ARCHIVES OF TOXICOLOGY: SUPPLEMENTAL MATERIAL

Time-matched analysis of DNA adduct formation and early gene expression as predictive tool for renal carcinogenesis in methylazoxymethanol acetate treated Eker rats

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SUPPLEMENTAL MATERIAL AND METHODS

Quantification of O^6-methyl-2'-deoxyguanosine and *N7*-methylguanine adducts

DNA isolation

To minimize formation of oxidative artifacts during isolation, 2,2,6,6-tetramethyl-piperidinoxyl (TEMPO, 20 mM final concentration, Sigma-Aldrich St. Louis, MO) was added to all solutions, and all procedures were performed on ice. Pooled frozen kidney cortex samples of three replicate animals were homogenized in PBS. After centrifugation at 1700 x g at 4 °C for 10 min, the nuclear pellets were incubated in cell lysis buffer (Qiagen, Valencia, CA, USA) overnight at 4 °C with proteinase K. Protein was precipitated with a protein precipitation solution (Qiagen, Valencia, CA, USA) and centrifugation at 2000 x g at 4 °C for 10 min. Nucleic acids were precipitated by mixing the supernatant with propanol for subsequent centrifugation at 2000 x g for 5 min at 4 °C. The DNA/RNA pellet was washed with 70 % ethanol and then centrifuged at 2000 x g for 3 min at 4 °C. After air drying it was resuspended in cell lysis solution and incubated with Ribonuclease A for 30 min at 37 °C. The enzyme was precipitated with protein precipitation solution. DNA was precipitated by propanol and washing with 70 % ethanol as described above. The washed and dried DNA was resuspended in sterilized double distilled water and stored at -80 °C.

O6-methyl-2'-deoxyguanosine adduct isolation and purification

DNA concentrations were determined by UV spectrometry, and concentrations were adjusted to 1 µg/µl. Fifty µl of 80 mM Tris-HCl, 20 mM $MgCl_2$ buffer (pH 7) and were added to 100 µg DNA solution, and the volume was adjusted with water to 350 µl. For accurate quantitation 200 fmol of [$^2H_3$]-O^6-methyl-2'-deoxyguanosine (a gift from Natalia Tretyakova, University of Minnesota, Minneapolis, MN) were added as internal standard. Enzymatic hydrolysis was started by addition of 50 U DNAse I and incubation at 37 °C for 10 min

followed by addition of 3.4 mU phosphodiesterase I and 2.5 U alkaline phosphatase with continued incubation at 37 °C for 60 min. The final volume of sample containing all reagents and internal standard was 400 µl. After hydrolysis, enzymes were removed by centrifugal filtration using pre-washed Centricon YM-10 microcentrifuge filters (Millipore, Bedford, MA).

O^6-methyl-2'-deoxyguanosine (O^6-me-dG) adducts were purified by reverse phase HPLC using an Ultrasphere ODS C18 4.6 × 250 mm 5 µm column (Beckman, Fullerton, CA). Chromatographic separation of the analyte was performed in 12 min under isocratic conditions. The mobile phase containing 25 % methanol and 75 % 10 mM ammonium formate (pH 4.3) was pumped at 1 ml/min by a 1200 HPLC system (Agilent, Santa Clara, CA) with UV detector set to 264 nm. The column oven, autosampler tray and fraction collector chamber temperatures were maintained at 30 °C, 4 °C and 4 °C, respectively. A 375 µL aliquot of the filtered DNA hydrolysis solution was injected. The retention time of O^6-me-dG was determined by using 2' deoxyguanosine (dG) as a retention time marker and multiplying its retention time by 2.4. Fractions were collected from 1.5 min before until 1.5 min after the predicted retention time. Solvents were removed by centrifugal evaporation in a SpeedVac concentrator, and the sample was re-dissolved in 20 µl HPLC grade water for subsequent analysis by LC-MS/MS.

The amount of O^6-me-dG adducts in each sample was normalized to the respective 2'-deoxyguanosine (dG) amount. The dG amount was determined by comparison with calibration standards.

N7-methylguanine adduct isolation and purification

The *N7*-methylguanine (*N7*MG) adduct was removed from the DNA backbone via neutral thermal hydrolysis (NTH). A 100 µg aliquot of DNA was spiked with 200 fmol [$^2H_3$]-*N7*MG internal standard (a kind gift from Lynn Pottenger (Dow Chemical Company, Midland, MI)

and diluted with water for a total volume of 400uL. A 40 µL aliquot was removed for separate dG quantitation. The remaining volume was subjected to NTH at 100 °C for 30 min then filtered through Alltech 0.2 µm regenerated cellulose micro-spin tubes for 10 min at 10.000 rpm. The hydrosylates containing *N7*MG were transferred to glass autosampler vials and dried by centrifugal evaporation in a SpeedVac concentrator. The samples were redissolved in 20 µL HPLC grade water for quantitation of *N7*MG by LC-MS/MS.

Quantitation O6-methyl-2'-deoxyguanosine- and N7-methylguanine by UPLC-MS/MS

The quantitative analysis of both O^6-me-dG and *N7*MG was performed with an UPLC (Waters, Milford, MA) coupled to a TSQ-Quantum Ultra triple-quadrupole mass analyzer (ThermoFinnigan, San Jose, CA) using heat assisted electrospray ionization (HESI) in positive mode.

For O^6-me-dG separation was performed on a 2.1 × 100 mm HSS T3 C18, 1.8 µm column (Waters, Milford MA) with gradient elution at a flow rate of 200 µL per min using 0.1 % acetic acid in water as mobile phase A and methanol as mobile phase B. The gradient started at 1 % B and increased linearly to 5 % B in 1 min. The amount of B was increased linearly to 40 % from 1 min to 10 min, was held at 40% for 2 min, decreased to 1 % in 0.5 min, and was held at 1 % for 2.5 min for column re-equilibration. The retention time of O^6-me-dG was 8.2 min, and the total run time was 15 min. The analyte and internal standard were detected in selected reaction monitoring (SRM) mode, monitoring the transitions of m/z 282.1 to 166.1 and m/z 285.1 to 169.1 for O^6-me-dG and [$^2H_3$]-O^6-me-dG, respectively. The mass spectrometer parameters were as follows: spray voltage of 3000 V, vaporizer (HESI) temperature of 200 ˚C, sheath gas flow rate 35, auxillary gas flow rate 30, capillary temperature of 285 ˚C and collision energy of 12 eV.

*N7*MG was separated on a 2.1 × 100 mm HSS T3 C18, 1.8 µm column (Waters, Milford MA) with gradient elution at a flow rate of 200 µL per min using 0.1 % acetic acid in water as

mobile phase A and methanol as mobile phase B. The gradient started at 1 % B and was increased linearly to 10 % B in 1 min. The amount of B was increased linearly to 50 % from 1 min to 4 min, was held at 50 % for 0.5 min, decreased to 1% in 0.5 min, and was held at 1% for 2 min for column re-equilibration. The retention time of *N7*MG was 3.2 min, and the total run time was 4.5 min. The analyte and internal standard were detected in selected reaction monitoring (SRM) mode, monitoring the transitions of m/z 166.1 to 149.1 and m/z 169.1 to 152.1 for *N7*-methylgaunine and [$^{2}H_{3}$]-*N7*MG, respectively. The mass spectrometer parameters were as follows: spray voltage of 3000 V, vaporizer (HESI) temperature of 200 ˚C, sheath gas flow rate 35, auxillary gas flow rate 30, capillary temperature of 285 ˚C and collision energy of 19 eV.

SUPPLEMENTAL RESULTS

Supplemental table 1: KEGG pathway enrichment analyses

Pathway ID	Description	Adjusted p-value	Number of genes	Genes
rno05212	Pancreatic cancer	0.00016	6	*Tgfbr2/ Smad3/ Tgfbr1/ Smad4/ Egfr/ Mapk9*
rno05210	Colorectal cancer	0.00162	5	*Tgfbr2/ Smad3 /Tgfbr1/ Smad4/ Mapk9*
rno05200	Pathways in cancer	0.00164	10	*Tgfbr2/ Gnai3/ Smad3/ Tgfbr1/ Hsp90ab1/ Smad4/ Nfkb2/ Prkacb/ Egfr/ Mapk9*
rno04520	Adherens junction	0.00164	5	*Tgfbr2/ Smad3/ Tgfbr1/ Smad4/ Egfr*
rno04010	MAPK signaling pathway	0.00173	8	*Tgfbr2/ Tgfbr1/ Pak2/ Nfkb2/ Prkacb/ Egfr/ Mapk9/ Cacng4*
rno04068	FoxO signaling pathway	0.00197	6	*Tgfbr2/ Smad3/ Tgfbr1/ Smad4/ Egfr/ Mapk9*
rno04933	AGE-RAGE signaling pathway in diabetic complications	0.00477	5	*Tgfbr2/ Smad3/ Tgfbr1/ Smad4/ Mapk9*
rno05142	Chagas disease	0.00477	5	*Tgfbr2/ Gnai3/ Smad3/ Tgfbr1/ Mapk9*
rno04971	Gastric acid secretion	0.01080	4	*Gnai3/ Kcnj15/ Kcnj1/ Prkacb*
rno05220	Chronic myeloid leukemia	0.01080	4	*Tgfbr2/ Smad3/ Tgfbr1/ Smad4*
rno04350	TGF-beta signaling pathway	0.01564	4	*Tgfbr2/ Smad3/ Tgfbr1/ Smad4*
rno04914	Progesterone-mediated oocyte maturation	0.01699	4	*Gnai3/ Hsp90ab1/ Prkacb/ Mapk9*
rno04915	Estrogen signaling pathway	0.01992	4	*Gnai3/ Hsp90ab1/ Prkacb/ Egfr*
rno04724	Glutamatergic synapse	0.03580	4	*Gnai3/ Shank3/ Prkacb/ Slc38a2*
rno05166	HTLV-I infection	0.04488	6	*Tgfbr2/ Smad3/ Tgfbr1/ Smad4/ Nfkb2/ Prkacb*
rno04510	Focal adhesion	0.04488	5	*Cav2/ Pak2 /Itga1/ Egfr/ Mapk9*
rno05230	Central carbon metabolism in cancer	0.04590	3	*Pdha1 /Egfr/ Pfkl*
rno04380	Osteoclast differentiation	0.04808	4	*Tgfbr2/ Tgfbr1/ Nfkb2/ Mapk9*

KEGG pathway enrichment analyses of 76 genes with significant expression changes at least at one time-point investigated following 1, 3, 7 or 14 days of MAMAc treatment, revealed a significant effect (adjusted p-value < 0.05) of MAMAc on 18 different pathways, which included 42 out of 76 genes, listed in the right column.

Supplemental table 2: Non-neoplastic renal pathology in short-term treated Eker rats

	Control (males)				MAMAc (males)			
Renal cortex								
	day 1	**day 3**	**day 7**	**day 14**	**day 1**	**day 3**	**day 7**	**day 14**
Pigment Deposits	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)
Necrosis	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.2 (1/3)	0.0±0.0 (0/3)	0.0±0.2 (1/3)	0.0±0.0 (0/3)	0.0±0.2 (1/3)	0.0±0.0 (0/3)
Apoptosis	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)
Karyomegally	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)
Vacuolization	0.0±0.2 (1/3)	0.0±0.2 (1/3)	0.0±0.2 (1/3)	0.0±0.0 (0/3)	0.0±0.2 (1/3)	0.0±0.0 (0/3)	0.0±0.2 (1/3)	0.0±0.0 (0/3)
Cell shedding	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)
Proteinaceous casts	0.0±0.2 (1/3)	0.5±0.2 (2/3)	0.5±0.2 (2/3)	0.0±0.0 (0/3)	0.5±0.2 (2/3)	0.0±0.0 (0/3)	0.5±0.2 (2/3)	0.0±0.0 (0/3)
Tubular dilatation	0.5±0.2 (2/3)	0.0±0.2 (1/3)	0.5±0.0 (3/3)	0.0±0.2 (1/3)	0.5±0.0 (3/3)	0.0±0.2 (1/3)	0.5±0.0 (3/3)	0.0±0.2 (1/3)
Calcium casts	0.5±0.2 (2/3)	0.0±0.2 (1/3)	0.0±0.2 (1/3)	0.5±0.2 (2/3)	0.0±0.2 (1/3)	0.5±0.2 (2/3)	0.0±0.2 (1/3)	0.5±0.2 (2/3)
Tubular regeneration	0.0±0.0 (0/3)	0.0±0.2 (1/3)	0.0±0.2 (1/3)	0.0±0.2 (1/3)	0.0±0.2 (1/3)	0.0±0.2 (1/3)	0.0±0.2 (1/3)	0.0±0.2 (1/3)
CPN	0.5±0.2 (2/3)	0.0±0.2 (1/3)	0.5±0.2 (2/3)	0.0±0.2 (0/3)	0.5±0.2 (2/3)	0.0±0.2 (0/3)	0.5±0.2 (2/3)	0.0±0.2 (0/3)
Inflammation	0.0±0.2 (1/3)	0.0±0.0 (0/3)	0.0±0.2 (1/3)	0.0±0.2 (0/3)	0.0±0.2 (1/3)	0.0±0.2 (0/3)	0.0±0.2 (1/3)	0.0±0.2 (0/3)
	Control (females)				**MAMAc (females)**			
Renal cortex								
	day 1	**day 3**	**day 7**	**day 14**	**day 1**	**day 3**	**day 7**	**day 14**
Pigment Deposits	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)
Necrosis	0.0±0.2 (1/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)
Apoptosis	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)
Karyomegally	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.2 (1/3)	0.0±0.0 (0/3)
Vacuolization	0.0±0.2 (1/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)
Cell shedding	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.5±0.2 (3/3)	0.5±0.2 (3/3)	0.0±0.0 (0/3)	0.5±0.3 (2/3)
Proteinaceous casts	0.5±0.2 (2/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.2 (1/3)	0.5±0.2 (2/3)	0.5±0.2 (2/3)	0.5±0.2 (2/3)	0.5±0.0 (3/3)
Tubular dilatation	0.5±0.0 (3/3)	0.0±0.2 (1/3)	0.5±0.0 (3/3)	0.5±0.3 (2/3)	0.0±0.2 (1/3)	0.0±0.2 (1/3)	0.5±0.2 (2/3)	0.5±0.2 (2/3)
Calcium casts	0.0±0.2 (1/3)	0.5±0.2 (2/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)
Tubular regeneration	0.0±0.2 (1/3)	0.0±0.2 (1/3)	0.0±0.0 (0/3)	0.0±0.2 (1/3)	0.0±0.2 (1/3)	0.0±0.2 (1/3)	0.0±0.2 (1/3)	0.0±0.2 (1/3)
CPN	0.5±0.2 (2/3)	0.0±0.2 (0/3)	0.5±0.0 (3/3)	0.5±0.2 (2/3)	0.5±0.2 (2/3)	0.5±0.2 (2/3)	0.5±0.2 (2/3)	0.5±0.0 (3/3)
Inflammation	0.0±0.2 (1/3)	0.0±0.2 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)	0.0±0.0 (0/3)

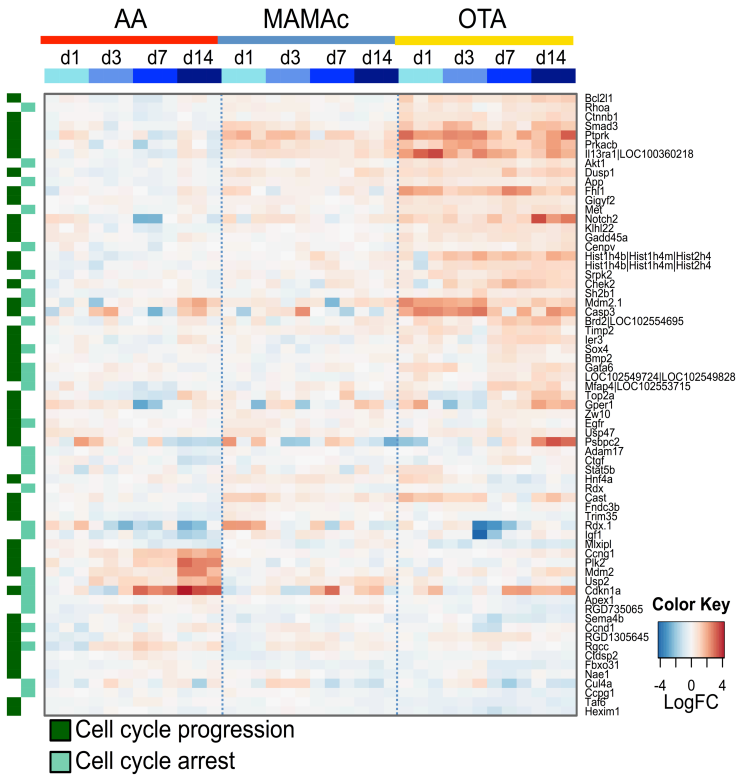
Non-neoplastic pathology of the renal cortex of male and female Eker rats treated with vehicle (controls) or MAMAc for 1, 3 7 and 14 days respectively (n = 3 per group). Histopathological changes were ranked from none (0) to severe (4) including intermediate classes. Values are presented as median ± median absolute deviation (MAD). Incidences are given in parenthesis. Non-parametric Mann-Whitney test revealed no significant changes in the kidney cortex, or the medulla / papilla region of the kidney (data not shown). CPN: Chronic progressive nephropathy.

Supplemental Table 3: Non-neoplastic pathology in vehicle or MAMAc treated Eker rats

	3 months treatment				6 months treatment			
	males		females		males		females	
	Control	**MAMAc**	**Control**	**MAMAc**	**Control**	**MAMAc**	**Control**	**MAMAc**
	N = 10	N = 10	N = 10	N = 10	N = 10	N = 10	N = 9	N = 10
Renal Cortex								
Glomerular changes	2.0 ± 1.2	1.0 ± 0.7	1.0 ± 0.4	1.0 ± 1.0	1.0 ± 0.6	1.0 ± 0.4	1.0 ± 0.6	1.0 ± 0.6
Pigment	0.0 ± 0.3	0.0 ± 0.3	0.0 ± 0.3	0.0 ± 0.3	0.0 ± 0.2	0.0 ± 0.4	0.0 ± 0.7	0.0 ± 0.2
Necrosis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.6	1.0 ± 1.0
Apoptosis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Vacuolization	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.5	0.0 ± 1.0	3.0 ± 0.4	3.0 ± 1.2
Cell shedding	2.0 ± 1.0	3.0 ± 0.6	2.0 ± 0.5	3.0 ± 1.3	2.0 ± 1.2	1.5 ± 0.9	2.0 ± 0.7	2.0 ± 0.8
Tubular dilatation	1.0 ± 0.9	1.0 ± 1.3	2.0 ± 1.0	1.0 ± 1.0	1.0 ± 1.6	3.0 ± 1.0	1.0 ± 1.0	1.5 ± 1.2
Protein casts	1.0 ± 1.0	0.5 ± 0.9	0.0 ± 0.7	0.5 ± 0.8	3.0 ± 0.6	2.0 ± 1.0	0.0 ± 1.1	1.5 ± 1.1
Calcium casts	0.0 ± 0.7	0.0 ± 0.0	0.0 ± 0.3	0.0 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Karyomegaly	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.5	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.2
Regeneration	1.0 ± 0.4	0.0 ± 0.7	0.5 ± 0.7	0.0 ± 0.6	0.0 ± 1.0	1.0 ± 1.1	1.0 ± 0.9	0.0 ± 1.2
CPN	1.5 ± 0.8	1.5 ± 1.2	1.0 ± 0.6	1.5 ± 0.9	3.0 ± 1.1	3.0 ± 0.7	2.0 ± 0.5	2.0 ± 1.3
Inflammation	1.5 ± 0.7	1.5 ± 1.1	1.0 ± 0.8	1.5 ± 1.0	3.0 ± 1.1	3.0 ± 0.5	2.0 ± 0.5	2.0 ± 1.3
Hypertrophy	0.0 ± 0.5	1.0 ± 0.6	1.0 ± 0.4	1.0 ± 0.5	0.5 ± 0.5	0.0 ± 0.6	1.0 ± 0.6	1.0 ± 0.4
Medulla / Papilla								
Pigment	0.0 ± 0.2	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2
Necrosis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.8
Apoptosis	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Vacuolization	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 1.1	0.0 ± 1.4
Cell shedding	0.0 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Tubular dilatation	0.0 ± 0.5	0.0 ± 0.0	0.0 ± 0.4	0.0 ± 0.0	0.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 1.2
Protein casts	1.0 ± 0.9	0.0 ± 1.0	0.0 ± 0.7	0.0 ± 0.8	2.5 ± 0.8	3.0 ± 0.8	2.0 ± 1.0	2.0 ± 0.9
Calcium casts	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0
Karyomegaly	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Regeneration	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0
CPN	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.6	0.0 ± 0.7	0.0 ± 0.5	0.0 ± 0.4
Inflammation	0.0 ± 0.0	0.0 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.9	0.0 ± 0.7	0.0 ± 0.4	0.0 ± 0.4
Hypertrophy	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

Non-neoplastic pathology in male and female Eker rats after 3 or 6 months of treatment with MAMAc or vehicle. Data represent the median ± Median absolute deviation (MAD). Non-parametric Mann-Whitney test revealed no statistical significance between MAMAc treated and control groups. CPN: Chronic progressive nephropathy.

Supplemental figure 1:



Supplemental Fig 1: Heatmap of genes involved in cell cycle regulation in kidney cortices of Eker rats treated for 1, 3, 7 and 14 days with aristolochic acid (AA), methylazoxymethanol acetate (MAMAc) or Ochratoxin A (OTA). N = 3 rats per group. Gene expression data from AA and OTA treated rats have been published previously (Stemmer et al. 2009), but data were re-analyzed to allow a direct comparison with the MAMAc treated groups, which shared the same control groups. Differentially expressed genes due to treatment or time were determined by the moderated t-test implemented in the R / Bioconductor package limma. P-values were corrected for multiple testing using the Benjamini-Hochberg method to control for the false discovery rate (FDR). Differentially expressed genes with an adjusted P-value < 0.05 were considered statistically significant.

Supplemental References

Stemmer K, Ellinger-Ziegelbauer H, Ahr HJ, Dietrich DR (2009) Molecular characterization of preneoplastic lesions provides insight on the development of renal tumors. Am J Pathol 175(4):1686-98 doi:10.2353/ajpath.2009.081071