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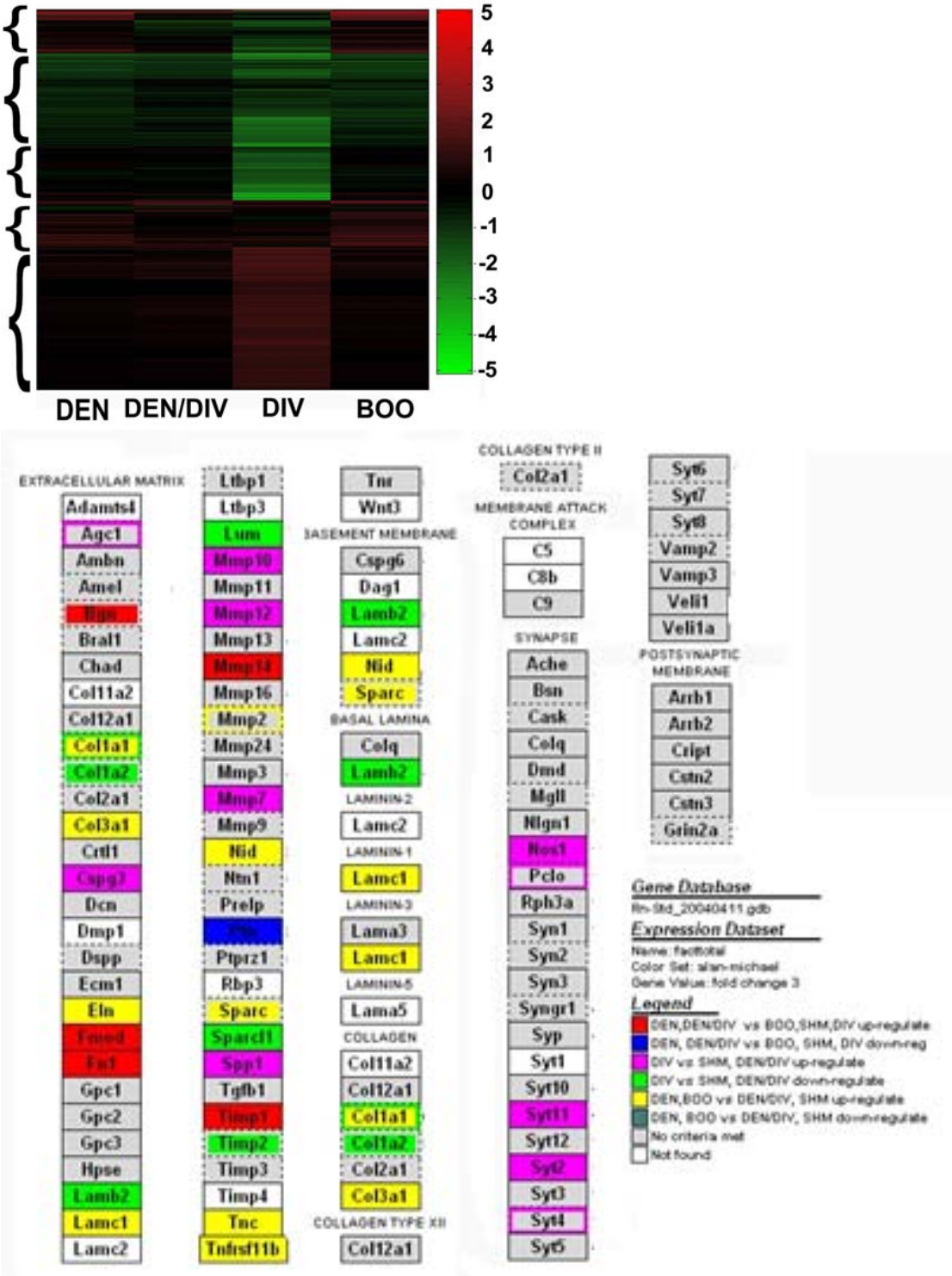
REGULATION OF BLADDER GENE EXPRESSION BY OUTLET OBSTRUCTION, DENERVATION AND URINARY DIVERSION REVEALED BY MICROARRAY ANALYSIS

Hypothesis / aims of study
Previous studies have shown that bladder hypertrophy but not denervation in the rat alters the muscarinic receptor mediating bladder contraction from M_3 toward M_2. We used Affymetrix 230A arrays to determine what additional genes may be regulated.

Study design, materials and methods
Whole urinary bladder RNA was pooled from 3 female Sprague Dawley rats per group 3 days after the following surgeries: sham operated controls, bilateral major pelvic ganglion denervation, bladder outlet obstruction, denervated with urinary diversion and urinary diversion alone. The RNA was analysed for gene expressino using the Affymetrex 230A GeneChip array system. Each of the 15,866 genes on the Affymetrix 230A GeneChip array are represented by 11 probe pairs. Each pair consists of a perfect sequence match and a mismatch to define non-specific hybridization. Affymetrix GCOS software was used to compare the experimental groups to sham operated controls. Genes regulated compared to sham in the hypertrophic tissues (denervated and obstructed) were compared to genes regulated compared to sham in the atrophic tissue (urinary diversion).

Results
Bladder hypertrophy developed in the denervated and obstructed animals. Atrophy developed in the diverted but not the denervated-diverted animals. No genes were increased in the atrophic tissue and decreased in both the hypertrophic tissues. The quantitation of the 5 muscarinic receptor subtypes genes and 2 houskeeping genes (GAPDH and L32) expression as detected by the Affymetrex GeneChip array system was confirmed using ribonuclease protection assays. There were 24 genes down regulated and 21 genes up regulated in both hypertrophic tissues as well as the atrophic tissue. Most of the 15 genes that were up regulated in the hypertrophic tissues and down regulated in the atrophic tissue are connective tissue and cytoskeletal genes (e.g., collagens, actin, tubulin, desmin and integrin). RMAexpress software (University of California, Berkeley) was used to process the Affymetrix array data. This software corrects the background and probe effect resulting in a data set size of 15,855 genes x 5 groups. We used the data from RMA software to calculate the mean difference for each gene. The genes were shuffled and the mean difference between these randomized gene pairs was calculated. From the randomized gene pairs, the thresholds for significant ($p<0.05$) increases and decreases is calculated for the non-shuffled data set.

Interpretation of results
This was a 2 X 2 factorial design with denervation and hypertrophy as the factors and included an additional atrophic group (urinary diversion). Data mining methodology has identified genes involved in various metabolic pathways that are differentially regulated by denervation, hypertrophy and the interaction between these 2 factors. There are 2,519 unique genes after the four data sets of genes were merged which are identified as up or down regulated genes by any of the factors. Hierarchical clustering of these genes shows that there are clearly 5 major clusters which may help to identify the co-regulated genes. These 2,519 genes were analyzed with Ingenuity and GenMapp software to identify pathways and functions which have genes that are significantly altered by the 2 factors (denervation and hypertrophy).



Concluding message

Microarray analysis of bladder gene expression provides a global overview of transcript regulation of nearly the entire rat genome following experimental pathologies.

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