Research Article

Carcinogen-Specific Gene Expression Profiles in Short-term Treated Eker and Wild-type Rats Indicative of Pathways Involved in Renal Tumorigenesis

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Abstract

Eker rats heterozygous for a dominant germline mutation in the *tuberous sclerosis 2* (*Tsc2*) tumor suppressor gene were used as a model to study renal carcinogenesis. Eker and corresponding wild-type rats were exposed to genotoxic aristolochic acid (AA) or non-genotoxic ochratoxin A (OTA) to elucidate early carcinogen-specific gene expression changes and to test whether Eker rats are more sensitive to carcinogen-induced changes in gene expression. Male Eker and wild-type rats were gavaged daily with AA (10 mg/kg body weight) or OTA (210 μg/kg body weight). After 1, 3, 7, and 14 days of exposure, renal histopathology, tubular cell proliferation, and Affymetrix gene expression profiles from renal cortex/outer medulla were analyzed. AA-treated Eker and wild-type rats were qualitatively comparable in all variables assessed, suggesting a Tsc2-independent mechanism of action. OTA treatment resulted in slightly increased cortical pathology and significantly elevated cell proliferation in both strains, although Eker rats were more sensitive. Deregulated genes involved in the phosphatidylinositol 3-kinase-AKT-Tsc2-mammalian target of rapamycin signaling, among other important genes prominent in tumorigenesis, in conjunction with the enhanced cell proliferation and presence of preneoplastic lesions suggested involvement of *Tsc2* in OTA-mediated toxicity and carcinogenicity, especially as deregulation of genes involved in this pathway was more prominent in the *Tsc2* mutant Eker rat. [Cancer Res 2007;67(9):4052–68]

Introduction

Eker rats, heterozygous for a loss-of-function mutation in the *tuberous sclerosis 2* (*Tsc2*) tumor suppressor gene, seem ideal models to study the etiology of renal carcinogenesis (1, 2). Heredity of the *Tsc2* mutation follows Mendelian genetics (1, 3), and heterozygous progeny are predisposed to spontaneous development of multiple bilateral renal neoplasms originating from the proximal tubular epithelium with complete penetrance by 1 year of age (4). Approximately 60% of the spontaneous renal tumors in Eker rats also exhibit a functional inactivation of the second *Tsc2* allele, suggesting, in accordance with Knudson's two-hit hypothesis, that a second somatic mutation might be the rate-limiting step for the development of renal cell carcinomas in Eker rats (5). Eker rats have been employed to elucidate the mechanism of renal carcinogens, primarily using histopathologic and statistical analyses of the number, multiplicity, and progression of renal lesions (6, 7). Accordingly, treatment of Eker rats with dimethylnitrosamine resulted in a 70-fold increase in the induction of renal adenomas and carcinomas, when compared with wild-type rats (6). No increased lesion incidence, albeit an advanced lesion progression, was observed in Eker rats subchronically treated with the tumor promoter sodium barbital (7). Although the latter data highlight that Eker rats are sensitive to genotoxic and non-genotoxic compounds, the involvement of Tsc2 protein (tuberin) in renal carcinogenesis remains to be established. Several studies suggest that functional Tsc2 promotes the GTP hydrolysis of the Ras homologue Rheb, thereby acting as a negative regulator of the phosphatidylinositol 3-kinase (PI3K)-Akt-Tsc1/2-Rheb-mammalian target of rapamycin (mTOR) pathway. Consequently, Tsc2 is suspected to play a central role in mediating growth factor, nutrient, and energy sensing to regulate cell growth, proliferation, migration, and differentiation (8).

The objective of this study was to elucidate whether short-term exposure of Eker and wild-type rats to a non-genotoxic and a genotoxic renal carcinogen would result in compound-specific changes in renal nonneoplastic and preneoplastic pathology and cell proliferation rates. Subsequently, the hypothesis was investigated, whether compound-specific changes in histopathology and cell proliferation can be associated with respective changes in gene expression, and whether Eker and wild-type rats respond differently. This should allow identification of deregulated genes involved in known and novel pathways possibly mediating carcinogen-induced renal tumorigenesis. Accordingly, Eker and wild-type rats were treated with daily doses of the genotoxic and the non-genotoxic renal carcinogen aristolochic acid (AA) and ochratoxin A (OTA), respectively, for which renal tumor induction in long-term *in vivo* studies was previously shown (refs. 9, 10; see Supplementary Fig. S1 for compound structures).

Indeed, intragastric administration of 10 mg AA/kg body weight/day [representing a mixture of structurally related nitrophenanthrene carboxylic acids (mostly AAI and AAII)] to rats over 3 months was shown to induce tumors in the forestomach, kidney, and the urinary bladder (9). DNA reactivity of AA was confirmed in that the most frequent and persistent dAdenin-AAI adduct could lead to mutation and activation of the H-*ras* oncogene in the forestomach but not in kidneys of rats (11, 12) or to p53 mutations in urothelial tumors of humans (13). Despite the lack of H-*ras* mutations, higher levels of AA adducts were found in renal tissues than in the forestomach of orally treated Wistar rats (5 mg AA/kg body weight/day) after only 1 week of exposure (14), suggesting an H-*ras*–independent pathway of renal tumor induction. The

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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genotoxic properties of AA are explained by the metabolic activation of AA by several phase I enzymes to a DNA-reactive aristolactam-nitriumion (15–17). Similarly, the mycotoxin OTA increased the incidence of renal adenoma and carcinoma in rats when exposed for up to 2 years to dietary OTA (18) or 210 μg OTA/kg body weight/day via gavage (10). However, as OTA has not been convincingly shown to covalently interact with DNA, a non-genotoxic mechanism of action is assumed (19, 20).

The comparison of cell proliferation, pathology, and expression profiles of AA- and OTA-treated Eker and wild-type rats should allow for a more in-depth understanding of the involvement of the Tsc2-mTOR pathway as well as of other early gene expression changes in the etiology of carcinogen-induced renal tumors.

Materials and Methods

Compounds. OTA (>98% purity, benzene-free) was kindly provided by Dr. M.E. Stack (U.S. Food and Drug Administration, Washington, DC). AA sodium salt mixture (41% AAI and 56% AAII) was purchased from Sigma-Aldrich.

Animals. Six- to 10-week-old genotyped heterozygous *Tsc2* mutant Eker rats ($Tsc2^{+/-}$, Long Evans) were purchased from the M.D. Anderson Cancer Center and maintained at the University of Konstanz animal research facility under standard conditions with food and water *ad libitum*. Male rats were randomly assigned to dose groups [three animals per compound (or vehicle) and time point] and allowed to acclimatize to laboratory conditions for 4 weeks. Two weeks before exposure, rats were handled daily to reduce non–compound-related stress during exposure.

Heterozygous Eker rats were bred, and wild-type ($Tsc2^{+/+}$) genotypes of the progeny were determined via PCR (21). Two weeks before exposure, 8- to 9-week-old genotyped male wild-type rats were randomly allocated to dose groups and accustomed to daily handling (see above).

Animal treatment and sample collection. Eker and wild-type rats were gavaged daily with OTA (210 μg/ kg body weight) or AA (10 mg/ kg body weight) dissolved in 0.1 mol/L sodium bicarbonate. Time-matched vehicle controls were gavaged with 0.1 mol/L sodium bicarbonate. Following 1, 3, 7, and 14 days of treatment, Narcoren (pentobarbital)–anesthetized rats were sacrificed by exsanguination subsequent to retrograde perfusion with PBS. Left kidneys were collected, cross-sectioned into 5-mm slices, and stored in RNAlater (Qiagen) or in PBS-buffered histology fixative buffer containing 2% paraformaldehyde and 1% glutaraldehyde for subsequent paraffin embedding and sectioning.

Histopathology. For histopathologic examinations, H&E-stained sections were randomized, and pathologic analysis was carried out by light microscopy at 40- to 400-fold magnification. Nonneoplastic changes were classified as none (0), mild (1), moderate (2), strong (3), and severe (4), including intermediate classes (e.g., 0.5, 1.5, etc.), whereas total numbers of preneoplastic and neoplastic lesions were counted.

Immunohistochemistry. Cell proliferation was evaluated by immunohistochemical staining for proliferating cell nuclear antigen (PCNA) using monoclonal primary anti-PCNA antibody (PC-10; DAKO) in paraffin-embedded kidney sections.

Sections were deparaffinized, rehydrated in a decreasing alcohol series, and washed with PBS. For antigen retrieval, slides were placed in 0.1 mol/L sodium citrate buffer (pH 6), microwaved to boiling point thrice, and cooled to room temperature for 20 min. Sections were denatured with 4 N HCl (20 min at 37°C) and washed with PBS (2× 5 min), and nonspecific protein binding was blocked by preincubation with casein solution (Power Block, BioGenex) for 20 min. Sections were incubated with PC-10 primary antibody (diluted 1:50 in Power Block) at 4°C for 16 h. Antigen-antiserum complexes were visualized using the super sensitive alkaline phosphatase–labeled, biotin streptavidin–amplified detection system and Fast Red as chromogen according to the manufacturer's instructions (BioGenex).

Cell proliferation was quantified on PCNA-stained sections and randomized across all treatment and control groups. Twenty microscopic fields (×10 ocular, ×40 objective) were randomly chosen in the outer cortex and inner cortex/outer medulla. All tubule cell nuclei were counted, concurrently differentiating between negative and positive PCNA staining. Nuclear labeling indices (LI %) for PCNA (PCNA-positive nuclei/total number of nuclei counted) were determined based on a minimum of at least 2,000 nuclei evaluated.

RNA isolation and expression profiling. RNA isolation from RNAlater-fixed kidneys was done as described previously (22). Starting with 5 μg of total RNA with a 28S/18S rRNA peak ratio >1.7, biotin-labeled cRNA was prepared and subsequently hybridized on Affymetrix Rat Genome RAE230A arrays according to the manufacturer's instructions (Affymetrix; GeneChip Expression Analysis 701194 Rev.1). This specific array contains 15,866 probe sets, corresponding to ~5,399 annotated rat genes and 10,467 expressed sequence tags.

Microarray data processing and statistical analysis. Microarray quality control was done as described previously (22), and gene expression data were submitted to the GEO repository (accession no. GSE5923).[3] Expressionist Analyst software (Genedata AG) was used for statistical analysis. Significantly deregulated genes per compound were selected based on the factors treatment and time as both single and interaction effects in a two-way ANOVA with P_{cutoff} of 0.005, combined with a 1.7-fold deregulation threshold for at least one time point. Significantly deregulated genes were divided into gene groups with distinct expression profiles over the time course using self-organizing map (SOM) analysis. SOM analysis also allowed deselection of genes showing inconsistent expression between the controls at different time points. Using the adjusted data sets, gene expression ratios of individual genes were calculated by dividing the respective expression values of single treated replicate samples by the mean expression value of all corresponding time-matched control samples. Heat maps were used to graphically display the relative expression data, after one-dimensional clustering of the genes (for the validation of microarray data, see Supplementary Information).

Functional analysis of microarray data. For functional analysis, each significantly deregulated gene was characterized according to the biochemical role of its encoded protein, whenever sufficient information from databases [e.g., NetAffx from Affymetrix (update from August 2006), Swissprot, Proteome, and Pubmed] was available. The consequence of the direction of deregulation was interpreted specifically with regard to possible downstream pathophysiologic effects. This allowed distribution of the deregulated genes into toxicologic categories (Supplementary Table S1) and facilitated the comparison of specific pathophysiologic pathways involved in the response of Eker and wild-type rats to AA and OTA treatment. In addition, the pathophysiologic pathways were compared with major pathways suspected to be involved in AA- and OTA-induced carcinogenesis (Tables 1 and 2) and histopathologic changes observed.

Statistical analysis. Statistical analysis of histopathologic and cell proliferation changes were carried out using GraphPad Prism 4 Software. Statistically significant differences in nuclear labeling indices (LI %) or total number of lesions in treated and control animals were analyzed by an unpaired (two tailed) *t* test. A statistically significant effect of the treatment time response was tested with a two-way ANOVA.

Results

Cell Proliferation Data

AA treatment resulted in no overt change in cell proliferation rate (Fig. 1*A* and *B*) in either strain, despite that occasional AA groups seemed to have a significantly lower proliferation then the corresponding controls. Conversely, OTA treatment increased the proliferation rate on day 14 (Fig. 1*C* and *D*; Fig. 2*A* and *B*) 3.8- and 3.4-fold above control in Eker and wild-type rats, respectively. Moreover, Eker rats seemed to be more sensitive to OTA as an increased cell proliferation rate was already observed at day 7 of treatment.

[3] http://www.ncbi.nlm.nih.gov/projects/geo

Table 1. Categories of genes differentially deregulated by AA in Eker and wild-type rats

Toxicological category	Accession no.	Gene title	Biochemical category	Fold deregulation (mean of three replicate animals per time point)							
				Ek d1	Ek d3	Ek d7	Ek d14	wt d1	wt d3	wt d7	wt d14
Metabolism and Biotransformation	AI233740	*AKR1B8* (aldo-keto reductase 1B8)	Biotransformation, phase I	**2.2**	**3.4**	**2.5**	**23.3**	**1.8**	**2.0**	**2.7**	**2.7**
	NM_012844	*EPHX1* (epoxide hydrolase 1; microsomal)	Biotransformation, phase I	**1.7**	**1.7**	**1.5**	**1.9**	**1.2**	**1.3**	**1.4**	**1.8**
	AW142784	*CYP4A10* (cytochrome *P*450 4a10)	Biotransformation, phase I	**2.0**	**1.6**	**1.6**	**2.1**	**1.6**	**1.8**	**1.9**	**1.8**
	J02679	*NQO1* (NADPH-quinone oxidoreductase)	Biotransformation, phase I	**2.4**	**1.9**	**1.6**	**3.1**	**2.0**	**2.1**	**1.5**	**2.0**
	U27518	*UGT2B8* (UDP-glucuronosyltransferase 2B8, microsomal)	Biotransformation, phase II	**6.1**	**4.5**	**3.3**	**4.7**	**3.1**	**2.5**	**1.7**	**1.7**
	NM_031980	*UGT2B12* (UDP-glucuronosyltransferase 2B12, microsomal)	Biotransformation, phase II	**2.0**	**2.1**	**1.9**	**2.2**	**2.2**	**2.0**	**2.0**	**1.9**
	M31109	*UGT2B3* (UDP-glucuronosyltransferase 2B3, microsomal)	Biotransformation, phase II	**4.1**	**4.6**	**4.5**	**6.7**	**2.7**	**2.0**	**4.7**	**3.6**
	M28241	*GSTM1* (glutathione *S*-transferase M1)	Biotransformation, phase II	**4.2**	**5.5**	**5.4**	**15.8**	**3.9**	**3.1**	**3.6**	**7.1**
	NM_031154	*GSTM3* (glutathione *S*-transferase M3)	Biotransformation, phase II	**1.1**	**2.0**	**1.6**	**2.0**	**1.3**	**1.3**	**1.4**	**2.1**
	NM_053293	*GSTT1* (glutathione *S*-transferase T1)	Biotransformation, phase II	**1.3**	**1.6**	**2.0**	**2.0**	**1.3**	**1.3**	**1.6**	**1.8**
	AA945082	*GSTA2* (glutathione *S*-transferase A2)	Biotransformation, phase II	**4.1**	**4.5**	**2.9**	**3.8**	**3.9**	**4.3**	**4.2**	**5.0**
	X02904	*GSTP1* (glutathione *S*-transferase P1)	Biotransformation, phase II	**4.8**	**5.7**	**3.9**	**4.8**	**4.9**	**4.9**	**5.3**	**6.0**
	NM_013215	*AKR7A3* (aldo-keto reductase family 7, member A3)	Biotransformation, phase I	**1.8**	**1.7**	**1.5**	**2.0**	1.5	1.4	1.5	1.7
	NM_017084	*GNMT* (glycine-*N*-methyltransferase)	Biotransformation, phase I	**1.4**	**1.6**	**1.8**	**1.6**	1.2	1.2	1.0	1.9
	NM_133586	*CES2* (carboxylesterase 2; intestine, liver)	Biotransformation, phase I	**−1.1**	**1.1**	**1.1**	**2.1**	−1.4	1.1	1.5	2.9
	AW142784	*CYP51* (cytochrome *P*450 51)	Biotransformation, phase I	**1.1**	**−1.4**	**−1.7**	**−1.1**	1.0	1.0	−1.6	−1.1
	BI285792	*GSTM7-7* (similar to glutathione transferase GSTM7-7)	Biotransformation, phase II	**1.5**	**1.7**	**1.5**	**2.1**	1.3	1.5	1.3	1.5
	AI169331	*GSTM2* (glutathione *S*-transferase M2)	Biotransformation, phase II	**1.1**	**1.2**	**1.7**	**2.1**	1.0	1.2	1.3	1.9
	AF461738	*UGT1A* (UDP-glucuronosyltransferase 1A, microsomal)	Biotransformation, phase II	**1.9**	**1.7**	**1.5**	**1.8**	1.5	1.6	1.6	1.5
	NM_133547	*SULT1C2* (sulfotransferase K1; SULTK1)	Biotransformation, phase II	**−1.3**	**−1.2**	**−1.3**	**−2.0**	1.0	−1.3	−1.1	−1.3
	AY082609	*MDR1B* (Multidrug resistance 1B; ABCB1b)	Drug transport	**1.1**	**1.2**	**1.2**	**5.5**	−1.1	1.1	1.6	2.5
	NM_012833	*MRP2* (Multidrug resistance-associated protein 2)	Drug transport	**1.5**	**1.5**	**1.2**	**4.6**	1.1	1.3	1.1	1.6
	L46791	*CES3* (Carboxylesterase 3)	Biotransformation, phase I	1.1	1.5	1.5	1.7	**1.4**	**1.6**	**1.6**	**2.3**

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Table 1. Categories of genes differentially deregulated by AA in Eker and wild-type rats (Cont'd)

Toxicological category	Accession no.	Gene title	Biochemical category	Fold deregulation (mean of three replicate animals per time point)							
				Ek d1	Ek d3	Ek d7	Ek d14	wt d1	wt d3	wt d7	wt d14
	NM_134349	*MGST1* (microsomal glutathione *S*-transferase 1)	Biotransformation, phase II	1.5	1.4	1.3	1.5	**1.6**	**1.4**	**1.5**	**1.8**
	AF072816	*MRP3* (multidrug resistance-associated protein 3)	Drug transport	4.1	−1.7	1.3	2.2	**2.3**	**1.3**	**1.9**	**7.8**
DNA damage response (incl. Oxidative stress response)	NM_012861	*MGMT* (O_6-methylguanine-DNA methyltranferase)	DNA repair	**−1.1**	**1.2**	**1.7**	**3.1**	**1.1**	**1.2**	**1.9**	**2.7**
	AW520812	*PHLDA3* (Pleckstrin homology-like domain family A3)	DNA damage response	**1.1**	**1.1**	**1.3**	**3.2**	**1.4**	**1.4**	**2.4**	**3.2**
	Q64315	*CDKN1A* (cyclin-dependent kinase inhibitor 1A; p21)	Cell cycle checkpoint	**1.5**	**1.1**	**6.9**	**14.2**	**1.1**	**2.7**	**2.8**	**9.8**
	NM_031821	*SNK* (serum-inducible kinase; PLK2)	Cell cycle checkpoint	**1.2**	**1.3**	**2.2**	**6.6**	**1.4**	**1.2**	**2.0**	**4.3**
	NM_012923	*CCNG1* (cyclin G1)	Cell cycle checkpoint	**1.1**	**1.5**	**2.3**	**3.8**	**1.1**	**1.6**	**2.1**	**4.1**
	BI296301	*MDM2* (ubiquitin E3 ligase Mdm2; predicted)	Cell cycle checkpoint	**1.0**	**1.7**	**1.4**	**4.9**	**−1.2**	**1.0**	**1.9**	**2.4**
	NM_022547	*FTHFD* (10-formyltetrahydrofolate dehydrogenase)	Cell cycle	**2.0**	**1.9**	**1.5**	**1.8**	**1.7**	**1.7**	**1.6**	**1.8**
	AI411345	*PRODH EST* [similar to Proline dehydrogenase (oxidase) 1]	Proapoptotic	**1.4**	2.5	1.7	2.0	1.2	1.7	1.8	1.9
	NM_057153	*OXR1* (oxidation resistance 1)	DNA damage response	−1.1	1.2	1.6	1.5	**1.2**	**−1.1**	**1.2**	**1.7**
Inhibited cell survival and proliferation	AA801395	*TNFAIP8* (tumor necrosis factor α–induced protein 8)	Antiapoptotic	**1.1**	**−1.3**	**−1.4**	**−2.5**	1.0	1.1	1.2	−1.4
	M14050	*HSPA5* (heat shock 70 kDa protein 5; GRP78)	Antiapoptotic	**1.5**	**−1.7**	**−1.6**	**−1.4**	1.0	1.2	1.1	−1.3
	AF106659	*USP2* (ubiquitin-specific protease 2)	Proapoptotic	**−1.1**	**1.9**	**1.6**	**3.5**	1.0	2.0	1.5	1.4
	BF288101	*SNN* (stannin)	Proapoptotic	**−1.2**	**1.1**	**1.1**	**1.9**	−1.1	1.1	1.0	1.8
	AI714002	*Ki-67* (predicted)	DNA replication	**−2.4**	**−2.2**	**−1.9**	**−1.2**	1.5	−1.4	1.0	−1.4
	AI072892	*FRZB* (Frizzled-related protein)	Signaling cascades	**−1.1**	**1.2**	**1.8**	**1.3**	1.6	1.2	1.0	1.3
	M15481	*IGF1* (insulin-like growth factor 1)	Regulation of proliferation	**−1.1**	**−1.2**	**−1.7**	**−2.4**	1.0	1.2	−1.1	−2.0
	NM_022266	*CTGF* (connective tissue growth factor)	Cell adhesion/migration	**1.2**	**−1.1**	**−1.1**	**−1.9**	1.4	1.1	1.2	−1.9
	BI275994	*TGM2* (tissue-type transglutaminase)	Cell adhesion/migration	**−1.7**	**−1.5**	**−1.5**	**−2.9**	−1.9	−2.0	−1.9	−1.9
	AI102530	*NAB2* (Ngfi-A binding protein 2; predicted)	Transcriptional corepressor	**−1.1**	**1.2**	**1.8**	**2.0**	1.1	1.0	1.0	1.7
	BF417638	*CDCA3* (cell division cycle-associated 3; TOME1)	Cell cycle (G_2-M)	1.1	−1.1	−1.3	1.3	**−1.2**	**−1.7**	**−1.6**	**−1.5**
	X64589	*CCNB1* (cyclin B1, G_2-M specific)	Cell cycle (G_2-M)	1.0	−1.3	−1.3	1.0	**−1.4**	**−2.2**	**−1.3**	**−1.6**
	AI171185	*HMMR* (hyaluronan-mediated motility receptor)	Cell cycle (G_2-M)	1.2	−1.4	−1.4	1.2	**−1.6**	**−2.0**	**−1.4**	**−1.7**

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Table 1. Categories of genes differentially deregulated by AA in Eker and wild-type rats (Cont'd)

Toxicological category	Accession no.	Gene title	Biochemical category	Ek d1	Ek d3	Ek d7	Ek d14	wt d1	wt d3	wt d7	wt d14
				Fold deregulation (mean of three replicate animals per time point)							
	NM_019296	*CDC2* (cell division cycle 2 protein kinase; CDK1, cyclin dependent kinase 1)	Cell cycle (G_2-M)	−1.2	−1.4	−2.5	1.2	**−1.5**	**−2.4**	**−2.0**	**−1.6**
	AA944180	*CKS2* (similar to cyclin-dependent kinases regulatory subunit 2)	Cell cycle (G_2-M)	1.0	1.1	−1.7	2.5	**−1.6**	**−2.3**	**−2.6**	**−1.3**
	BF396602	*SFRP2* (secreted frizzled-related protein 2)	Antiapoptotic	−1.1	−1.2	−1.6	−1.4	**−1.1**	**−1.1**	**−1.4**	**−1.7**
	NM_012593	*KLK7* (Kallikrein 7)	Antiapoptotic	1.2	−1.3	−1.8	−2.2	**−1.4**	**−1.1**	**−1.2**	**−1.8**
Enhanced cell survival/ proliferation	NM_021854	*TSC1* (tuberous sclerosis 1)	Tumor suppressor gene	**−1.9**	**−2.1**	**−1.3**	**−2.3**	−1.1	−1.8	1.0	−1.5
	BE098555	*WWOX* (WW-domain oxidoreductase; predicted)	Proapoptotic	−1.4	−1.5	−1.5	−1.7	**−1.5**	**−1.5**	**−1.9**	**−1.9**
	NM_134330	*KEG1* (kidney-expressed gene 1)	Regulation of proliferation	1.2	1.0	1.8	1.3	**1.7**	**1.2**	**1.8**	**2.2**

NOTE: For the major toxicologic categories, the associated genes are listed together with their Genbank accession number. The main biochemical functions or pathways in which these genes are involved are indicated in column 4. For Eker and wild-type rats, the fold deregulation ratios of genes that were significantly deregulated over all time points according to one-way ANOVA are shown in the last eight columns. Genes meeting the significance criteria are indicated in bold.
Abbreviations: Ek, Eker; wt, wild-type.

Preneoplastic and Neoplastic Pathology

Although no preneoplastic or neoplastic lesions (23) were observed in any of the wild-type rat controls, the Eker rat control groups presented with atypical tubules (Fig. 2*C*), atypical hyperplasia (Fig. 2*D*), and an occasional adenoma (see Supplementary Table S2). Neither AA nor OTA treatment induced an increase in preneoplastic or neoplastic lesions in wild-type rats. Similarly, AA treatment of Eker rats resulted in no-significant increase in atypical tubules or atypical hyperplasia. However, two carcinomas were observed in the day 14 AA-treated Eker group and an adenoma in the day one group. In contrast, OTA treatment of Eker rats resulted in a significant increase of atypical tubules on day 14 (Fig. 1*E*). However, no significant increase in atypical hyperplasia or neoplastic lesions was observed.

Nonneoplastic Pathology

Overall, only marginal, strain-independent renal pathologic changes were observed (see Supplementary Tables S3 and S4). AA treatment of Eker and wild-type rats seemed to induce a slightly higher inflammatory response in the cortex, corroborating earlier reports by Chen et al. (24). Similarly, OTA treatment of Eker and wild-type rats led to the previously reported (25) increased prevalence and severity of apoptosis, karyomegaly, cell shedding, and tubular regeneration, primarily in the P3 portion of the proxima (Fig. 2*E* and *F*). In addition, an increased cell proliferation response was observed.

Gene Expression Profiles

Oral treatment of Eker and wild-type rats with AA and OTA, respectively, led to a significant deregulation of gene expression already after 1 day of exposure. Compared with the respective time-matched controls, the number of significantly deregulated genes increased with the duration of exposure in both strains. At all time points, compound-treated Eker rats consistently showed a higher number of significantly deregulated genes compared with their wild-type counterparts (Fig. 3).

Overall, AA treatment led to the significant deregulation of 111 nonredundant genes in Eker rats compared with 81 genes in wild-type rats. However, the visualization of the expression profiles of the union of the Eker- and wild type–selected genes revealed a qualitatively comparable profile in AA-treated Eker and wild-type rats. In contrast, treatment with OTA resulted in 375 significantly deregulated, nonredundant genes in Eker rats compared with 141 genes in wild-type rats, with a strikingly different expression profile of at least half of the genes (Fig. 3).

Functional Analysis of Significantly Deregulated Genes

Many of the nonredundant genes deregulated by AA (Table 1) or OTA (Table 2) treatment in Eker and wild-type rats, respectively, could be associated with major pathophysiologic processes involved in renal toxicity and regeneration (see Supplementary Table S1).

Genes deregulated by AA

Metabolism and bioactivation. Treatment of Eker and wild-type rats with AA led to a prominent up-regulation of genes encoding phase I or phase II biotransformation enzymes or drug transporters (Table 1). Most of the genes were either constantly up-regulated

Table 2. Categories of genes differentially deregulated by OTA in Eker and wild-type rats

Toxicological category	Accession no.	Gene title	Biochemical category	Fold deregulation (mean of three replicate animals per time point)							
				Ek d1	Ek d3	Ek d7	Ek d14	wt d1	wt d3	wt d7	wt d14
Metabolism and Biotransformation	NM_031565	*CES1B* (carboxylesterase RL1)	Biotransformation phase I	**1.0**	**−1.7**	**−2.7**	**−1.9**	**−1.1**	**−1.5**	**−2.2**	**−2.2**
	L46791	*CES3* (carboxylesterase 3)	Biotransformation phase I	**1.0**	**−1.4**	**−2.7**	**−3.4**	**−1.2**	**−1.7**	**−2.7**	**−1.9**
	NM_020538	*AADAC* (arylacetamide deacetylase)	Biotransformation phase I	**−1.4**	**−1.3**	**−1.9**	**−2.5**	**1.0**	**−1.2**	**−1.5**	**−2.0**
	AW142784	*CYP4A12* (cytochrome *P*450 4a12)	Biotransformation phase I	**1.0**	**1.6**	**1.5**	**1.9**	**1.7**	**1.8**	**2.0**	**1.9**
	NM_133558	*CML1* (Camello-like 1)	Biotransformation phase II	**−1.1**	**−1.3**	**−2.6**	**−2.9**	**−1.2**	**−1.1**	**−2.0**	**−2.1**
	NM_022635	*NAT8* (*N*-acetyltransferase 8)	Biotransformation phase II	**−1.2**	**−2.1**	**−3.3**	**−2.9**	**−1.3**	**−1.5**	**−2.4**	**−2.9**
	AI072042	*GGT6* (γ-glutamyl transpeptidase type VI)	Biotransformation phase II	**−1.4**	**−2.0**	**−2.9**	**−2.7**	**−1.3**	**−1.3**	**−1.6**	**−1.8**
	NM_134349	*MGST1* (microsomal glutathione *S*-transferase 1)	Biotransformation phase II	**−1.3**	**−1.4**	**−1.8**	**−1.8**	**−1.1**	**−1.3**	**−1.7**	**−1.7**
	NM_134369	*CYP2T1* (cytochrome *P*450 2T1)	Biotransformation phase I	**1.0**	**−1.1**	**−2.0**	**−1.9**	1.1	−2.0	−1.3	−1.3
	BF283000	*WBSCR21* (Williams-Beuren syndrome chromosome region 21)	Biotransformation phase I	**1.1**	**−1.4**	**−1.7**	**−1.9**	−1.1	−1.1	−1.3	−1.4
	AI407458	*ALDH6A1* (aldehyde dehydrogenase 6A1)	Biotransformation phase I	**1.8**	**2.0**	**1.6**	**2.1**	1.1	1.1	−1.1	−1.1
	NM_022270	*OCTN1* (organic cation carnitine transporter 1)	Drug transport	**−1.1**	**−1.1**	**−2.1**	**−1.8**	1.0	−1.1	−1.5	−1.2
	NM_017224	*OAT1* (organic anion transporter 1; SLC22A6)	Drug transport	**−1.1**	**1.1**	**−1.9**	**−1.7**	1.1	−1.2	−1.3	−1.6
	NM_019303	*CYP2F4* (cytochrome *P*450 2F4)	Biotransformation phase I	1.3	1.0	−1.4	−1.8	**1.2**	**−1.8**	**−1.8**	**−1.1**
	NM_017084	*GNMT* (glycine-*N*-methyltransferase)	Biotransformation phase II	1.0	−1.4	−1.4	−1.6	**−1.4**	**−1.5**	**−1.9**	**−1.5**
	U76379	*OCT1* (organic cation transporter 1; SLC22A1)	Drug transport	1.1	−1.3	−1.4	−1.7	**−1.1**	**−1.4**	**−1.7**	**−1.7**
DNA damage response (incl. Oxidative stress)	BM388545	*SUPT16H* (suppressor of Tyr^{16} homologue; predicted)	DNA damage repair	**1.0**	**1.8**	**1.4**	**1.8**	**1.6**	**1.8**	**2.1**	**2.4**
	NM_053677	*CHEK2* (checkpoint kinase 2)	Cell cycle checkpoint	**1.0**	**1.8**	**2.8**	**2.2**	1.0	−1.1	1.1	−1.1
	BF548539	*MDM2* (ubiquitin E3 ligase Mdm2; predicted)	Cell cycle checkpoint	**5.3**	**4.4**	**1.8**	**2.4**	−1.4	−1.4	−1.4	1.0
	AI178158	*RBBP6* (retinoblastoma binding protein 6; predicted)	Cell cycle checkpoint	1.0	1.1	1.0	1.0	**1.2**	**1.5**	**2.0**	**1.5**
Oxidative stress response	NM_019192	*SEPP1* (Selenoprotein P)	Oxidative stress response	**1.1**	**3.5**	**3.4**	**3.9**	**4.1**	**4.2**	**4.9**	**4.2**

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Table 2. Categories of genes differentially deregulated by OTA in Eker and wild-type rats (Cont'd)

Toxicological category	Accession no.	Gene title	Biochemical category	Fold deregulation (mean of three replicate animals per time point)							
				Ek d1	Ek d3	Ek d7	Ek d14	wt d1	wt d3	wt d7	wt d14
	NM_019235	*GGTLA1* (γ-glutamyltransferase-like activity 1)	Oxidative stress response	**1.4**	**2.6**	**1.8**	**4.2**	1.3	−1.4	2.1	1.0
	NM_031614	*TXNRD1* (Thioredoxin reductase 1, cytoplasmic)	Oxidative stress response	**1.7**	**1.7**	**1.4**	**1.7**	−1.1	1.0	−1.3	−1.1
Enhanced oxidative stress	AI231438	*CN1* (Carnosine dipeptidase 1)	Oxidative stress response	**−1.2**	**−1.2**	**−2.9**	**−2.5**	**−1.2**	**−1.5**	**−1.8**	**−1.3**
	BM386741	*HSP40-3* (heat shock protein hsp40-3; predicted)	Protein folding in the cytosol	**−1.3**	**−1.8**	**−1.7**	**−1.4**	1.0	1.0	−1.5	−1.1
	BF414210	*KEAP1* (Kelch-like ECH-associated protein 1)	Regulation of transcription	**1.7**	**1.6**	**2.0**	**2.4**	1.1	1.0	−1.1	1.0
	NM_053307	*MSRA* (methionine sulfoxide reductase A)	Oxidative stress response	1.4	1.4	1.0	−1.3	**1.0**	**−1.6**	**−2.0**	**−1.8**
Cellular stress	BM387750	*DUSP11* (dual-specificity phosphatase 11)	MAPK pathway	**−1.1**	**−1.7**	**−1.7**	**−1.5**	**−1.5**	**−1.5**	**−1.4**	**−1.8**
	BE110108	*DUSP1* (dual-specificity protein phosphatase 1)	MAPK pathway	**1.9**	**1.6**	**2.3**	**2.3**	−1.1	−1.3	−1.3	1.0
	NM_031032	*GMFB* (glia maturation factor β)	MAPK pathway	**2.4**	**4.0**	**5.9**	**5.6**	1.5	1.3	2.7	1.5
	AAH61870	*JNK2* (c-Jun NH_2-terminal kinase 2)	MAPK pathway	**1.8**	**1.3**	**1.4**	**1.3**	1.2	−1.4	−1.1	−1.3
	AA851481	*BRE* (brain and reproductive organ-expressed protein)	MAPK pathway	**1.8**	**1.7**	**1.5**	**1.7**	1.1	−1.1	−1.2	−1.2
	L48060	*PRLR* (prolactin receptor)	MAPK pathway	**1.5**	**2.1**	**1.3**	**1.7**	1.1	1.2	1.2	1.2
	NM_023090	*HIF2α* (hypoxia-inducible factor 2α)	HIF pathway	**2.5**	**1.8**	**2.0**	**1.7**	1.2	1.1	−1.2	1.0
	BF403837	*NFE2L1* [nuclear factor (erythroid-derived) 2 like 1]	Regulation of gene expression	**1.4**	**1.3**	**1.3**	**1.7**	1.0	−1.3	−1.1	−1.1
	AI598399	*RBM3* (RNA-binding motif protein 3)	RNA metabolism	1.0	1.0	−1.3	1.0	**1.4**	**1.1**	**1.2**	**1.8**
Reduced cell survival/proliferation	BF281976	*POLD4* [polymerase (DNA-directed), delta 4]	DNA replication	**2.0**	**−2.2**	**−2.0**	**−2.1**	**−1.1**	**−1.4**	**−1.6**	**−2.0**
	NM_057138	*CFLAR* (CASP8 and FADD-like apoptosis regulator)	Antiapoptotic	**−1.3**	**−1.9**	**−2.9**	**−4.3**	**1.2**	**−3.6**	**−2.9**	**−3.4**
	AW253957	*ENDOG* (endonuclease G)	Proapoptotic	**1.1**	**1.9**	**2.3**	**1.9**	**1.3**	**1.4**	**1.8**	**2.5**
	AA944698	*BAT3* (HLA-B–associated transcript 3)	Proapoptotic	**6.5**	**4.4**	**3.9**	**5.0**	1.2	1.3	1.1	−1.1
	NM_019208	*MEN1* (multiple endocrine neoplasia 1)	Tumor suppressor gene	**1.9**	**1.2**	**1.6**	**1.9**	−1.1	−1.2	1.1	−1.1
	AI113091	*TSSC4* (tumor-suppressing subchromosomal transferable fragment 4; predicted)	Tumor suppressor gene	**1.8**	**1.4**	**1.2**	**2.2**	−1.1	1.0	−1.1	1.0

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Table 2. Categories of genes differentially deregulated by OTA in Eker and wild-type rats (Cont'd)

Toxicological category	Accession no.	Gene title	Biochemical category	Fold deregulation (mean of three replicate animals per time point)							
				Ek d1	Ek d3	Ek d7	Ek d14	wt d1	wt d3	wt d7	wt d14
	AA996685	*PKIA* (cyclic AMP–dependent protein kinase inhibitor α)	Signaling cascades	**3.0**	**3.1**	**2.5**	**2.4**	−1.3	1.2	1.3	2.6
	BI290885	*FSTL* (Follistatin-like 1)	Signaling cascades	**1.9**	**1.2**	**1.7**	**1.9**	1.1	−1.1	1.1	1.3
	BG663107	*AKAP12* (a kinase anchor protein 12; gravin)	Signaling cascades	**1.7**	**1.4**	**2.0**	**1.5**	−1.1	−1.2	−1.1	−1.2
	M86708	*ID1* (inhibitor of DNA binding 1)	Regulation of transcription	**1.1**	**−1.3**	**1.4**	**1.0**	**−1.3**	**−1.1**	**−1.3**	**−1.9**
	NM_031546	*RGN* [regucalcin (SMP-30)]	Antiapoptotic	**−1.4**	**1.9**	**−3.7**	**−4.5**	**1.2**	**−1.7**	**−1.9**	**−2.6**
Increased cell survival/ proliferation	BE108969	*IGFBP-4* (insulin-like growth factor binding protein 4)	(IGF)-PI3K-AKT pathway	**−1.3**	**−1.4**	**−3.0**	**−3.8**	**1.0**	**−1.3**	**−3.0**	**−2.8**
	AI145815	*MKRN-1* (Makorin-1)	Protein metabolism	**−1.1**	**−1.6**	**−1.9**	**−1.9**	**−1.3**	**−1.7**	**−1.7**	**−1.6**
	BM986536	*HIST1H4I* (histone 1 H4i)	Nucleosome assembly	**1.4**	**3.1**	**2.7**	**3.0**	1.3	2.2	2.5	2.3
	NM_022265	*PDC4* (programmed cell death 4)	Antiapoptotic	**1.5**	**2.1**	**1.7**	**1.9**	1.0	−1.1	−1.3	1.1
	BE112895	*PEA15* (phospho-protein–enriched in astrocytes 15)	Antiapoptotic	**−1.5**	**1.3**	**1.9**	**1.9**	1.2	1.1	1.4	1.1
	NM_021846	*MCL1* (myeloid cell leukemia 1)	Antiapoptotic	**1.6**	**1.4**	**1.5**	**1.8**	1.1	−1.3	−1.1	−1.4
	NM_022943	*MERTK* (C-Mer proto-oncogene tyrosine kinase)	Proto-oncogene	**3.3**	**4.9**	**4.3**	**2.9**	−1.3	1.0	1.2	1.1
	NM_012807	*SMOH* (smoothened)	Proto-oncogene	**1.8**	**1.3**	**1.6**	**1.5**	−1.1	1.0	−1.3	1.0
	NM_022264	*V-KIT* [Hardy-Zuckerman 4 feline sarcoma viral oncogene homologue (c-Kit)]	Proto-oncogene	**2.9**	**3.7**	**2.4**	**2.5**	−1.2	−1.3	−1.5	1.2
	NM_012843	*TMP* tumor-associated membrane protein (EMP1 Epithelial membrane protein 1)]	Proto-oncogene	**2.8**	**1.7**	**2.2**	**4.3**	1.0	−1.3	−1.2	−1.3
	AA943541	*OVCA2* (candidate tumor suppressor OVCA2; predicted)	Tumor suppressor gene	**−1.3**	**−1.4**	**−1.6**	**−1.8**	−1.3	−1.1	−1.4	−1.6
	NM_053481	*PIK3CB* (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic β subunit)	(IGF)-PI3K-AKT pathway	**2.0**	**2.9**	**−1.3**	**2.0**	1.3	1.0	1.0	−1.1
	AI102030	*AKT1S1* (AKT1 substrate 1; proline-rich)	(IGF)-PI3K-AKT pathway	**2.4**	**4.0**	**2.0**	**2.1**	1.0	1.0	−1.1	−1.1
	AI105076	*AKT2* (Thymoma viral proto-oncogene 2)	(IGF)-PI3K-AKT pathway	**1.5**	**1.5**	**1.2**	**1.9**	1.0	1.1	−1.1	−1.2
	AW434982	*SBF1* (similar to SET binding factor 1)	(IGF)-PI3K-AKT pathway	**2.4**	**3.5**	**2.2**	**3.6**	1.2	1.0	−1.1	1.0

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Table 2. Categories of genes differentially deregulated by OTA in Eker and wild-type rats (Cont'd)

Toxicological category	Accession no.	Gene title	Biochemical category	Fold deregulation (mean of three replicate animals per time point)							
				Ek d1	Ek d3	Ek d7	Ek d14	wt d1	wt d3	wt d7	wt d14
	NM_012593	*KLK7* (glandular kallikrein 7)	Protein metabolism	**1.8**	**1.3**	**1.9**	**1.6**	−1.1	−1.2	−1.2	−1.1
	BI300565	*ADAM10* (A disintegrin and metalloprotease domain 10)	Protein metabolism	**1.6**	**1.8**	**1.3**	**1.4**	1.1	−1.3	−1.3	−1.3
	NM_053665	*AKAP1* [a kinase (PRKA) anchor protein 1]	Signaling cascades	**5.9**	**3.8**	**4.1**	**7.8**	1.0	1.2	−1.1	1.0
	NM_017094	*GHR* (growth hormone receptor)	Signaling cascades	**1.7**	**2.8**	**1.2**	**2.0**	1.2	−1.1	1.0	1.1
	NM_012850	*GHRHR* (growth hormone releasing hormone receptor)	Signaling cascades	**1.2**	**1.3**	**1.8**	**1.7**	1.6	1.4	1.1	−1.1
	BI294916	*KLF2* (Kruppel-like factor 2)	Regulation of gene expression	**1.6**	**1.4**	**1.9**	**1.9**	1.1	−1.1	−1.2	1.2
	NM_131904	*MGEA5* (meningioma-expressed antigen 5)	Protein metabolism	**2.1**	**1.7**	**1.8**	**1.4**	−1.1	−1.1	−1.5	−1.7
	AF080594	*VEGF* (vascular endothelial growth factor)	Angiogenesis	**2.3**	**2.0**	**1.6**	**2.6**	1.1	−1.3	1.0	1.0
	AW524517	*VEZF1* (vascular endothelial zinc finger 1)	Angiogenesis	**1.9**	**1.6**	**1.2**	**1.4**	1.1	1.0	−1.1	1.1
	NM_133569	*ANGPTL2* (angiopoietin-like 2)	Angiogenesis	**1.3**	**1.9**	**1.2**	**1.9**	1.2	−1.1	−1.1	1.1
	NM_017089	*EPHB1* (Ephrin B1)	Angiogenesis	**1.9**	**1.6**	**1.6**	**1.9**	1.1	1.0	−1.3	1.0
	AB035507	*MCAM* (melanoma cell adhesion molecule)	Angiogenesis	**2.1**	**1.5**	**1.6**	**1.3**	1.0	−1.3	1.1	−1.1
Cell cycle progression and mitosis	NM_022615	*TOP1* (DNA topoisomerase 1)	DNA replication	**1.5**	**1.8**	**1.6**	**2.2**	1.0	−1.1	1.0	1.1
	BM385181	*SMARCA4* (SWI-SNF–related matrix-associated actin-dependent regulator of chromatin subfamily A member 4)	Chromatin remodeling	**1.3**	**1.1**	**1.7**	**1.7**	−1.1	1.4	1.0	1.1
	U75920	*MAPRE1* (microtubule-associated protein RP/EB family member 1)	Mitotic spindle formation	**2.4**	**2.6**	**2.0**	**2.8**	1.1	1.0	−1.1	1.0
	AJ306292	*AJUBA* (Ajuba protein)	Mitotic spindle formation	**1.4**	**1.3**	**1.3**	**1.8**	1.0	−1.2	−1.1	−1.2
	BE118382	*NEK9* (NIMA–related kinase 9)	Mitotic spindle formation	**2.1**	**1.9**	**1.8**	**2.4**	1.0	1.0	1.0	−1.1
	U77583	*CSNK1A1* (Casein kinase 1, α 1)	Mitotic spindle formation	**1.6**	**1.5**	**1.6**	**1.8**	1.1	1.0	−1.1	1.0
	NM_057148	*SEPT2* (Septin 2)	Mitotic spindle formation	**2.5**	**2.2**	**2.1**	**2.5**	−1.1	−1.1	1.0	−1.1

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Table 2. Categories of genes differentially deregulated by OTA in Eker and wild-type rats (Cont'd)

Toxicological category	Accession no.	Gene title	Biochemical category	Fold deregulation (mean of three replicate animals per time point)							
				Ek d1	Ek d3	Ek d7	Ek d14	wt d1	wt d3	wt d7	wt d14
		PPP4R1 (protein phosphatase 4, regulatory subunit 1)	Mitotic spindle formation	**2.1**	**1.7**	**1.3**	**2.0**	1.0	1.0	−1.2	−1.1
	NM_013194	*NMMHC-A* (Non-muscle myosin heavy chain A)	Mitotic spindle formation	**1.6**	**1.6**	**1.7**	**2.0**	1.3	−1.1	1.1	1.2
Cell structure remodeling	AA955773	*PCDH1* (protocadherin 1)	Cell adhesion molecule	**−1.1**	**−1.7**	**−1.6**	**−1.9**	**−1.3**	**−1.7**	**−2.2**	**−1.8**
	BE097805	*PCDH4* (protocadherin 4)	Cell adhesion molecule	**2.7**	**1.2**	**3.6**	**2.3**	1.5	1.3	1.6	1.3
	AA955091	*ITGA6* (integrin α_6)	Cell adhesion molecule	**2.0**	**1.3**	**1.7**	**1.5**	1.0	1.0	−1.3	−1.4
	NM_031699	*CLDN1* (Claudin 1)	Cell adhesion molecule	**1.6**	**1.2**	**2.0**	**1.8**	1.0	−1.2	−1.3	−1.2
	NM_031329	*OCLN* (Occludin)	Cell adhesion molecule	**2.5**	**1.9**	**1.3**	**2.2**	1.4	1.0	−1.1	1.0
	NM_013217	*AF-6* (Afadin)	Regulation of cell adhesion/migration	**1.6**	**1.7**	**1.7**	**1.9**	1.0	1.0	−1.2	−1.2
	BF284125	*IQGAP1* (IQ motif containing GTPase activating protein 1)	Regulation of cell adhesion/migration	**1.3**	**1.3**	**1.8**	**1.8**	1.0	−1.1	−1.3	−1.2
	AB020726	*PODXL* (Podocalyxin)	Regulation of cell adhesion/migration	**1.6**	**1.3**	**1.5**	**1.8**	1.2	1.0	1.1	−1.1
	NM_020085	*RPTPK* (receptor-like protein tyrosine phosphatase κ extracellular region)	Signaling cascades	**5.6**	**5.7**	**3.2**	**5.4**	1.0	−1.4	−1.8	−1.4
	NM_057115	*PTPN12* (protein tyrosine phosphatase, non-receptor type 12)	Signaling cascades	**1.9**	**1.5**	**2.7**	**3.6**	−1.1	−1.3	−1.2	−1.2
	NM_031034	*GNA12* (guanine nucleotide-binding protein α 12)	Signaling cascades	**2.2**	**2.2**	**1.6**	**2.4**	1.1	1.2	1.1	−1.1
	BE115857	*PARVA* (Parvin α)	Cytoskeleton organization	**2.4**	**2.1**	**1.6**	**2.1**	1.0	1.0	1.1	−1.1
	NM_024401	*AVIL* (Advillin; Pervin)	Cytoskeleton organization	**1.2**	**1.3**	**1.9**	**2.6**	1.0	1.1	1.5	1.8
	AF054618	*CTTN* (Cortactin)	Cytoskeleton organization	**1.8**	**1.5**	**1.9**	**2.0**	1.1	1.0	1.1	1.1
	NM_032613	*LASP1* (LIM and SH3 protein 1)	Cytoskeleton organization	**3.1**	**1.8**	**1.5**	**2.0**	1.1	1.0	−1.2	1.1
	NM_023982	*ARHGEF11* [Rho guanine nucleotide exchange factor (Rho-GEF) 11]	Cytoskeleton organization	**1.7**	**2.5**	**2.8**	**3.1**	1.7	2.1	2.1	1.4
	AI170442	*DSTN* (Destrin)	Cytoskeleton organization	**1.5**	**1.5**	**1.7**	**2.0**	1.0	1.1	1.0	−1.1
	NM_030873	*PFN2* (Profilin 2)	Cytoskeleton organization	**1.6**	**2.5**	**2.1**	**3.4**	1.3	−1.1	−1.2	1.2
	AA875047	*CCTZ* (Chaperonin containing T-complex 1 zeta)	Cytoskeleton organization	**9.0**	**2.0**	**6.7**	**5.0**	−2.2	2.4	−1.5	−1.1

(*Continued on the following page*)

Table 2. Categories of genes differentially deregulated by OTA in Eker and wild-type rats (Cont'd)

Toxicological category	Accession no.	Gene title	Biochemical category	Fold deregulation (mean of three replicate animals per time point)							
				Ek d1	Ek d3	Ek d7	Ek d14	wt d1	wt d3	wt d7	wt d14
	V01217	*ACTB* (actin-β)	Cytoskeleton constituent	**2.2**	**2.1**	**2.1**	**3.1**	1.0	−1.1	−1.1	1.0
	NM_016990	*ADD1α* (adducin)	Cytoskeleton constituent	**2.0**	**1.3**	**1.5**	**2.1**	1.2	1.2	−1.1	1.1
	X70706	*PLS3* (Plastin 3 T-isoform; T-plastin)	Cytoskeleton constituent	**2.0**	**2.4**	**1.8**	**2.9**	1.0	1.1	1.0	1.2
	NM_030863	*MSN* (Moesin)	Cytoskeleton constituent	**1.7**	**1.8**	**1.6**	**1.9**	1.3	−1.2	1.0	1.1
	BF392456	*SPNB2* (Spectrin β 2)	Cytoskeleton constituent	**1.9**	**1.9**	**1.5**	**2.4**	1.1	1.1	1.0	1.0
	BI284344	*KRT2-7* (Keratin complex 2, basic, gene 7; predicted)	Cytoskeleton constituent	**−1.1**	**−1.4**	**−1.8**	**−2.0**	1.0	−1.3	−1.4	−1.6
	BI295970	*TPM3* (Tropomyosin 3 γ; Tropomyosin α3)	Cell morphology/ motility	**1.7**	**1.7**	**1.6**	**2.1**	1.0	1.0	−1.1	−1.1
	NM_012678	*TPM4* (Tropomyosin 4)	Cell morphology/ motility	**2.2**	**1.8**	**2.3**	**2.0**	1.0	−1.1	−1.4	1.1
	NM_053986	*MYO1B* (myosin IB; Brush border myosin I)	Cell morphology/ motility	**1.7**	**1.3**	**1.5**	**1.8**	−1.1	1.1	−1.1	−1.1
	NM_023092	*MYO1C* (myosin 1C; unconventional myosin Myr2 I heavy chain)	Cell morphology/ motility	**1.5**	**1.4**	**1.7**	**1.7**	1.1	1.1	1.0	1.1
	BG380723	*CAP1* (Adenylyl cyclase-associated protein 1)	Cell morphology/ motility	**2.0**	1.5	1.9	2.3	−1.1	1.2	1.0	1.0
	AA997129	*LAMC1* (Laminin γ 1)	Extracellular matrix component	**2.2**	1.5	1.6	1.6	1.1	−1.2	1.0	1.3
	AW435213	*NANS* (*N*-acetylneuraminic acid synthase; sialic acid synthase; predicted)	Cell adhesion/ migration	**1.4**	1.3	1.5	1.7	1.0	1.1	−1.3	−1.2
	U61261	*LAMA3* (Laminin α3; Laminin-5 α3)	Extracellular matrix component	1.2	1.1	1.2	−1.3	**−1.2**	**−1.7**	**−1.6**	**−2.0**
	BF284673	*FGD1* (Faciogenital dysplasia homologue)	Cell adhesion/ migration	1.1	1.3	1.2	1.5	**1.5**	**1.2**	**1.9**	**2.2**
EMT/Fibrosis	NM_013085	*PLAU* (urokinase-type plasminogen activator)	Cell adhesion/ migration	**−1.3**	**−1.3**	**−2.1**	**−1.9**	**−1.3**	**−2.0**	**−1.7**	**−2.1**
	NM_013143	*MEP1A* (Meprin A α-subunit)	Protein degradation	**−1.2**	**1.0**	**−2.5**	**−2.9**	**−1.2**	**−1.6**	**−1.6**	**−2.1**
	L09653	*TGFBR2* (TGF-β receptor type II)	TGF-β family pathway	**3.9**	**5.5**	**5.9**	**3.4**	1.0	−1.4	−1.1	1.0
	NM_013130	*SMAD1* (MADH1; Mothers against decapentaplegic homologue 1)	TGF-β family pathway	**2.1**	**1.5**	**1.6**	**1.4**	1.0	1.1	1.0	1.7
	NM_013095	*SMAD3* (MADH3; Mothers against decapentaplegic homologue 3)	TGF-β family pathway	**2.1**	**3.5**	**1.6**	**2.8**	1.2	1.1	−1.1	1.1

(*Continued on the following page*)

Table 2. Categories of genes differentially deregulated by OTA in Eker and wild-type rats (Cont'd)

Toxicological category	Accession no.	Gene title	Biochemical category	Fold deregulation (mean of three replicate animals per time point)							
				Ek d1	Ek d3	Ek d7	Ek d14	wt d1	wt d3	wt d7	wt d14
	NM_019275	*SMAD4* (MADH4; Mothers against decapentaplegic homologue 4)	TGF-β family pathway	**1.7**	**1.7**	**1.3**	**1.6**	−1.2	−1.2	−1.3	−1.6
	NM_053357	*CTNNB1* (β-catenin)	Regulation of proliferation	**1.7**	**1.6**	**1.3**	**1.9**	1.0	−1.2	−1.1	1.0
	NM_031317	*PDGFC* (platelet-derived growth factor C)	Regulation of proliferation	**2.1**	**1.7**	**1.4**	**2.4**	1.0	−1.4	−1.1	−1.1
	BE095528	*HAI-1* (hepatocyte growth factor activator inhibitor 1)	Regulation of proliferation	**3.9**	**9.2**	**5.4**	**3.0**	1.0	1.0	−1.1	1.0
	AF172255	*NPHS1* (nephrosis 1 homologue; nephrin)	Cell adhesion/ migration	**1.7**	**2.3**	**2.0**	**1.8**	−1.1	1.0	1.0	1.1
	NM_012886	*TIMP3* (tissue inhibitor of metalloproteinase 3)	Cell adhesion/ migration	**1.8**	**1.4**	**1.4**	**1.8**	1.0	−1.1	−1.4	−1.7
	BI278545	*DPT* (dermatopontin)	Cell adhesion/ migration	**1.6**	**1.5**	**1.7**	**1.8**	1.1	1.0	1.2	1.2
	NM_012924	*CD44* (antigen; ECMR-III, extracellular matrix receptor-III)	Cell adhesion/ migration	**2.3**	**3.3**	**1.2**	**1.6**	−1.2	−1.1	1.1	1.2
	NM_024358	*NOTCH2* (Notch homologue 2)	Differentiation/ organogenesis	**2.4**	**2.2**	**3.2**	**7.4**	1.0	1.0	−1.1	−1.1
	AA997458	Similar to CSRP2 cysteine and glycine-rich protein 2	Differentiation/ organogenesis	**1.0**	**−1.5**	**−1.8**	**−1.6**	−1.3	−1.5	−1.6	−1.6
	NM_012901	*AMBP* (α-1-microglobulin/ bikunin precursor)	Extracellular transport	**1.1**	**2.0**	**2.6**	**1.9**	1.0	1.3	1.8	1.9
	NM_017309	*CNB* (Calcineurin B regulatory subunit isoform 1)	Signaling cascades	**4.5**	**7.6**	**1.8**	**5.8**	−1.1	−1.4	−1.3	−1.3

NOTE: For the major pathophysiologic categories, the associated genes are listed together with their Genbank accession number. The main biochemical functions or pathways in which these genes are involved are indicated in column 4. For Eker and wild-type rats, the fold deregulation ratios of genes that were significantly deregulated over all time points according to one-way ANOVA are shown in the last eight columns. Genes meeting the significance criteria are indicated in bold.
Abbreviations: MAPK, mitogen-activated protein kinase; ECM, extracellular matrix.

over the whole exposure time frame or increasingly up-regulated with prolonged duration of exposure in both Eker and wild-type rats. In addition to the genes significantly deregulated in both strains, some genes met the significance criteria only in one or the other strain. Yet, the direction of deregulation was mostly comparable (e.g., *GSTM2* or *CES3*, apparently specific for Eker or wild-type rats, respectively).

DNA damage response. p53 is inducible by DNA damage and oxidative stress. The up-regulation of several *p53* pathway genes, as observed in Eker and wild-type rats, was therefore summarized as DNA damage response (including oxidative stress). Most genes involved in this category showed a time-dependent increase with the highest deregulation values after 14 days of treatment and met the significance criteria in both strains.

Inhibited cell survival and proliferation. In conjunction with the DNA damage response described above, down-regulation of antiapoptotic genes and genes involved in DNA replication and cell cycle progression as well as the up-regulation of proapoptotic genes were observed. These genes showed a comparable time-dependent increase in expression as the genes representing the DNA damage response (see above), with the highest deregulation values after 14 days of treatment. In contrast to the DNA damage response genes, many of these genes showed an apparently strain-specific deregulation, yet again with a qualitatively similar expression pattern for most of them in both strains. An exception to the latter observation were genes directly involved in the G_2-M transition of the cell cycle, which were specifically and consistently down-regulated in wild-type rats.

Enhanced cell survival/cell proliferation. Only three genes were assigned to this category. The *Tsc1* tumor suppressor gene, which is known to be associated with Tsc2 (8), was significantly down-regulated in Eker but not wild-type rat. Enhanced cell survival and proliferation induced by AA was suggested by the observed up-regulation of a positive regulator of cell proliferation (*KEG1*) and the down-regulation of a proapoptotic gene (*WWOX*).

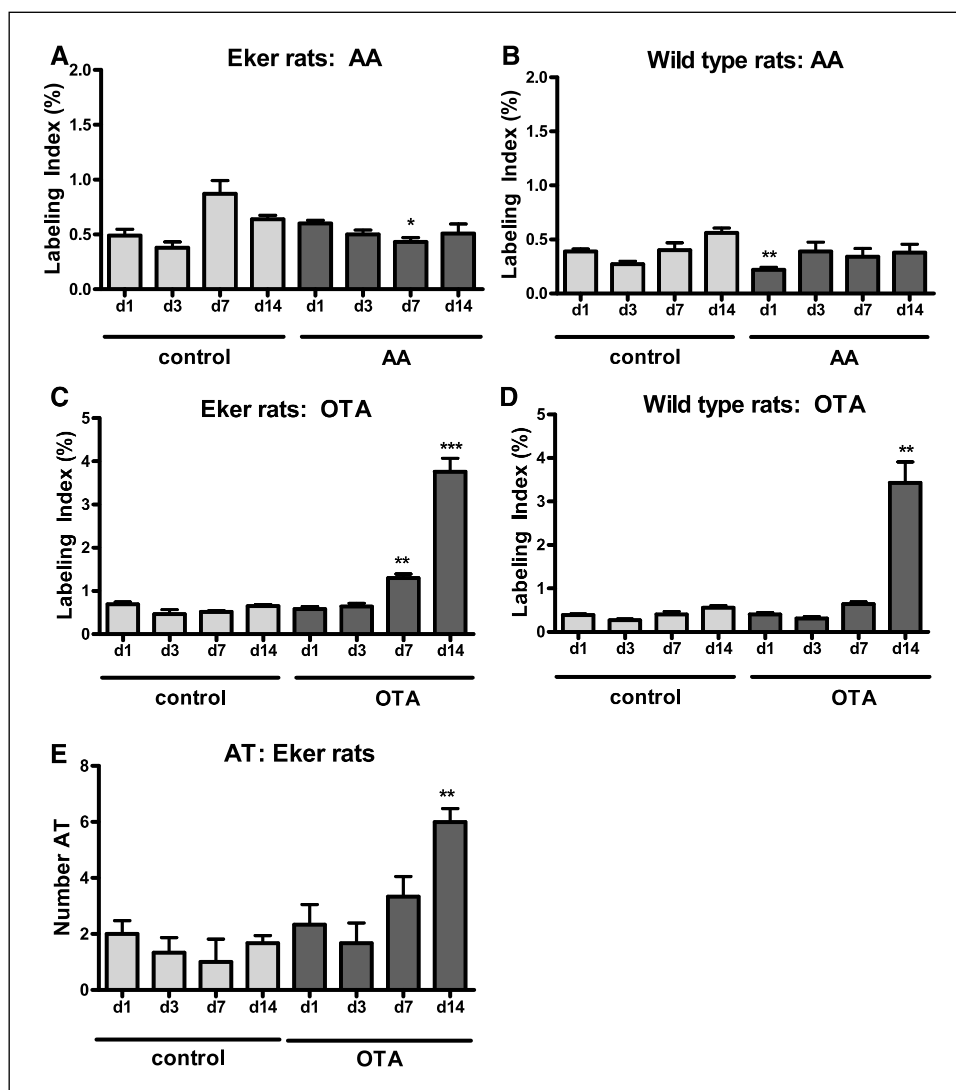

Figure 1. *A* to *D,* comparison of PCNA S-phase labeling indices (LI %) in the renal cortex of AA- or OTA-treated Eker and wild-type rats, respectively, at various treatment time points. *E,* mean number of atypical tubules (*AT*; preneoplastic lesion) per animal in control and OTA-treated Eker rats. *Columns,* mean of $n = 3$ animals per group; *bars,* SE. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, significant differences between treated and time-matched control groups (two-tailed unpaired *t* test).

Genes deregulated by OTA

Biotransformation. OTA treatment down-regulated the expression of several phase I and phase II enzymes and drug transporter genes in both strains (Table 2). Although this effect seemed constant over time for some genes, most deregulated genes showed an enhanced down-regulation with increasing exposure time. Besides the down-regulation of numerous genes coding for components of the biotransformation machinery, OTA treatment resulted in an increased up-regulation of the phase I gene *CYP4A12* in both strains and a consistent up-regulation of phase I *ALDH6A1* in Eker rats.

DNA damage response (including oxidative stress). OTA treatment led to significant up-regulation of *p53* pathway genes in both strains. However, the up-regulation of these genes differed between the two strains. Eker rather than wild-type rats showed an up-regulation of genes known to be involved in oxidative stress responses as well as down-regulation of genes that code for products with extracellular antioxidant activities. The latter genes and the up-regulated *KEAP1*, which suppresses the transactivation of antioxidant responsive elements, were categorized as "enhanced oxidative stress."

Cellular stress. A general stress response, as indicated primarily by the up-regulation of several components of the stress-inducible mitogen-activated protein kinase pathway, was predominantly detectable in Eker rats because the latter genes were not significantly deregulated in wild-type rats.

Inhibited cell survival and proliferation. Predominantly OTA-treated Eker rats presented with an up-regulated expression of tumor suppressor, negative cell proliferation control, and proapoptotic response genes. However, in both strains, an inhibited cell survival response could be inferred from the up-regulation of a proapoptotic gene and the down-regulation of an antiapoptotic and a DNA replication gene.

Enhanced cell survival and proliferation. Numerous genes coding for regulators of cell survival signaling pathways [e.g., the insulin-like growth factor (IGF)-PI3K-PKB pathway], antiapoptosis, mitosis, growth, and proliferation (including proto-oncogenes) were primarily up-regulated in Eker rats, whereas a tumor suppressor gene and a gene coding for a signal cascade inhibitor (*OVCA2*) was down-regulated. In comparison, the latter genes were not or only marginally deregulated in the wild-type rats treated with OTA.

Cell cycle progression and mitosis. Up-regulation of genes, more directly involved in the cell cycle progression, was categorized separately as they seemed as an entity distinct from the category "enhanced cell survival and proliferation." Remarkably, gene deregulation that would further cell cycle progression was exclusively observed in Eker rats.

Cell structure remodeling. Similar to the effects observed for genes involved in enhanced cell survival and proliferation (see above), genes coding for components and regulators of the cytoskeleton altered cell-cell adhesion or communication, and components of the Rac and Rho signaling were almost exclusively up-regulated in Eker rats.

Epithelial-mesenchymal transition/fibrosis. This category includes regulators of cell proliferation, growth factor activation, cell adhesion, and extracellular matrix known to be associated in the process of epithelial-mesenchymal transition (EMT) and/or fibrosis and that could be associated with the progression of renal and urothelial tumors. Such increased expression of components of the transforming growth factor-β (TGF-β) pathway and of a hepatocyte growth factor activator-inhibitor gene in conjunction with a down-regulated expression of extracellular matrix protease genes were predominantly deregulated in Eker rats.

Discussion

As expected for the short-term duration and dose regimen employed (9, 10), neither AA nor OTA induced pronounced nonneoplastic renal pathology in either strain of rats. Although AA-treated rats presented with a slightly higher inflammatory response than the corresponding controls, OTA-treated rats responded with the typical pathologic changes (e.g., karyomegaly, apoptosis, cell shedding, regenerative proliferation, and an inflammatory response in the renal cortex), as reported earlier (10, 25). However, the AA- and OTA-induced nonneoplastic pathology was clearly distinct from one another, and this was also reflected in the cell proliferation assessment (Fig. 1). Lack of overt cell necrosis and subsequent cell shedding and regeneration in the tubuli of AA-treated rats coincided with absence of increased cell proliferation, showing that AA had neither a cytotoxic nor a mitogenic effect on the kidneys of either strain of rats. In contrast,

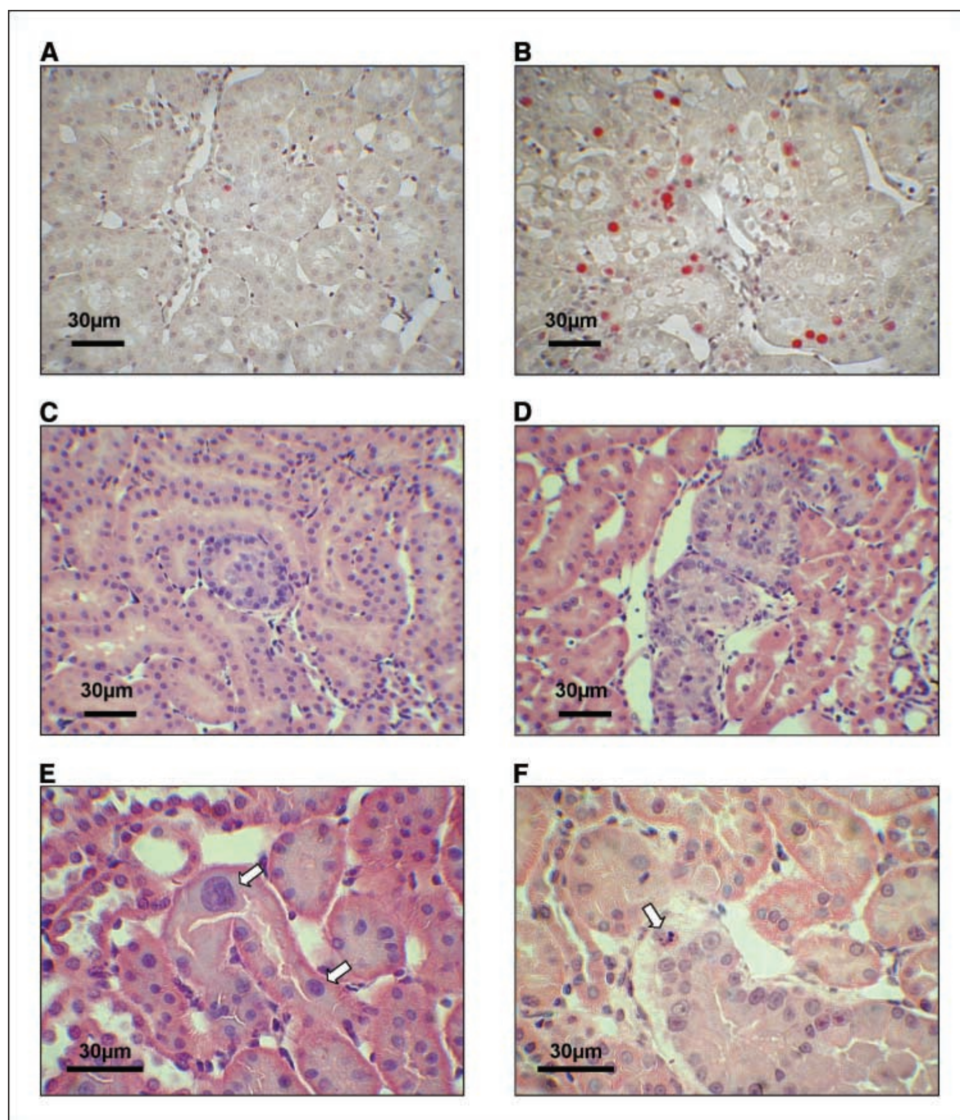

Figure 2. Representative PCNA staining patterns of control (*A*) and OTA-treated (*B*) Eker rats. *C,* atypical tubule. *D,* atypical hyperplasia. Karyomegally (*E*; *arrows*) and apoptotic nuclei (*F*; *arrow*) observed in OTA-treated rats.

OTA-induced tubular toxicity and possibly mitogenic activity were reflected in the overt cell regeneration observed with the histopathologic and immunohistologic assessments. Moreover, the observed effects increased with the duration of treatment and were comparable in extent of effect in both strains. Based on above observations, the age of animals used, and the duration and type of exposure, the expectation was confirmed that no increased prevalence (number of animals affected) or number (per animal) of preneoplastic and neoplastic lesions were to be encountered in the wild-type control and AA-treated rats. Moreover, AA treatment of the arguably more susceptible Eker rats did not increase the prevalence or number of lesions, suggesting that the *Tsc2* mutation was not critical for AA-induced effects. The two carcinomas observed on day 14 in AA-exposed Eker rats cannot be conclusively associated with AA treatment, as adenomas were also observed in the corresponding Eker rat control and AA treatment groups already on day 1 (see Supplementary Table S2). As the number of animals employed for this experiment limited statistical evaluation, a progression of already present adenomas to carcinomas due to AA treatment cannot be excluded.

Similar to AA, OTA treatment was not associated with an increased prevalence or number of lesions in wild-type rats despite the clearly enhanced cell proliferative response in the renal cortex. The latter observation corroborates numerous earlier findings (10, 25), suggesting that non-genotoxic compound-induced renal carcinogenesis can only be observed after a prolonged compound exposure period. In contrast to the situation in the wild type, Eker rats presented with a significantly increased prevalence and number of atypical tubules on day 14 of OTA treatment (Fig. 1*E*; Supplementary Table S2). The latter finding suggests that the increased cell proliferative stimulus (Fig. 1*C*) provided for an enhanced manifestation of the predisposition for renal neoplasia mediated by the *Tsc2* mutation and thus a direct or indirect interaction of OTA with Tsc2 (tuberin) pathway.

At the outset of this experiment, it was assumed that each compound would induce a distinct gene expression profile, which is reflected by the short-term pathology but also displays characteristic genes representative for pathways involved in the compound-specific type of renal carcinogenesis. Consequently, the genotoxic AA was expected to induce a gene expression profile, most likely involving genes of cell cycle arrest and DNA damage repair but not cell proliferation and most likely would not involve *Tsc2*. Indeed, the expression profiles of AA-treated Eker and wild-type rats (Fig. 3) were not distinctly different and were similar to expression profiles obtained with AA in kidneys of Big Blue transgenic F344 rats (24). Several phase I genes were deregulated by AA treatment from the first day of exposure. The gene product of one of the up-regulated phase I genes (*NQOI*) was previously shown to be capable of reducing the nitro group of AAI leading to metabolic activation. *NQOI* up-regulation could, therefore, be at least partly responsible for DNA adduct formation (16). Although DNA adduct formation could not be measured with the study design employed, previously published reports support the assumption that AA DNA adducts are formed (14). Indeed, up-regulation of several p53 pathway genes, including p53 target genes carrying a p53 consensus sequence in the promoter region, was most prominent on days 7 and 14 of exposure, indicating a DNA damage response upon bioactivation of AA. This interpretation is further supported by the fact that several of the p53 target genes (e.g., *MDM2, p21*, or *CCNG1*) have also been shown to be up-regulated in rat liver after short-term exposure to different known genotoxic compounds (22). DNA damage and activation of p53

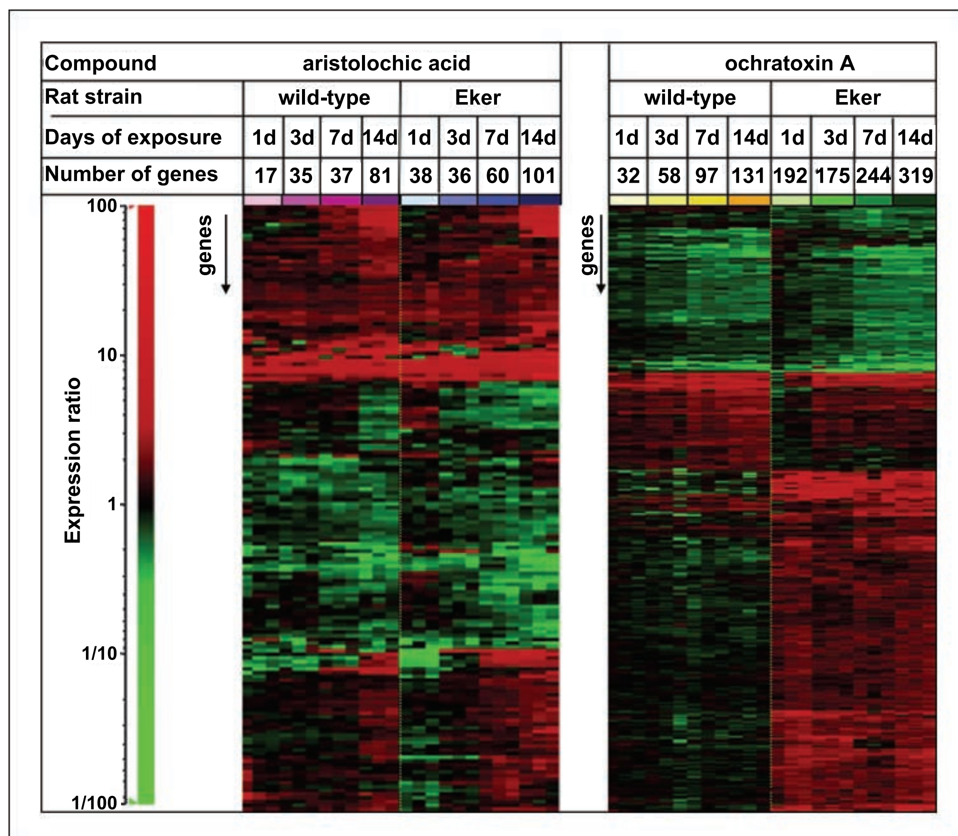

Figure 3. Heat map of compound-specific unions of genes found significantly deregulated (*red*, up-regulated; *green*, down-regulated) from the corresponding control in AA- or OTA-induced renal gene expression profiles of Eker or wild-type rats after 1, 3, 7, or 14 d of treatment (n = 3 per time point). Color scale (*left*): gene expression ratio.

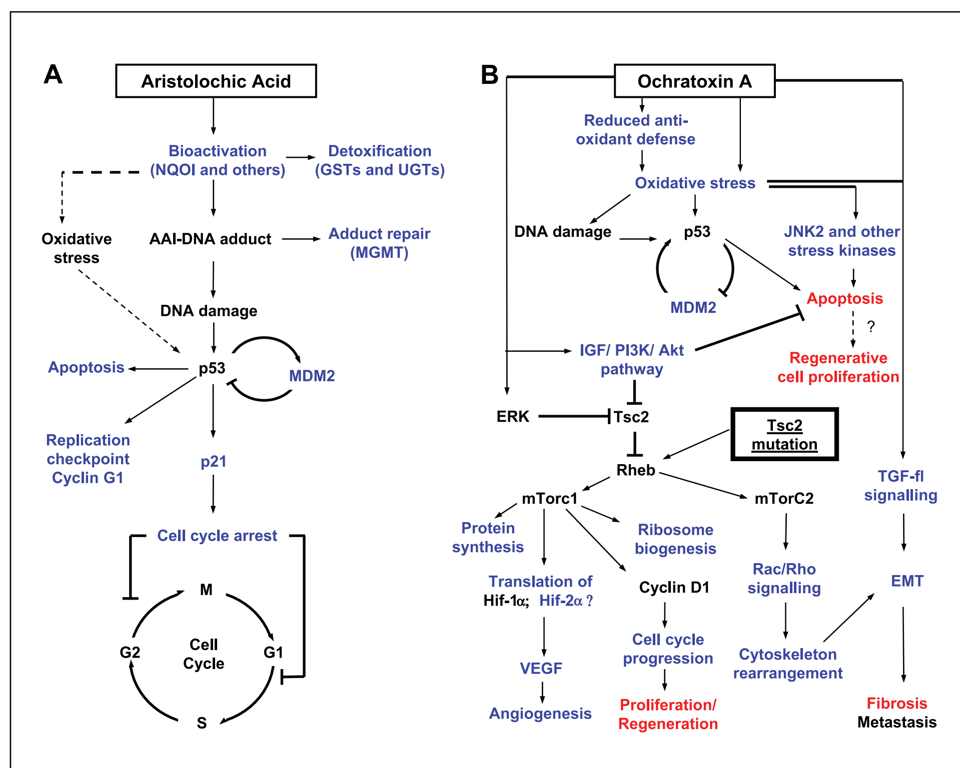

Figure 4. Postulated mechanistic pathways of AA-induced (*A*) and OTA-induced (*B*) toxicity in Eker and wild-type rats and potential influence of Tsc2 in the manifestation of short- and long-term effects discussed. *Black,* pathways and processes suggested from the literature; *blue,* pathways and processes implicated by gene expression analysis; *red,* pathways and processes implicated by histopathology and corroborated by gene expression analysis.

pathway genes are expected to result in cell cycle arrest (Fig. 4*A*) followed by damage repair or programmed cell death (26). AA treatment led to the deregulation of several genes involved in apoptosis, with a comparable time profile to the p53 pathway and target genes. Although cell cycle components are predominantly regulated on the protein level, down-regulation of genes crucial for the G_2-M transition (e.g., *CDC2, Cyclin B, TOME1*, and *CKS2*) and down-regulation of genes required for mitotic spindle formation, like *TUBA1* or *HMMR*, suggest a G_2 arrest. Deregulation of the latter genes in wild-type rats only may be explained by 12-fold lower PI3K mRNA expression compared with Eker rats (27). Increasing evidence suggest that constitutive activation of the PI3K pathway could lead to defects in DNA damage checkpoint control (28). Consequently, AA-induced G_2-M arrest would, therefore, not be readily detectable in Eker rats, although they responded with up-regulation of proapoptotic genes as well as down-regulation of *Ki-67*, an observation also supported by the cell proliferation and pathologic analysis (Fig. 1*A* and *B*).

In contrast to AA, the gene expression profiles of OTA-treated Eker and wild-type rats were distinctly different (Fig. 3). However, in both strains, up-regulation of *CYP4A12* and down-regulation of other phase I and II genes could provide for increased generation of reactive oxygen species and enhanced oxidative DNA damage (19, 20), which could lead to cellular damage and regenerative cell proliferation. Indeed, enhanced proximal tubular damage and cell regeneration/proliferation (Fig. 1*C* and *D*) was observed in both rat strains, corroborating the above interpretation. OTA-induced oxidative stress and ensuing DNA damage in combination with enhanced cell proliferation could increase the likelihood of neoplastic transformation (29). The presence of oxidative DNA damage, as also suggested by earlier findings (30–32), is supported by the up-regulation of the p53 pathway genes *SUPT16H* in both strains, *MDM2* and *CHEK2* in Eker rats, and *RBBP6* in wild-type rats as well as by the time-dependent down-regulation of *HSP 40-3, CN1, MSRA*, and *MGST1*, responsible for the protection of cells against oxidative stress. Indeed, recent findings showed that overexpression of glia maturation growth factor β (*GMFB*) resulted in reduced antioxidant enzyme activities, subsequent accumulation of H_2O_2, and finally enhanced oxidative injury of renal proximal tubular cells (33). The 5.9-fold up-regulation of *GMFB* in Eker rats, shown here, and the known reduced 8-oxoguanine-DNA glycosylase expression in Eker rats (34) may suggest that Eker rats are more susceptible to oxidative stress than wild-type rats. This interpretation is supported by the observation that Eker rats responded with increased cell proliferation already on day 7 (Fig. 1*C*) and increased formation of atypical tubules (Fig. 1*E*). However, OTA-induced regenerative proliferation may not have been the sole contributor to the propagation of preneoplastic lesions. Indeed, OTA has previously been assumed to have mitogenic properties (35, 36), a hypothesis supported by down-regulation of *IGFBP-4*, a negative regulator of the (IGF)-PI3K-AKT mitogenic pathway, in Eker and wild-type rats. Despite this, only Eker rats showed a significant increase of preneoplastic lesions. The latter can be explained with the renal gene expression profile of OTA-treated Eker rats that contains deregulated genes characteristic of the most pertinent hallmarks involved in cancer progression (37): (*a*) the evasion of programmed cell death via up-regulation of antiapoptotic genes (e.g., *PDC4, PEA15*, or *MCL1*); (*b*) insensitivity to growth inhibitory signals via up-regulation of proto-oncogenes (e.g., *MERTK, SMOH, V-KIT*, and *TMP*); (*c*) self-sufficiency in growth signals [e.g., via an activated (IGF)-PI3K-AKT pathway (*PIK3CB, AKT1S1, AKT2*, or *SBF1*)]; (*d*) limitless replication potential [e.g., via an up-regulation of genes involved in DNA replication (*TOP1*), chromatin remodeling (*SMARCA4*), or mitotic spindle formation (*MAPRE1, AJUBA, NEK9*)]; (*e*) sustained angiogenesis via up-regulation of *VEGF, VEZF1*, and *ANGPTL2*; and (*f*) tissue invasion

and metastasis via a broad set of deregulated genes involved in cell structure remodeling (e.g., Rho guanine nucleotide exchange factor *ARHGEF11* or *IQGAP1*, an effector for CDC42 and RAC1) and EMT (*TGFβR-II, SMADs, CD44*, and *TIMP3*).

As shown, OTA treatment of Eker rats led to the expression of genes that could be intricately involved in, or are the result of, activated mTOR signaling (38). Thus, OTA treatment and the *Tsc2* mutation may have acted in concert or separately on the (IGF)-PI3K-AKT pathway, thus resulting in a co-joint activation of mTOR signaling. Additional stimulation of the mTOR pathway could be possible by OTA-mediated extracellular signal-regulated kinase (ERK) activation (35). Tsc2 was shown to be a direct substrate of ERK (39). As Eker rats exhibit only one functional allele of the *Tsc2* gene, OTA-mediated ERK-dependent inactivation of Tsc2 could lead to more drastic and therefore earlier detection of proliferative effects and neoplastic transformation in Eker rats (Fig. 4*B*).

In summary, gene expression profile comparisons with histopathologic findings from AA- and OTA-treated Eker and wild-type rats discussed here highlight that gene expression analysis subsequent to short-term *in vivo* assays may have the potential to identify deregulated genes involved in compound- and strain-specific pathology. Moreover, deregulation of genes, for which a similar direction of deregulation has been reported for various types of cancers, suggests that pathways linked to tumorigenesis may be deregulated already after short-term carcinogen exposure. Whether these changes in gene expression are transient or can be causally linked to a compound-specific tumorigenicity cannot be determined without gene expression profile analysis of the respective preneoplastic and neoplastic lesions in rats chronically treated with AA or OTA. This analysis, as currently carried out in this laboratory using laser-capture microdissection (40), will provide further information as to the relevance of the pathways identified in short-term experiments for the understanding of the mechanisms underlying AA- and OTA-induced renal carcinogenesis.

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