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# Upregulation and function of GADD45γ in unilateral ureteral obstruction

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**We performed differential display analysis to determine transcriptional activity in the rat kidney, following unilateral ureteral obstruction and found a 12-fold increase in the expression of Growth Arrest and DNA Damage-45γ (GADD45γ), a stress-responsive molecule that interacts with cell-cycle proteins. GADD45γ was strongly expressed in as little as 6 h following ureteric obstruction in the renal tubules, and was also found in kidney tissue of patients with chronic glomerulonephritis. Adenovirus-mediated expression of GADD45γ in cultured renal tubular cells activated p38 along with a significant upregulation of C–C and C–X3–C chemokine ligands and fibrosis-related factors such as several matrix metalloproteinases, transforming growth factor-β1, decorin, and bone morphogenetic protein 2. Silencing of GADD45γ expression significantly blunted the upregulation of these inflammatory and fibrogenic mediators and monocyte infiltration in the ureteral obstructed rat kidney. Our study shows that GADD45γ is quickly upregulated in the kidney with an obstructed ureter, enhancing the production of factors regulating the pathogenesis of kidney disease.**

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Growth Arrest and DNA Damage-45 (GADD45) family of genes has three isoforms, GADD45α, GADD45β, and GADD45γ. GADD45α (=GADD45) was first identified after ultraviolet light-mediated DNA damage to Chinese hamster ovary cells,[1] and two other human isoforms were isolated afterwards and were designated as GADD45β (=MyD118) and GADD45γ (=CR6).[2] The GADD45 proteins are evolutionarily conserved and are about 55–58% identical at the amino-acid level.[2] These proteins have been located mainly in the cell nucleus and less abundantly in the cytoplasm.[2,3] They are induced in response to DNA damage and environmental stresses and interact with various proteins involved in cell-cycle regulation.[4] With regard to kidney diseases, data on the role of GADD45 proteins are scarce. Here, we provide evidence that GADD45γ is upregulated in unilateral ureteral obstruction (UUO) and it regulates various molecules implicated in the pathogenesis of kidney diseases.

## RESULTS

### Differential display analysis

We cloned and sequenced 37 genes that were differentially expressed in the kidney 1 day post-UUO and found that 18 genes were relevant to rat DNA (data not shown). We performed a gene-specific polymerase chain reaction (PCR) for those rat genes using multiple kidney samples, and results were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression levels. We found that GADD45γ is a molecule that is significantly upregulated in the cortex of the UUO kidneys.

### GADD45γ mRNA expression during the course of UUO

We serially examined the changes of GADD45γ expression during the course of UUO from 6 h through 5 days. The PCR analysis showed that GADD45γ was significantly increased as early as 6 h post-UUO (~12-fold), gradually declined thereafter, and remained elevated up to 3 days post-UUO compared with sham-operated controls (Figure 1). GADD45γ gene expression in the contralateral unobstructed kidneys was similar to that in the sham-operated kidneys at all time points of the experiment. We then performed *in situ* hybridization to localize GADD45γ mRNA in the kidney tissue 1 day post-UUO. We found that GADD45γ was

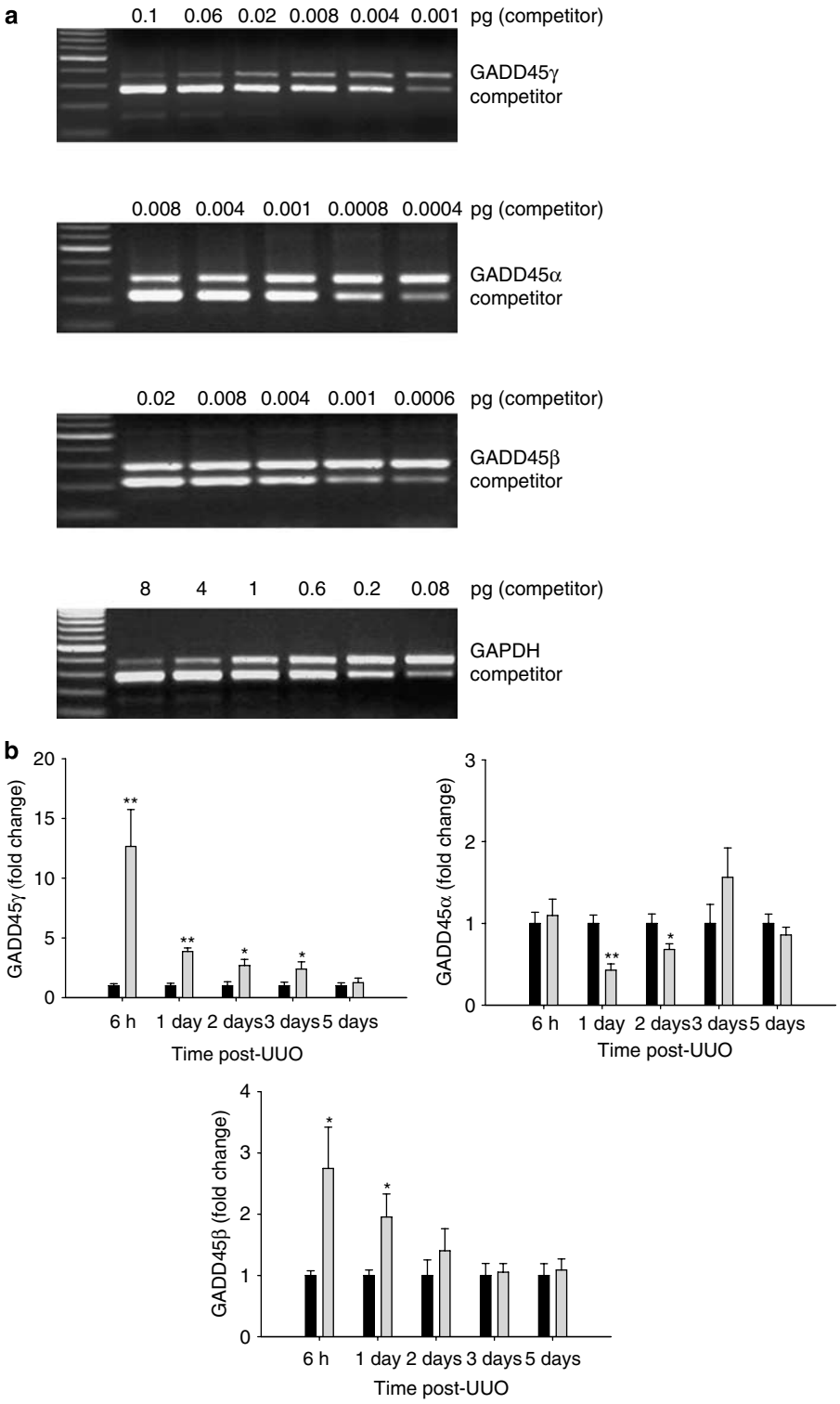


**Figure 1 | Quantification of mRNA expression of GADD45-family genes in kidneys after UUO by competitive PCR.** (**a**) Competitor constructs were designed to be flanked by sequences recognized by a pair of target gene-specific primers and to contain an intervening sequence that differed in size from the target DNA. (**b**) Rats with UUO (gray bars) were compared with sham-operated controls (black bars) for each corresponding time. Target gene product amounts were normalized to those of GAPDH, and the results were expressed as fold changes compared with the controls, $n = 11$–14 for each experimental group. Data are mean ± s.e.m.; $^{*}P < 0.05$, $^{**}P < 0.01$ compared with controls. UUO, unilateral ureteral obstruction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

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