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New horizons at the caudal embryos; coordinated urogenital/reproductive organ formation by growth factor signaling

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Summary

The cloaca/urogenital sinus and its adjacent region differentiate into the urogenital/reproductive organs. Caudal regression syndrome (CRS; including Mermaid syndrome), a type of severe cloacal malformation displays hindlimb fusion and urogenital organ defects, thus suggesting that such defects are caused by several morphogenetic alterations during early development. The attenuation of Bone Morphogenetic Protein (Bmp) signaling at the posterior primitive streak of embryos leads to the caudal dysmorphogenesis including the cloaca and fusion of both hindlimbs. Genetic tissue lineage studies indicate the presence of coordinated organogenesis. Hedgehog (HH)-responding cells derived from peri-cloacal mesenchyme (PCM) contribute to the urogenital/reproductive organs. These findings indicate the existence of developmental programs for the coordinated organogenesis of urogenital/reproductive tissues based on growth factor function and crosstalk.

Introduction

Research on urogenital/reproductive organ formation is an interdisciplinary field for molecular developmental biology. Such organs involved need to develop coordinated architectures, including several cavitated and tubular structures inside the pelvic cavity. Concomitantly, appendicular structures, i.e., external genitalia and hindlimbs, and tail regions develop adjacent to such pelvic organs (Figure 1). The cloacal cavity is located in the center of these organogeneses. It can be subdivided into urogenital sinus (US) that participates in organogenesis inside the pelvic cavity. The cloaca forms at the caudal end of the hindgut [1-5]. The cloaca/US and its adjacent tissues differentiate into many urogenital/reproductive organs, including the urinary bladder (the bladder; Figure 1) [6]. The cloaca also constitutes the boundary region for the reproductive tracts, such as those for Wolffian Duct (WD) and Müllerian Duct (MD).

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Various congenital malformations, ductal abnormalities of connections and fistula formations have been reported for the ureter, vagina, cloaca, perineal region development. Anorectal malformations (ARM) range from simple defects such as an imperforate anus to more complex syndromes [7,8]. Abnormalities in the cloacal derived regions have often been suggested to be associated with more widespread alterations [9], as it is the case for the exstrophy-epispadias complex (exstrophy of cloaca or bladder and abnormal dorsal external genitalia with a defective body wall) (Figure 1) [3,10]. These malformations may suggest that coordinated developmental programs regulate their morphogenesis (Table 1). However, the developmental contribution of the transient embryonic cloacal region to the formation of urogenital/reproductive organs inside and outside the pelvic cavity remains still obscure.

Coordinated development of the urogenital/reproductive organs; genetic lineage study for hedgehog responding tissues

The epithelial and mesenchymal structures within tissues develop “in coordination” through reciprocal interactions that result in integrated organ architectures. The application of mouse molecular genetics has successfully been used to analyze the developmental context of urogenital/reproductive organ formation [11-14]. The elucidation of growth factor systems not only reveals their functions but also provides hints to understand their involvement in coordinating organ development. Hedgehog (HH) proteins, a family of growth factors known to be involved in endoderm, limb patterning, and the formation of other organs, exert fundamental functions on mesenchymal tissues in the posterior part of embryos [15-17]. The expression of HH family member *Shh* in the Cloacal Membrane (CM) is necessary for cloacal formation before the Genital Tubercle (GT), anlage of external genitalia, outgrowth [16,18]. The Distal Urethral Epithelium (DUE), which is part of the CM, has been suggested to regulate GT outgrowth. DUE expresses *Fgf8* and other *Fgfs* [19,20], and the GT ectoderm expresses several Wnt ligands (unpublished observation). Canonical Wnt/ β -catenin signaling, which is observed in the distal region including the DUE, regulates GT development [21] (unpublished observation). Recent works have furthermore indicated a crosstalk of *Shh*, Wnt/ β -catenin and *Bmp* signaling in the initiation of GT outgrowth, an event related to the DUE and GT ectoderm interaction [16,21,22] (unpublished data). *Shh* expression persists in the developing urethral plate (UP), pelvic urethra and in the bladder epithelium (Figure 2) and HH signaling is also important for the formation of those tissues [15,16,23].

Using the responsiveness to HH signals as read-out to analyze the fate of the peri-cloacal mesenchyme (PCM), it has been shown that HH-responsive PCM contributes to both the dorsal GT and mesenchyme for bladder smooth muscles (Figure 2) [15]. Thus, the coordinated formation of the bladder, internal pelvic urethra and external genitalia is orchestrated in part by HH responding cells. As several Wnt signal mutants show early embryo patterning defects including the formation of the caudal embryo [24-26], analysis of the crosstalk between the HH-Wnt/ β -catenin signals at the cloaca stage will provide new insights for understanding the coordinated development of subsequent structures. The PCM region, which has been partly described as the infra-umbilical mesenchyme [27], is located (at least partly) in the upper (anterior) part of the cloacal field contributing to urogenital tissues and thus is connected to EEC (exstrophy epispadias complex) syndromes. Noteworthy is that the HH responsive midline is derived from the prechordal plate during craniofacial formation [28] indicating that organogenesis of midline derived structures both in the anterior and posterior part of embryo involves specific group of HH responsive cells.

Apart from organogenesis, the contribution of HH signaling to mesenchymal differentiation is at large not elucidated. Previous studies have suggested a possible function of HH signaling for smooth muscle differentiation [29], where the immature mesenchyme can respond and “interpret” epithelially derived *Shh* signals. However, the precise role of epithelial derived HH

signaling and subsequent mesenchymal responses remain unclear. *Bmp4* is one of the candidate downstream regulators. *Bmp4* is normally expressed in the PCM adjacent to the cloaca [15], and *Bmp4* heterozygote mutants display hydronephrosis due to defective ureter and renal pelvis formation [30]. It remains unclear how subsequent signals within and from the urogenital mesenchyme are orchestrated and relayed. The identification of mesenchymal growth factors and other regulators for the differentiation of smooth muscle is a priority for regenerative medicine [31]. Regulatory mechanism that drives immature mesenchymal cells to smooth muscle differentiation need to be elucidated, and it will be interesting to see what functions HH signaling has in the regulation of smooth muscle progenitors.

Regulation of the Epithelial-Mesenchymal Transition (EMT) at the VER (Ventral Ectodermal Ridge); the cutting edge of the caudal developmental coordination

Malformations associated with cloacal defects involve dysmorphogenesis of the vertebral column, kidneys, urinary tract and hindlimbs and extrophy of the cloaca. Cloacal exstrophy with hindlimb defects may be related to abnormalities in the caudal mesoderm [32]. Because these defects often involve the more posterior parts of the body, some studies proposed that the cloacal defects induce several caudal defects including the most extreme form, namely caudal regression syndrome (CRS) [33].

Developmental coordination also implies proper contribution from early tissue progenitors to several organ components. EMT (Epithelial-Mesenchymal Transition) is an important process in embryogenesis [34-36] and regulates cell-mass supply in many developing regions. It has been recently suggested that defects in formation of the primitive streak (PS) during the late gastrula stage could lead to caudal body malformations including part of the urogenital/reproductive organs as well as the hindlimbs [37]. The late PS contributes to the ventral ectodermal ridge (VER), the thickened ectodermal tissue located ventrodistally at the tailbud [38-40]. Development of the cloaca and the tail are associated [1,37,41] and the tailbud-derived mesenchyme may play a role in controlling ureter morphogenesis [42]. The histological similarity between the VER and the apical ectodermal ridge (AER), the signaling epithelia for limb development, suggests that the VER is the signaling epithelia for tail development [41, 43]. Several findings indicate that a regulated supply of cells through the PS and VER is critical for caudal embryo formation. Dysregulation of the EMT at this point can induce symptoms similar to mermaid syndrome, known as Sirenomelia, which is characterized by leg fusion. Such EMT is regulated by Bmp signaling at the caudal end of embryos, suggesting that dysregulation of Bmp signaling results in the hypoplasia of the perineal region with hindlimb fusion [37]. The modulation of Bmp signaling by *Bmp7* and twisted gastrulation (*Tsg*) results in similar phenotypes [44].

Cells supplied from the above EMT may also influence the perineal region and possibly affect the development of HH-responding PCM cells around the cloacal regions. However, a correlation between HH responsive tissue lineage contribution and cell contribution by EMT has not been shown. Influences from the upper (anterior) lower body wall on perineal development should regulate the expression of several genes in the lower body wall and in the pelvic region. Influences from the lower (posterior) tail and VER are also supposed to lead similarly to coordinated effects. The PCM region, which is regulated by HH signaling, should thus be coordinated by the above developmental processes. Recent studies suggest that the PCM is located, at least in part, on the anterior side of cloaca. The developmental characterization of other PCM regions lateral to the cloacal cavity or lower to the cloacal cavity requires further investigation to obtain a comprehensive understanding of the caudal development.

Hormonal modulation of organogenesis; an epilogue of coordinated organogenesis

One of the major characteristics of urogenital/reproductive organ formation is its sexually dimorphic development [45-49]. Hormonal control of sexual development in organogenesis has been studied for several decades. Sexual differentiation is a remarkably complex process, which depends on the orchestration of the signaling network. However, the involvement of non-gonadal and locally produced masculine factors (or “effector molecules”) which can potentially interact with hormonal signaling in the sexually developing organs has not yet been elucidated. The GT development serves as a good model to study sexual dimorphisms. Intriguingly, Wnt/ β -catenin signaling regulates such sexually dimorphic development [45] as sexually dimorphic Wnt/ β -catenin signaling is observed in the bilateral mesenchymal region adjacent to the UP epithelium. This is possibly due to augmented *Dkk2* expression in the female GT leading to altered Wnt/ β -catenin signaling. In line with this, genetic female (XX) mice with constitutive active β -catenin display adult male-like “sex reversal” of the external genitalia indicating that the Wnt/ β -catenin pathway acts as a locally expressed masculine effector. As Wnt/ β -catenin signaling is also involved in the female gonadal developmental pathway [50-55], it appears that the Wnt/ β -catenin pathway is involved in sexual differentiation in a developmental context-dependent manner with different activities for the male and female pathways. To dissect context-dependent functions of growth factors, analysis of genetic interactions and the coordination between hormones and locally expressed growth factor signaling during development of urogenital/reproductive organs will be further necessary.

Conclusions

Reflecting the complex nature of their genesis, urogenital/reproductive organs are one of the most frequent sites for developmental malformations. As such, urethral birth defects are among the frequently observed congenital defects observed in humans [56-58]. However, the genetic basis for most of the congenital malformations is poorly understood. Increasing our understanding of growth factor crosstalk, coordinated organ formation and its modulation for sexually dimorphic development will help us understanding many birth defects observed in urogenital/reproductive organs.

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Figure 1. A schematic illustration showing “the developmental coordination” at the level of caudal embryos

Externally growing appendicular anlage, such as the hindlimb (purple) and external genitalia (green) and also internally developing organs (from the cloaca (red cavity) towards pelvic organs) develop in coordination. Defects in the coordination may be involved for the onset of some syndromes with abnormal organogenesis (a lower arrow). An example of a syndrome displaying such abnormalities including bladder and external genital defects (EEC as an example; exstrophy and epispadias complex). Pathological mechanisms of EEC is largely unknown. The defects in the developmental coordination of PCM derived cells may constitute one of the causal factors. Developmental coordination also includes proper regulation of cell-supply through EMT from the developing VER (ventral ectodermal ridge) and the PS, which are close to the tail bud. Dysregulation of such cell supply has been suggested to cause hindlimb fusion and reduction of the “cloacal field”. PCM, peri-cloacal mesenchyme.

Figure 2. Contribution of HH responding cells in the formation of urogenital organs
 (A) One of the key epithelial growth factors expressed at the cloacal membrane (CM) is *Shh*. *Shh* expression is first detected prominently in the endoderm including the cloaca. (B) Its expression continues to several epithelial regions derived from cloaca, towards the urethral plate (UP), the pelvic urethra and the bladder lumen. (C) Genetic lineage analysis revealed the tissue contribution from the PCM towards the dorsal external genitalia and bladder mesenchyme (smooth muscle) [15]. PCM, peri-cloacal mesenchyme; CM, cloacal membrane; GT, genital tubercle; UP, urethral plate; DUE, distal urethral epithelium.

Table1

Congenital diseases and candidate developmental regulators

Congenital diseases	Feature	Candidate developmental regulators	Reference
Exstrophy epispadias complex (EEC)	An anterior midline defect with the infraumbilical abdominal wall including the pelvis, urinary tract, and external genitalia.	Hedgehog (Shh) signaling (?)	[15]
Anorectal malformation (ARM)	A congenital defects of urorectal septum (URS), which divide cloaca into the urethra and hind gut.	Shh, Gli, Bmp7, Wnt5a	[59,60,61]
Caudal regression syndrome (CRS)	A congenital caudal anomalies affecting the caudal spine and spinal cord, the hindgut, the urogenital system, and the hind limbs.	Bmp7,Tsg, Hlxb9, Retinoic Acid signaling	[44,62,63]
Hand-foot genital syndrome	Abnormal development of the hands and feet, the urinary tract, and the reproductive system.	Hoxa13, (Hoxd13)	[64,65]