Are we closer to de-mystifying the mechanism of ureteral dilatation with pre-stenting?

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INTRODUCTION

Ureteral stents are an integral part of any Urological practice. Pre-stenting of the ureter causes passive ureteric dilatation and better access to the urinary system during subsequent procedures. Ureteric peristalsis is regulated by myogenic and neurogenic factors. The placement of a stent disrupts the physiological ureteral peristalsis which in turns leads to ureteral dilatation. However, the molecular signalling pathway that accounts for ureteral dilatation in humans remains unknown.

OBJECTIVES

To investigate and identify the molecular signalling pathways that may account for ureteral dilatation.

MATERIALS & METHODS

We harvested and performed analysis on the ureters of the 4 female Yorkshire-Landrace pigs— which had undergone unilateral ureteric stenting.

- Pig 1 and 2 were unilaterally stented on the right ureter for 5 days.
- Pig 3 and 4 were unilaterally stented on the left ureter for 3 days.

Measurements

On Day 1, 3 and 5, an intravenous pyelogram (IVP) (Fig 1) was performed to obtain ureteral measurements. After anaesthesia was given, an elastic body binder was applied to compress the ureters at the lower flanks to improve contrast collection in the renal pelvis. An iodine-based contrast was given as a bolus infusion through an intravenous cannula. The body binder was released and a series of IVPs were obtained after bolus infusion was given.

The ureter was measured at 3 consistent levels using the midline vertebral body as reference. These 3 measurements were averaged to obtain the mean ureteral diameter of each side on Day 1, 3 and 5. The difference in each ureteral diameter was analysed using mixed linear model.

RNA Sequencing Analysis

The pigs were then euthanized and ureters were harvested. RNA was extracted, analysed for quality control before they were subjected to RNA sequencing analysis.

- Raw data was statistically analysed using Partek Genomics Suite. Comparisons of differential gene expression between treatment (stenting), duration and batch were measured with ANOVA.
- A list of genes ($p<0.05$ and fold change of >2) was generated and was overlaid onto a global molecular network developed from information contained in the Ingenuity Pathway Analysis (IPA) knowledge base
- For network analysis, IPA computed a score (p-score=-log10(p-value)) based on the list of genes that were submitted and associate them with a list of biological functions stored in the knowledge base
- The network identified was presented as a graph indicating the molecular relationships between the differentially expressed genes/gene products. The functional analysis identified the biological functions and the canonical signaling pathways that were most significant to the input data set.
- The significance of the association between the input data set and the functions or pathways was determined based on two parameters:
  - (1) a ratio of the number of genes from the data set that map to the function/pathway divided by the total number of genes that map to the function/pathway and
  - (2) a P-value calculated using Fischer's exact test to determine the probability that the association between the genes in the dataset and the function/pathway is explained by chance alone.
- The molecular pathway of significance was then correlated to the stented / non-stented ureters macroscopically and radiologically.

RESULTS

Measurements

Table 1 gives the mean bilateral ureteral measurements across 3-5 days for each ureter of each pig. The mean change in ureter diameter is invariably increased in the stented side ($p = 0.01$). The non-stented side shows increase in mean ureteral diameters in Pig 2 and 3.

Table 1. Mean change of ureteral diameters between non-stented or stented

Pig		Mean ureteral diameter (mm)	
		non-stented	stented
1	Day 1	8.53	8.01
	Day 3	7.7	8.78
	Day 5	8.12	9.98
	Mean change	**-0.41**	**1.97**
2	Day 1	7.55	8.82
	Day 3	8.11	10.06
	Day 5	9.41	11.69
	Mean change	**1.86**	**2.87**
3	Day 1	4.91	6.35
	Day 3	7.08	8.33
	Mean change	**2.18**	**1.98**
4	Day 1	8.85	7.17
	Day 3	8.62	8.75
	Mean change	-0.24	1.58

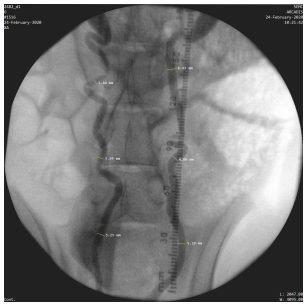

Fig 1. IVP of Pig 2 on Day 1

RNA Sequencing Analysis

To aid visualization of high dimensional data, a Principal Components Analysis (PCA) of differentially expressed genes were performed, where each point represents an aggregate observation on a sample and where the distance between any two points is related to the similarity between the two observations/samples.

The PCA diagram indicates a clear difference in gene expression between stented and non-stented sides.

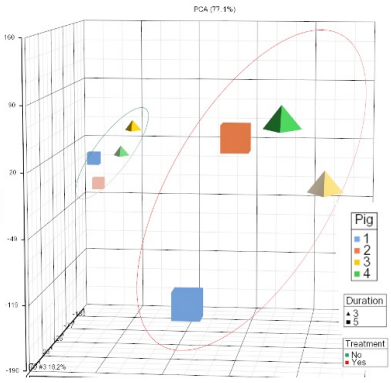

Fig 2. PCA of differential gene expression

Differential Gene Expression

Pathways with molecules associated with survival and proliferation appear be the top canonical pathways responsible for ureteral dilatation e.g. Kinetochore Metaphase Signalling Pathway, Cardiac Hypertrophy Signalling Pathway.

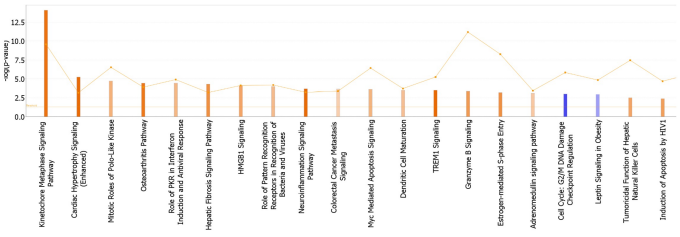

Fig 3. Top pathways with differential gene expression for Day 3 and 5

DISCUSSION

- Ureteral stenting leads to ureteral dilatation in all models.
- There is a clear difference in gene expression between the stented and non-stented sides.
- This appears to be a result of an increase in gene expression of molecules associated with cell survival and proliferation