

TRANSGENIC FEMALE MICE WITH ORNITHINE DECARBOXYLASE (ODC) OVER-EXPRESSION RESTRICTED TO UROTHELIUM HAVE OAB VOIDING PHENOTYPE AND INCREASED URINARY CYTOKINES: A TRANSLATIONALLY RELEVANT MURINE MODEL OF OAB

Hypothesis / aims of study

Prior preclinical OAB animal models (e.g. intraperitoneal cyclophosphamide, intravesical acetic acid or protamine, bladder outlet obstruction) have lacked translational relevance to the idiopathic female OAB seen in the clinics. Because human urothelial cells from female subjects with OAB urgency incontinence, had increased ornithine decarboxylase (ODC) expression and increased intracellular polyamines (the products of ODC enzymatic action) (1,2), the question is whether urothelial overexpression of ODC in an animal could recapitulate an OAB micturition phenotype. Therefore, we constructed a transgene vector containing a UPII promoter (urothelial restriction) linked to an truncated ODCt gene (ODCt is more stable than ODC) downstream. This transgene was inserted into the C57BL6 mouse (WT) genome to create a urothelial restricted ODC overexpressing transgenic mouse (ODC+). We present for the first time that the ODC+ female mice have an OAB phenotype.

Study design, materials and methods

Three cohorts of 12-16 week old female mice, ODC+, ODC- (non-transgene carrying siblings in litter) and WT animals, were used. Whole bladders underwent H&E staining and q-RT-PCR for ODC specifically in the urothelium was performed. For voiding behaviour study, 18 mice (6 in each cohort) were used. Voiding behaviour was measured over 4 hours in the dark cycle and void spot assays (VSA) were performed per algorithm (3). Urine specimens were collected in another set of animals (n=4 for each cohort) and analysed with 32-plex murine cytokine ELISA.

Results

ODC+ animals were heavier (by 20%) and had shorter tails. There was more intraabdominal fat. H&E staining of bladder revealed intracellular inclusion bodies in umbrella cells. qPCR of the urothelium of the ODC+ animal showed 20x higher ODC mRNA expression compared to WT and ODC- (Fig. 1). The ODC+ animal voided significantly more frequently (Fig. 2A, 2D) with significantly more spots in the center of the filter paper (Fig. 2B, 2E). The voided volume was not different between the 3 cohorts of animals (Fig. 2C, 2F). Urinary cytokine analyses revealed 6 of the 32 cytokines had a significant elevation in the ODC+ animals: G-CSF, IL-1 α , IL-1 β , KC (CXCL1), LIX (CXCL5) and VEGF (Fig. 3).

Interpretation of results

The ODC+ female mice have an OAB voiding behaviour phenotype. This included more frequent voiding and more voids in areas that are not normally seen (centre part of filter paper). Because the voided volume was not different between the 3 cohorts, the increased voiding frequency in ODC+ animals was not due to polyuria. Six urinary cytokines in OAB+ animals were significantly elevated suggesting urothelial dysregulation of these cytokines.

Concluding message

We created a translationally relevant OAB transgenic animal model based on prior data from clinical OAB specimens. The ODC+ animal not only had an OAB voiding behaviour, it also had significant increases in several urinary cytokines. This transgenic animal is a valuable tool to study urothelial dysregulation contribution to OAB bladder behaviour phenotype and represents a beside to bench paradigm for studying OAB pathophysiology.

FIGURE 1 – ODC mRNA expression levels are increased by 20-fold in ODC+ urothelium

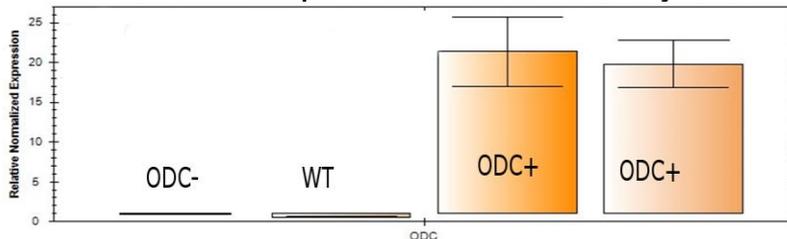
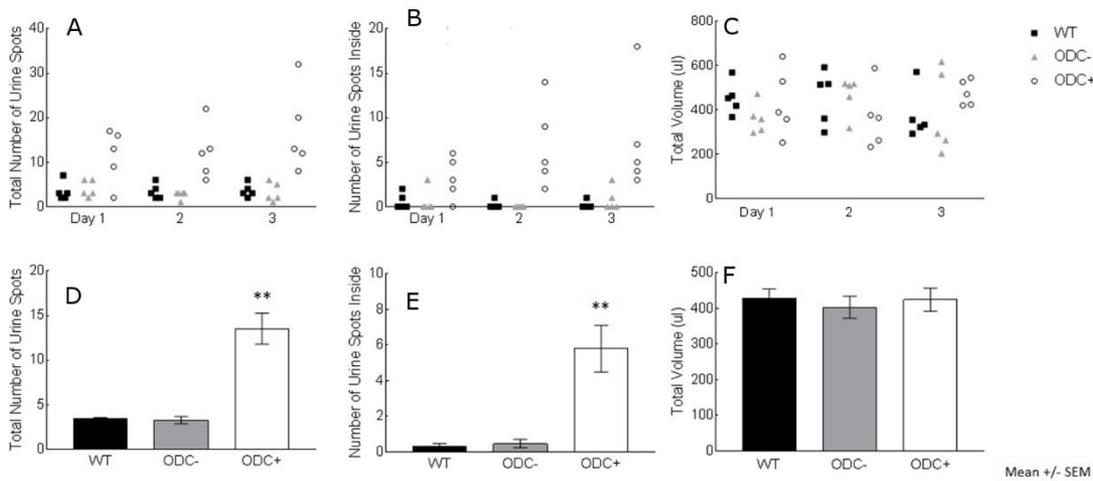
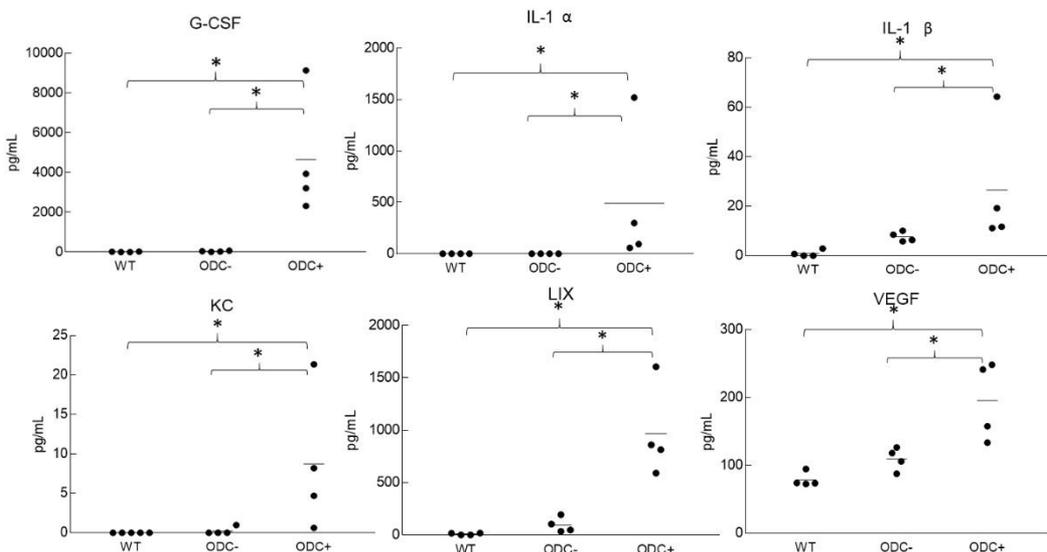


FIGURE 2 – ODC+ animals' voiding behaviour is an OAB phenotype



LEGEND FIG. 2: VSA was performed and analysed according to published protocol (3: total void spots on entire filter paper, B: void spots in pre-defined centre of filter paper, C: total volume voided, D: mean of total void spots, E: mean void spots in centre, F: mean total volume)

FIGURE 3 - ODC+ urines have significantly elevated cytokine levels



LEGEND FIG. 3: Urines were collected and murine cytokines were measured using ELISA. Six cytokines were significantly elevated in urines from ODC+ animals, G-CSF (granulocyte colony stimulating factor), IL-1 α (interleukin 1 α), IL-1 β (interleukin 1 β), KC (keratinocyte-derived cytokine, homologue of human CXCL1), LIX (LPS induced CXC chemokine or CXCL5), and VEGF (vascular endothelial growth factor).

References

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Disclosures

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