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Bmp7 expression and null phenotype in the urogenital system suggest a role in re-organization of the urethral epithelium

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ABSTRACT

Signaling by Bone morphogenetic proteins (Bmps) has multiple and diverse roles in patterning and morphogenesis of the kidney, eye, limbs and the neural tube. Here, we employed the *Bmp7*^{lacZ} strain to perform a detailed analysis of *Bmp7* expression and the null phenotype during development of the mouse urogenital system. The urethral compartment originates in mid-embryogenesis from the ventral part of the cloaca, a transient cavity at the caudal end of the hindgut. At mid-gestation, *Bmp7* expression was detected within several specific domains in the cloacal epithelium and mesenchyme. In late embryogenesis, *Bmp7* expression was present in the urethra, rectum, the urethral glands, corpus cavernosum, and in the male and female genital ducts. Importantly, loss of *Bmp7* resulted in arrest in cloacal septation, and severe defects in morphogenesis of the genital urethra and mesenchyme. Together, our analysis of *Bmp7* expression and the null phenotype, indicates that *Bmp7* may play an important role in re-organization of the epithelium during cloacal septation and morphogenesis of the genital tubercle.

1. Results and discussion

The mammalian urogenital system, the bladder, urethra and external genitalia, develop from the epithelium and mesenchyme of the cloaca, a transient embryonic cavity at the caudal end of the hindgut (Fig. 1A). Urogenital malformations occur at high frequency in humans, and often combine malformations of the genital tubercle (GT), the penis in males and clitoris in females, and defects in separation of the urethral and rectal compartments (Hendren, 1992, 1996, 1998; Kurzrock et al., 1999; Baskin et al., 2001; Levitt & Pena, 2005; Mo et al., 2001). Morphogenetic mechanisms that direct development of the urogenital system are not well understood. The caudal homeobox genes, *Hoxa13* and *Hoxd13*, are expressed in the cloacal epithelium and mesenchyme, and are essential for morphogenesis of all cloacal derivatives (Mortlock & Innis, 1997; Warot et al., 1997; Morgan et al., 2003). Expression of *p63* (Cheng et al., 2006) and *Sonic hedgehog* (*Shh*) (Haraguchi et al., 2001, 2007; Mo et al., 2001; Seifert et al., 2008) in the cloacal and urethral epithelium is also important both for septation of the cloaca, and development of the bladder and external genitalia. In addition, *Fibroblast Growth Factors 8* and *10, Bmp4 and 7*, and *Wnt5a* have been implicated in development of the GT (Petiot et al., 2005; Suzuki et al., 2008; Yamada et al., 2006; Yamaguchi et al., 1999), but their role in morphogenesis of the cloaca has not been explored. In this study, we employed the *Bmp7*^{lacZ} reporter strain (Godin et al., 1998) to perform a detailed analysis of *Bmp7* expression and the null phenotype during development of the urogenital system in males and females.

1.1. LacZ activity accurately reflects expression of Bmp7 in the urogenital system

Comparative analysis of *Bmp7* expression by in situ hybridization with an antisense *Bmp7* RNA probe (Lyons et al., 1995), and by X-gal staining of tissues in *Bmp7*$^{lacZ/+}$ background has shown that LacZ activity accurately reflects the expression of the wild type *Bmp7* allele in many embryonic tissues, including the kidney (Godin et al., 1998; Dudley et al., 1999) and the urethra (Fig. 4C and D). In addition, our analysis of *Bmp7*$^{lacZ/+}$ GT (Figs. 1–3) showed LacZ activity in the urethral plate and genital mesenchyme in complete agreement with previous reports on *Bmp7* expression (Morgan et al, 2003; Suzuki et al., 2003). Thus, we conclude that LacZ activity in *Bmp7*^{lacZ} strain is an accurate readout of the activity *Bmp7* promoter in the lower urogenital system and the hindgut.

1.2. Bmp7 expression and null phenotype in the cloacal area at E11.5–E13.5

The cloacal cavity is defined as the caudal end of the hindgut when, at embryonic day 9.5 (E9.5) in mice, it comes in contact with the ectoderm just anterior to the tail at the site of the future anal

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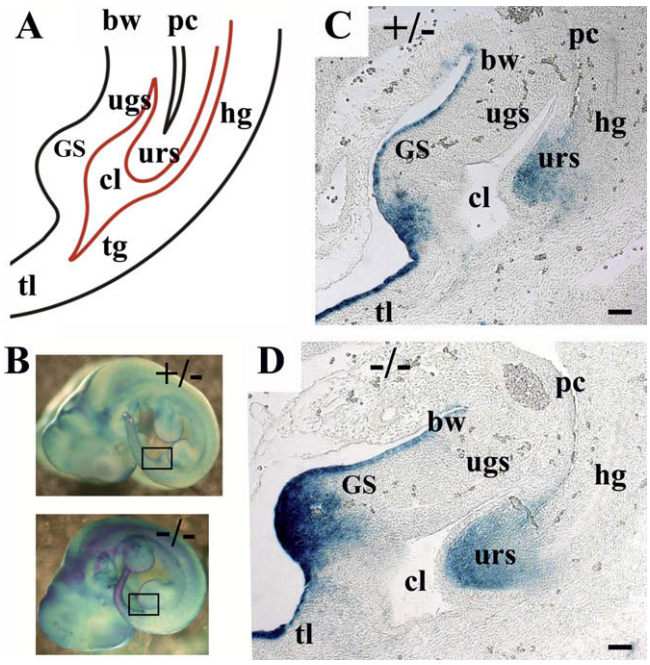

Fig. 1. *Bmp7* expression in the cloacal area at E11.5. (A) Schematic depiction of the cloacal. The genital swellings (GS), cloaca (cl), urorectal septum (urs), urogenital sinus (ugs), hindgut (hg), tail gut (tg), peritoneal cavity (pc), body wall (bw) and tail (tl) are indicated. (B) Whole mount X-gal stained $Bmp7^{lacZ/+}$ and $Bmp7^{lacZ/lacZ}$ embryos at E11.5. Areas of sections in (C and D) are indicated with frames. (C and D) Sagittal sections of $Bmp7^{lacZ/+}$ (C) and $Bmp7^{lacZ/lacZ}$ (D) embryos show strong LacZ-activity in the urorectal septum (urs), the mesenchyme of the genital swellings (GS) and the ventral ectoderm of the body wall and the tail. Scale bar: 50 mm.

opening (Perriton et al., 2002; Hynes and Fraher, 2004a,b,c; Sasaki et al., 2004). Starting at E10, the ventral end of the cloaca grows anteriorly giving rise to the embryonic urogenital sinus (UGS), the primordium of the pelvic urethra and the bladder (Fig. 1). Studies in mouse models indicate that septation of the cloaca is driven by the caudal growth of the lateral mesenchyme known as the urorectal septum at E10.5–E13.5, and is completed with disintegration of the cloacal membrane to uncover the anal and the caudal urethral orifices at E13.5–E14.0 (Kluth et al., 1995; Hynes and Fraher, 2004a,b,c; Sasaki et al., 2004; Seifert et al., 2008). Interestingly, analysis of $Bmp7^{lacZ/+}$ heterozygous embryos showed that *Bmp7* is expressed specifically in the urorectal septum (URS) at E11.5 (Fig. 1C) and E12.5 (Fig. 2F), and expression is dramatically reduced at E13.5 (Fig. 3C and D). We further asked whether *Bmp7* function may be required for cloacal septation. From E11.5 to E13.5, heterozygous embryos showed normal descent of the URS (Figs. 1C, 2E, and 3C; Hynes and Fraher, 2004a,b,c; Sasaki et al., 2004), and progressive separation of the urethral and hindgut compartments from anterior to caudal (Figs. 2G–I and 3E–G; Hynes and Fraher, 2004a,b,c; Sasaki et al., 2004). *Bmp7* null embryos showed normal invagination of the URS into dorsal cloaca at E11.5 (Fig. 1D). However, further septation was delayed at E12.5 (Fig. 2K and L compare to G and H) and E13.5 (Fig. 3I and J compare to E and F). At E12.5 and E13.5, transverse sections of *Bmp7* null GT showed persistent cloaca, whereas corresponding sections of heterozygous animals showed separate urethral and hindgut compartments (Figs. 2 and 3).

1.3. Bmp7 expression and null phenotype in the GT at E11.5–E13.5

The initial stages of development of the GT at E11–E15 are gender-independent, and begin with the appearance of genital swellings laterally to the cloaca at E10.5 (Perriton et al., 2002). At E11.5, *Bmp7* expression was detected in the mesenchyme of the genital swellings, and the ectoderm of the ventral body wall and the tail (Fig. 1C). Distal outgrowth of the genital swellings is accompanied by the extension of the urethral epithelium and lumen (Kurzrock et al., 1999; Yamada et al., 2003, 2006; Seifert et al., 2008). At E12, the distal urethral walls adhere to form a solid urethral plate (Fig. 2H and I; Kurzrock et al., 1999; Yamada et al., 2003, 2006; Seifert et al., 2008). Analysis at E12.5 showed *Bmp7* expression in the urethral plate (Fig. 2G and H), the adjacent mesenchyme (Fig. 2H), and the ventral ectoderm (Fig. 2E). In the null, LacZ activity marked the urethral plate, and was present in a broad domain in the GT mesenchyme (Fig. 2K and L). Distal extension of the urethral plate appeared 50% shorter in the null (Fig. 2D, H and L), most likely due to unzipping of the urethral folds at the ventral side of the GT (Fig. 2C, and K, L and insets) and formation of a large urethral groove (Fig. 2C).

At E13.5, *Bmp7* expression appeared in the dorsal GT mesenchyme (Fig. 3C and D) which may contribute to the corpus cavernosum (Yamada et al., 2003). *Bmp7* expression was maintained in the ventral mesenchyme, but decreased in the URS (Fig. 3C and D). In the genital urethra, *Bmp7* was expressed in a gradient with the strongest expression in the distal urethral epithelium (DUE) at the tip of the GT, which also expresses *Fgf8* (Haraguchi et al., 2000), and the weakest at the urorectal septum (Fig. 3F and G). In the null, LacZ activity was lost in the medial and proximal parts of the urethral plate, but maintained in the DUE (Fig. 3K and L). In the ventral GT mesenchyme, *Bmp7* expression was restricted to the urethral plate in heterozygous (Figs. 2H, 3G and H). In contrast, in the null, LacZ-positive mesenchyme spread laterally (Figs. 2K and L, 3K and L).

1.4. Bmp7 expression and null phenotype in the male urogenital system at E17.5 and P0

Starting at E15.5 in mice, the external genitalia undergo sex-specific differentiation largely controlled by androgens in the male (Drews et al., 2002; Kim et al., 2002; Yucel et al., 2003, 2004; Buckley et al, 2006). In E17.5 male embryos, *Bmp7* expression was detected in the urethra (Figs. 4D and 5B, D and H), the bulbourethral and preputial glands (Figs. 4D, 5H), the vas deferens (Fig. 4D, inset) and the corpus cavernosum (Figs. 4D, 5E, F and H). LacZ activity was not detected in the bladder, nor in the testes (Fig. 4D, and inset). To precisely localize LacZ activity to the epithelial and mesenchymal compartments of the GT, we embedded X-gal stained tissues in paraffin and carried out analysis of histological sections (Fig. 5). Some of the sections were co-immunostained for cytokeratin 14 (CK14) which marked the squamous epithelium in the urethra and the rectum (Fig. 5). *Bmp7* expression was detected in the dorsal and ventral epithelium of the urethra (Fig. 5B and H), in the preputial glands (Fig. 5D and H), and in the crypts of the intestine (Fig. 5H). *Bmp7* was also present in the mesenchyme of the urethra (Fig. 5D and K), the rectum (Fig. 5K), and in the corpus cavernosum (Figs. 4D, 5E, F and H). Normal morphogenesis of the penile urethra involves closure of the original caudal orifice (Perriton et al., 2002; Yamada et al., 2003), fusion of the ventral urethral groove (Fig. 5C and D; Yamada et al., 2003), and displacement of the ventral urethral seam by the genital mesenchyme (Fig. 5A; Kurzrock et al., 1999; Yamada et al., 2003; Seifert et al., 2008). In heterozygous GT, *Bmp7* was expressed in the mesenchyme surrounding the ventral epithelial fusion (Fig. 5D). LacZ-positive mesenchyme also localized to the abnormal genital urethra in *Bmp7* null (Fig. 5P–T, bellow). Further analysis showed that *Bmp7* null males developed complex rectourethral malformations, including rectourethral fistula and severe hypospadia (Figs. 4E and F, 5P–T, 8B). In the null, the hindgut lacked a separate anal opening (Figs. 4E and F, 8B), and a narrowed rectum opened directly into