken (Dieterlen-Lievre, 1975). The new source for adult hematopoiesis was found in the mouse embryo from an area including the dorsal aorta, genital ridge/gonads, and pro/mesonephros (aorta-gonad-mesonephros area [AGM]) (Medvinsky *et al.,* 1993; Medvinsky and Dzierzak, 1996). The hematopoietic precursors appear simultaneously in both the AGM region and yolk sac at late embryonic day 9 but, unlike yolk sac precursors, the stem cells from AGM are highly proliferative and can give rise complete hematopoiesis in the adult mice after irradiation. Thus, the definite hematopoiesis seems to be initiated at AGM region, and fetal liver and bone marrow are later seeded by these hematopoietic stem cells (Medvinsky and Dzierzak, 1996).

In accordance with this experimental data, expression of erythropoietin, a regulator of red blood cell production, has been found in the developing bovine mesonephros (Wintour *et al.,* 1996). Hematopoietic transcription factor GATA-3 is expressed in placenta and T-lymphocytes (Yang *et al.,* 1994), but also both by mouse (George *et al.,* 1994; Lakshmanan *et al.,* 1999) and human (Labastie *et al.,* 1998) mesonephric tubules, where its function is not resolved. An interesting possibility is that GATA-3 expression in mesonephros is regulating the differentiation of lymphocytes. The presumptive omentum (dorsal mesogastrium) of E13 mouse embryo is a region between anterior limbs, foregut and mesonephros. Surprisingly, cells in the presumptive omentum may differentiate into Thy-1-positive lymphocytes, and thus omentum is a stem cell source for developing lymphocytes (Kubai and Auerbach, 1983). As the dorsal mesogastrium lies next to mesonephros and is actually in contact with it, an interesting possibility is that the hematopoietic cell lineage of this tissue might be derived from the AGM.

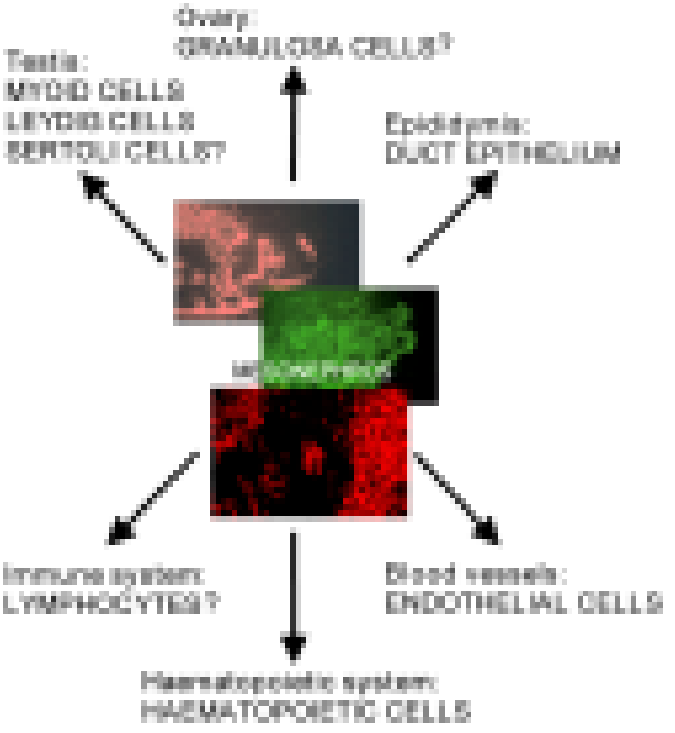

Fig. 2. Summary of the precursor cells migrating from the mesonephric area and their final destination in the embryo. *Whole-mount immunohistochemistry of the mesonephros detecting cytokeratin- and laminin-positive tubular structures (upper and middle) and podocalyxin-positive podocyte-like structure and endothelial cells (lower).*

Summary

Mesonephric kidney is an embryonic organ that in all mammalian species disappears when the permanent kidney, the metanephros, starts its function. In adult males, however, the epididymal ducts and in females the rete ovarii are derived from the mesonephros. During the last two decades increasing amount of evidence suggests that mesonephros is something else than purely a transitory organ with a short life span and minimal, if any, functional significance (see Fig. 2).

The regulation of mesonephric differentiation is not well understood at molecular level. However, some molecules regulating the metanephric development, such as WT-1 and Pax-2, are also important in the mesonephric differentiation. Besides the epithelial tubules, mesonephros contains the stromal cells. They are now suggested to be a source of multiple precursor cells. First, mesonephric stromal cells invade the genital ridge of both sexes. In males, at least part of the endothelial cells and Leydig cells are derived from the mesonephros. Some pre-Sertoli cells, based on morphological criteria, might be of mesonephric origin. Thus far there is no molecular evidence to support this assumption, but mesonephric cells are at least needed for the proper differentiation of Sertoli cells and the testicular cord formation. The Leydig cell precursors also seem to invade the presumptive female gonad but there the differentiation and steroid production by these cells is suppressed by Wnt-4. The fate of the pre-Leydig cells invading the female genital ridge is not clear. They may deterio-

rate apoptotically, but an interesting option is that these mesonephros-derived cells take another developmental pathway in the ovary.

The AGM region has taken over many functions that had previously been dedicated to the yolk sac. As a part of the AGM region, mesonephros is also a site of origin for intraembryonic hematopoiesis. Erythropoietin and members of the GATA transcription factors and their activators (Friend of GATAs; FOG) that are all involved in multiple steps of hematopoiesis are expressed in the urogenital area, where they could regulate the hematopoietic differentiation of the mesonephric stem cells. The other possibility is that GATAs and their activators are regulating the epithelial differentiation of the mesonephros.

Taken together, we have a good reason to consider the mesonephric area as a stem cell factory with multiple functions during embryonic development. The central location of mesonephros in the body cavity and the rather long existence of the pro- and mesonephos during organogenesis render this region an optimal source for several stem cell lineages, and still more to be found.

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