**Dlx5 and Dlx6 expression in adult steroidogenic organs suggests their potential implication in the control of spermatogenesis and adrenal function**

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**Introduction**

Dlx genes encode a highly conserved family of homeodomain-containing proteins that play central morphogenetic roles during embryonic development and are expressed in embryonic Leydig cells. Deletion of the coding and intergenic regions of Dlx5 and Dlx6 with a single targeting event in the mouse embryo results in altered differentiation of Leydig cells with reduced levels of testosterone and feminization at birth. Dlx5/6 regulates StAR gene expression by activating transcription factor GATA-4 in Leydig cells. Dlx5/6 expression in the adrenal gland is unknown.

In order to address the potential roles of Dlx5 in adult steroidogenic organs we analyzed the expression of this gene in the adult testis and adrenal gland.

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**MATERIALS AND METHODS**

Testis and adrenal glands were obtained from adult B6D2F1 (P60 or 1 year old). RT-PCR were performed on tissue extracts (mini Kit RNA PureLink) followed by reverse transcription (Invitrogen). in situ hybridization was performed as described in Bielle et al. 2005 Nat. Neuroscience 8, 1002. For immunohistochemistry, after inhibition of endogenous peroxidases with 0.5% H2O2 solution, PGTA (PBS + Triton 0.25% + 0.2% gelatin + 0.5% Azide), slides were incubated with primary antibody (anti-Dlx5, Invitrogen, AB_2898061, 1 : 500) overnight at 4°C. Staining was revealed with peroxidase-coupled secondary antibodies (Anti-rabbit, 1 :300).

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**TESTIS**

*Distribution of Dlx5 in adult mouse testis.* – A) Control with no primary antibody. B) Transverse section of the testis, note that anti-Dlx5 staining is different in each transverse tubule section possibly corresponding to synchronized stages of the spermatogenic cycles. D-F) Dlx5 is expressed during most stages of spermatogenesis from spermatogonia to late spermatids (blue arrow) and in Sertoli cells. Very low levels of expression were observed in adult Leydig cells. Bar: 50 µm in B; 10 µm C, D; 25 µm A, E, F.

**ADRENAL GLAND**

In the adult adrenal gland, Dlx5 expression is observed both in cortex and in medulla. (blue arrow)

**SINGLE-CELL RNA SEQ**

Single-cell RNaseq analysis of adult human testis. Dlx5 is expressed at most stages of spermatogenesis from spermatogonia to late spermatids and in Sertoli cells, but is virtually absent in adult Leydig cells.

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**Conclusions & Perspectives**

- Our findings suggest that, besides steroidogenesis, Dlx5 might be directly involved in the processes of spermatogenesis and corticosteroid secretion.
- We are generating conditional mutant mice in order to explore the role of Dlx5/6 in these processes.