以上の結果、NFT の腎発がん機序には遺伝毒性メカニズムの関与が強く示唆され、ヒトに対しても潜在的なリスクを有する可能性があると考えられた。また、NFT の遺伝毒性発現機序と *p53* 遺伝子の関連性を検索するため、*p53* 遺伝子欠損およびその野生型 *gpt* delta マウスを用い NFT、AHD および NFA の *in vivo* 変異原性試験を行うための予備試験を実施した。腎臓における *gpt* assay の結果、両遺伝子型において NFT 投与群で *gpt* MF が増加する傾向が認められたが、遺伝子型の違いによる変化は認められなかった。今後は今回得られた結果をもとに本試験を実施し、更に詳細な検討を行う予定である。

4. 牛脊柱からの背根神経節の除去に関する研究

第 1 頚神経から第 9 胸神経の背根神経節が除去されやすいのは、硬膜から背根神経節までの背根の長さが短いことと、背根神経節がある程度大きいことに起因すると思われる。

E. 結論

1. 巨大核出現に関与する標的遺伝子の探索

ラット 28 日間反復投与試験の枠組みで、発がん標的細胞が増殖亢進を示した発がん物質に共通して、M 期異常を反映すると考えられる核局在 Cdc2、Aurora B 及び p-Histone H3 陽性細胞が有意に増加する分子発現変化を見出した。また、G_1/S 期の $p21^{Cip1}$ と M 期分子である HP1α が標的臓器により異なる反応性を示した。次いで、異なる発がん標的に対する発がん過程促進早期におけるこれらの分子の反応性を検索した結果、増殖病変内で M 期異常を反映する分子発現変化を示した。このことは、これらの分子は、増殖性病変の形成にも関与することを示唆し、亜急性毒性試験の枠組みの中で、発がん物質の早期スクリーニング指標となり得る可能性を示唆した。

2. CYP1A inducer の複合投与によるラット肝発がんイニシエーション・プロモーション作用の修飾

CYP1A inducer である OPZ 及び BNF を併用投与することで、ラットにおいて肝発がんプロモーション作用が増強されることが明らかになった。しかし BNF と CYP1A/2B inducer である PBO、または CYP1A inducer である I3C と CYP2B/1A inducer である PB と併用した場合には、明らかな増強作用は示さなかった。加えて、CYP2B inducer 同士である PB と ORPH の併用投与では、ラットにおいて肝発がんプロモーション作用が増強されることが明らかとなった。これらのことから、誘導する CYP が同じ化合物同士の併用投与ではそのラット肝発がんプロモーション作用が増強することが示唆された。今後、発がんプロモーションに対する ROS の関与や、誘導する CYP family を考慮し、様々な組み合わせにおける CYP inducer 併用の肝発がんに対する影響について更なる検討が必要である。

3. ニトロフラン類の安全性評価法の確立

NFT はラット腎臓に対して *in vivo* 変異原性を有し、その機序に酸化的 DNA 損傷の関与の可能性が明らかとなった。また、雄ラット特有尿中蛋白 α_{2u}-globulin 沈着により生じる細胞増殖活性の亢進がその発がん機序に大きく寄与していると考えられた。一方、発がん性の認められない雌ラットにおいても同様に *in vivo* 変異原性を有することが明らかとなり、ヒトに対しても潜在的な発がんリスクを有する可能性があると考えられた。

4. 牛脊柱からの背根神経節の除去に関する研究

背根神経節の脊柱からの除去率の平均は 92%であったが、除去率の極端に低い第 10 胸神経以降の背根神経節の除去率が向上しない限り、前方 3/4 の背根神経節の完全除去達成は困難である。

牛の脊柱をゼラチンや牛エキスの原材料として利用するには、背根神経節の完全除去が必須だが、現時点ではその目標に達しておらず、更なる技術の改良が必要である。

F. 健康危機情報

特になし

G. 研究発表

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H. 知的財産権の出願・登録状況

１．特許所得

なし

２．実用新案登録

なし

３．その他

なし

Table 1.
Sequence of primers used for real-time RT-PCR analysis

Accession no.	Symbol	Forward primer [a]	Reverse primer
NM_130812	*Cdkn2b*	CCCTCACCAGACCTGTGCAT	CAGGCGTCACACACATCCA
NM_080782	*Cdkn1a*	ACCAGCCACAGGCACCAT	CGGCATACTTTGCTCCTGTGT
NM_171992	*Ccnd1*	GCGAGCCATGCTTAAGACTGA	CCCTCTGCACGCACTTGA
NM_053702	*Ccna2*	TGTCTCTGGTGGGTTGAGAAGA	ACCACAGCATGCCCAACAG
XM_001064075	*Ccne2*	TCTCCACAAGAAGCCCAGATAATT	GGTGATCTCCTCTGTTCTTTTTTTG
NM_001025682	*Cdr2*	CAAGGCCTCACAGCAGAAAATC	GAGGTGATCAATGTTGGTTTGC
NM_001012742	*Wee1*	CGGCAAACTCCTCAAGTGAATATT	CACTGTCCTGAGGAATGAAGCAT
NM_001107790	*Tpx2*	CCCAAGAGACCACCTGTTAAGC	ACTCTCGCTCATGAATTCGTTTCT
NM_024127	*Gadd45a*	CACCATAACTGTCGGCGTGTA	GGCACAGGACCACGTTGTC
NM_019296	*Cdk1*	GGTCGCCAGAGGTGTTGCT	TCTGCAAATATGGTCCCTATGCT
NM_171991	*Ccnb1*	TGTCCCACACGGAAGAATCTCT	GGCCACGGTTCACCATGA
NM_053749	*Aurkb*	CGGATGCATAATGAGATGGTAGAT	TCCCCACCATCAGTTCATAGC
NM_153296	*Aurka*	AAGAGAGTCATCCACAGAGACATCAA	CGATCTTCAACTCCCCATTTG
NM_030989	*Tp53*	CATGAGCGTTGCTCTGATGGT	GATTTCCTTCCACCCGGATAA
NM_001108099	*Mdm2*	GAAGGAGGACACACAAGACAAAGA	ATGGCTCGATGGCGTTCA
NM_001011991	*Ndrg1*	GTCACACCTTGTCTCCCATTATTG	CCAGGTGAGAGACATTCAGTTATCA
NM_031642	*Klf6*	GCGCCATCCAGTTTGCAT	GATCAGGAGTCGGAGCAGAAA
NM_031144	*Actb*	CCCTGGCTCCTAGCACCAT	AGAGCCACCAATCCACACAGA

Aurk, aurora kinase; Actb, actin beta; Ccn, cyclin; Cdk, cyclin-dependent kinase; Cdkn, cyclin-dependent kinase inhibitor; Cdr2, cerebellar degeneration-related 2; Gadd45a, growth arrest and DNA-damage-inducible, alpha; Klf6, Kruppel-like factor 6; Mdm2, p53 binding protein homolog (mouse); Ndrg1, N-myc downstream regulated 1; RT-PCR, reverse transcription polymerase chain reaction; Tp53, tumor protein p53; Tpx2, microtubule-associated, homolog (*Xenopus laevis*); Wee1, wee 1 homolog (*S. pombe*).
[a] The primer sets were designed using the Primer Express® software (Version 3.0; Applied Biosystems Japan Ltd.).

Table 2.
Antibodies used in the present study

Antigen	Host species	Clonality	Dilution	Antigen retrieval[a]	Manufacturer (City, State, Country)
Ki-67	Mouse	Monoclonal	1:50	Autoclaving	Dako (Glostrup, Denmark)
Cdc2 p34	Mouse	Monoclonal	1:100	None	Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA)
Histone H3 (Ser 10 phosphorylated)	Rabbit	Polyclonal	1:50	Autoclaving	Santa Cruz Biotechnology, Inc.
HP1α	Rabbit	Polyclonal	1:200	Microwaving	Cell Signaling Technology, Inc. (Danvers, MA, USA)
Aurora B	Rabbit	Polyclonal	1:200	None	Abcam (Cambridge, UK)
Incenp	Rabbit	Polyclonal	1:500	Autoclaving	Abcam
p53	Rabbit	Polyclonal	1:100	Autoclaving	Santa Cruz Biotechnology, Inc.
$p21^{Cip1}$	Mouse	Monoclonal	1:100	Microwaving	Abcam
$p27^{Kip1}$	Rabbit	Polyclonal	1:100	None	Cell Signaling Technology, Inc.
$p16^{Ink4a}$	Mouse	Monoclonal	1:100	None	Santa Cruz Biotechnology, Inc.
Wee1 (Ser 53 phosphorylated)	Rabbit	Polyclonal	1:100	Microwaving	Assay Biotechnology Co. Inc. (Sunnyvale, CA, USA)

[a] Antigen retrieval was applied to immunohistochemistry. Retrieval conditions were either autoclaving at 121°C for 10 min or by microwaving at 90 °C for 10 min in 10 mM citrate buffer (pH 6.0).

Table 3.

Primers used for real-time RT-PCR

Accession no.	Gene description	Symbol	Forward primer	Reverse primer
NM_012540	Cytochrome P450, family 1, subfamily a polyoeptide 1	*Cyp1a1*	gccttcacatcagccacaga	ttgtgactctaaccacccagaatc
NM_012541	Cytochrome P450, family 1, subfamily a polyoeptide 2	*Cyp1a2*	aagcgccggtgcattg	tgcaggaggatggctaagaag
NM_012940	Cytochrome P450, family 1, subfamily b, polypeptide 1	*Cyp1b1*	cttggccattgatcggaaa	caaggcgagcgaagtacaaagt
NM_001198676	Cytochrome P450, family 2, subfamily b, polypeptide 2	*Cyp2b2*	gggacactgaaaaagagtgaagct	aatgccttcgccaagacaaat
NM_022407	Aldehyde dehydrogenase 1 family, member A1	*Aldh1a1*	agtgccccttcggtggat	gctcagtgtactcataaagaccatgtt
NM_031972	Aldehyde dehydrogenase 3 family, member A1	*Aldh3a1*	tggagcctcatcctggcttat	gaatttggaggagtgaggtgaga
NM_017000	NAD(P)H dehydrogenase, quinone 1	*Nqo1*	tccgcccccaacttctg	tctgcgtgggccaataca
NM_001039691	UDP glucuronosyltransferase 1 family, polypeptide A6	*Ugt1a6*	tggctaccccaaaacgatct	ataccatgggaaccggagtgt
NM_001024285	Aryl-hydrocarbon receptor repressor	*Ahrr*	gctgctggagtctctcaatgg	gcccaggtagtccacaattgtt
NM_013215	Aldo-keto reductase family 7, member A3	*Akr7a3*	ccgcttctttgggaatccat	ggcgatgccattgaagtgt
NM_183403	Glutathione peroxidase 2	*Gpx2*	accgatcccaagctcatcat	tctcaaagttccaggacacatctg
NM_001159739	Glutathione S-transferase Yc2 subunit	*Yc2*	aagctgagcagggctgatgt	acaatgcctgggtccatctc
NM_012600.2	Malic enzyme 1, NADP(+)-dependent, cytosolic	*Me1*	cgaccagcaaagctgagtgtt	ctgccgctggcaaagatc
NM_017232	Cyclooxygenase-2	*Cox-2*	ttcgacttttccaggatggaa	gagtgtctttgactgtgggaggat
NM_012620	Serpin peptidase inhibitor, clade E , member 1	*Serpine1*	tggctcagaacaacaagttcaac	ggcagttccaggatgtcgtact
NM_053963	Matrix metallopeptidase 12	*Mmp12*	gcgaggctgacattacgatactt	taaggtaccacctttgccatca
NM_012589	Interleukin 6	*Il6*	cccaccaggaacgaaagtca	cttgcggagagaaacttcatagc
NM_130752	Fibroblast growth factor 21	*Fgf21*	gccaacaaccagatggaactc	tccttaagcagcagctctctga
XM_342346	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	*Nfkb1*	gaagtacagaggaaacgcaccagaag	ccgccgccgaaactg
NM_001105720	Rattus norvegicus nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	*Nfkbia*	gcctagccccgagcattc	aatgatctgtttccccaaatttca
NM_012675	Tumor necrosis factor	*Tnf*	acaaggctgccccgactat	ctcctggtatgaagtggcaaatc
NM_053677	Checkpoint kinase 1	*Chek1*	tggcagctggcaaagga	aatcccagtcttccacaaaagg
NM_001012742	Wee 1 homolog (S. pombe)	*Wee1*	cggcaaactcctcaagtgaatatt	cactgtcctgaggaatgaagcat
NM_012603	Myelocytomatosis oncogene	*Myc*	cgcctctgggaaactttgc	tcctggctcgcagattgtaa
NM_031144	Actin, beta	*Actb*	ccctggctcctagcaccat	agagccaccaatccacacaga

Table 4.

Final body and liver weights of rats after 28-day treatment with hepatocarcinogens or hepatotoxicants

Group	No. of animals examined	Final body weight (g)	Liver weight	
			Absolute (g)	Relative (g/100g BW)
Untreated controls	10	210 ± 16 [a]	7.93 ± 0.87	3.68 ± 0.17
TAA	10	141 ± 13**	7.08 ± 0.87*	4.94 ± 0.24**
FB	10	208 ± 14	8.62 ± 0.68	4.04 ± 0.14**
PBO	10	164 ± 10**	11.89 ± 0.77**	7.16 ± 0.18**
MEG	10	175 ± 13**	10.09 ± 0.89**	5.68 ± 0.31**
APAP	10	173 ± 9**	7.17 ± 0.55*	4.07 ± 0.15**
ANIT	10	111 ± 5**	6.43 ± 0.44**	5.70 ± 0.21**

[a] Mean ± SD

* $P < 0.05$ versus untreated controls.

** $P < 0.01$ versus untreated controls.

Table 5.

Representative cell cycle-related genes with known functional annotations that were upregulated in the livers of rats treated with TAA (≥2-fold)

Accession No.	Gene title	Symbol	TAA
XM_001054052	Anaphase promoting complex subunit 4	Anapc4	2.30
NM_153296	Aurora kinase A	Aurka	2.22
NM_053749	Aurora kinase B	Aurkb	4.03
XM_001080790	Cancer susceptibility candidate 5	Casc5	2.71
NM_053702	Cyclin A2	Ccna2	2.26
NM_171991	Cyclin B1	Ccnb1	4.16
NM_001009470	Cyclin B2	Ccnb2	2.45
NM_171992	Cyclin D1	Ccnd1	2.54
XM_001077331	Cyclin E1	Ccne1	3.80
XM_001064075	Cyclin E2	Ccne2	3.94
NM_012923	Cyclin G1	Ccng1	6.18
NM_019296	Cyclin-dependent kinase 1	Cdc2	2.52
XM_001068286	Cell division cycle associated 2	Cdca2	5.10
NM_001007648	Cell division cycle associated 3	Cdca3	2.93
NM_001025693	Cell division cycle associated 7	Cdca7	7.72
NM_001025050	Cell division cycle associated 8	Cdca8	2.92
NM_001012035	Cyclin-dependent kinase-like 2 (CDC2-related kinase)	Cdkl2	2.06
NM_080782	Cyclin-dependent kinase inhibitor 1A	Cdkn1a	5.96
NM_130812	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	Cdkn2b	2.52
NM_001025682	Cerebellar degeneration-related 2	Cdr2	5.90
XM_001069485	Centromere protein A	Cenpa	2.72
XM_001077739	Centromere protein E	Cenpe	2.78
NM_001008366	Centromere protein N	Cenpn	3.60
NM_001014215	Centromere protein Q	Cenpq	2.28
NM_001025646	Centrosomal protein 55	Cep55	2.20
NM_001017470	Centrosomal protein 70	Cep70	2.88
XM_001067027	Centrosomal protein 76	Cep76	3.34
XM_001076228	Centrosomal protein 135	Cep135	2.58
NM_080400	CHK1 checkpoint homolog (S. pombe)	Chek1	2.60
XM_001058264	Claspin homolog (Xenopus laevis)	Clspn	2.29
XM_001073486	Discs, large (Drosophila) homolog-associated protein 5	Dlgap5	2.68
XM_001070442	Excision repair cross-complementing rodent repair deficiency complementation group 6-like	Ercc6l	2.11
XM_001067790	Extra spindle poles like 1 (S. cerevisiae)	Espl1	2.30
XM_001065873	F-box protein 5	Fbxo5	2.06
XM_001075601	Fizzy/cell division cycle 20 related 1 (Drosophila)	Fzr1	2.20
NM_024127	Growth arrest and DNA-damage-inducible, alpha	Gadd45a	2.54
NM_001008321	Growth arrest and DNA-damage-inducible, beta	Gadd45b	2.73
XM_001078275	G-2 and S-phase expressed 1	Gtse1	14.53
XM_001074188	Inner centromere protein	Incenp	3.37
XM_001065116	Kinesin family member 2A	Kif2a	4.79
NM_001085369	Kinesin family member 2C	Kif2c	2.10
XM_001061764	Kinesin family member 20A	Kif20a	2.43
XM_001070728	Minichromosome maintenance complex component 3	Mcm3	2.94
XM_001068436	Similar to mcdc21 protein; minichromosome maintenance complex component 4	Mcm4	2.50
NM_012603	Myelocytomatosis oncogene	Myc	3.30
XM_001055166	NIMA (never in mitosis gene a)-related expressed kinase 2	Nek2	2.07
NM_182953	NIMA (never in mitosis gene a)-related kinase 6	Nek6	2.34
NM_177931	Origin recognition complex, subunit 1-like (yeast)	Orc1l	2.10
NM_199092	Origin recognition complex, subunit 4-like (yeast)	Orc4l	2.53
NM_001033690	Origin recognition complex, subunit 6 like (yeast)	Orc6l	2.19
NM 017198	p21 protein (Cdc42/Rac)-activated kinase 1	Pak1	2.90
NM_017100	Polo-like kinase 1 (Drosophila)	Plk1	3.24
NM_031821	Polo-like kinase 2 (Drosophila)	Plk2	3.41
NM_001007754	Ras association (RalGDS/AF-6) domain family member 1	Rassf1	2.43
XM_001055763	Retinoblastoma-like 1 (p107)	Rbl1	2.60
XM_001077474	SPC24, NDC80 kinetochore complex component, homolog (S. cerevisiae)	Spc24	4.70
NM_001009654	SPC25, NDC80 kinetochore complex component, homolog (S. cerevisiae)	Spc25	5.73
NM_022183	Topoisomerase (DNA) II alpha	Top2a	2.20
NM_001107790	TPX2, microtubule-associated, homolog (Xenopus laevis)	Tpx2	3.18
NM_001012742	Wee 1 homolog (S. pombe)	Wee1	6.38
NM_019376	Tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, gamma polypeptide	Ywhag	2.30

Values are fold change with the expression level in the untreated control group set as 1.

Table 6.
Validations in transcript levels measured by real-time RT–PCR in the livers of rats treated with TAA or FB

Gene symbol	Real-time RT-PCR normalized by *Actb*	
	TAA [a]	FB [a]
Cdkn2b	4.32 ± 0.20 [b,**]	2.03 ± 0.57**
Cdkn1a	3.49 ± 1.02**	2.32 ± 0.69**
Ccnd1	2.73 ± 0.52**	1.79 ± 0.48*
Ccna2	3.06 ± 0.61**	1.56 ± 0.52
Ccne2	6.60 ± 2.11**	1.48 ± 0.33*
Cdr2	7.94 ± 2.01**	1.44 ± 0.25*
Wee1	4.99 ± 2.83*	1.18 ± 0.54
Tpx2	3.38 ± 0.28**	1.16 ± 0.28
Gadd45a	2.76 ± 0.80**	1.60 ± 0.62
Cdk1	2.69 ± 0.61**	1.64 ± 0.44*
Ccnb1	2.28 ± 0.59**	1.57 ± 0.50
Aurkb	2.75 ± 0.65**	1.34 ± 0.21
Aurka	2.85 ± 0.42**	1.27 ± 0.27

Aurk, aurora kinase; Ccn, cyclin; Cdk, cyclin-dependent kinase; Cdkn, cyclin-dependent kinase inhibitor; Cdr2, cerebellar degeneration-related 2; Gadd45a, growth arrest and DNA-damage-inducible, alpha; Tpx2, microtubule-associated, homolog (*Xenopus laevis*); Wee1, wee 1 homolog (*S. pombe*).
[a] Numbers of animals examined were 6 in each group.
[b] Mean ± SD
* $P < 0.05$ versus untreated controls.
** $P < 0.01$ versus untreated controls.

Table 7.
Quantitative data for GST-P$^+$ liver cell foci after promotion with PBO or MP for 6 weeks

Group	No. of animals examined	GST-P$^+$ foci	
		Number (No./cm^2)	Area (mm^2/cm^2)
DEN-alone	11	5.57 ± 1.63[a]	0.31 ± 0.15
DEN + PBO 20,000 ppm	10	13.80 ± 2.62**	1.85 ± 0.40**
DEN + MP 1,000 ppm	11	26.72 ± 6.75**	1.96 ± 0.72**

[a] Mean ± SD.
** $P < 0.01$ vs. the DEN-alone group (Tukey's or Steel-Dwass multiple comparison test).

Table 8.
Quantitative data for p-Erk1/2$^+$ FFCHs after promotion with SDM for 4 weeks

Group	No. of animals examined	p-Erk1/2$^+$ FFCHs	
		Number (No./mm^2)	Area (μm^2/1,000 μm^2)
DHPN-alone	12	0.01 ± 0.03[a]	0.01 ± 0.02
DHPN + SDM 1,500 ppm	12	0.68 ± 0.27**	8.50 ± 3.77**

[a] Mean ± SD.
** $P < 0.01$ vs. the DHPN-alone group (Student's or Welch's *t*-test).

Table 9.

Final body and liver weights, food intake, BNF intake, number/area of GST-P positive foci, PCNA positive cells, ROS production and TBARS in the liver of male F344 rats given OPZ and/or BNF for 6 weeks after DEN treatment

Final body weight (g)	DEN-alone	Low OPZ	High OPZ	Low BNF	High BNF	OPZ+BNF
No. of animals examined	11	12	12	9	12	11
Final body weight (g)	278.13 ± 9.65	258.86 ± 11.93$^{**,\#\#}$	255.59 ± 14.83$^{**,\#\#}$	267.62±11.69$^{\#\#}$	260.68 ± 14.79$^{**,\#\#}$	233.01 ± 14.97**
Absolute liver weight (g)	8.25 ± 0.40	9.32 ± 0.67$^{**,\#\#}$	10.17 ± 0.84$^{**,\#\#}$	10.44±0.91$^{**,\#}$	11.23 ± 0.89**	11.98 ± 1.40**
Relative liver weight (g% body weight)	2.97 ± 0.10	3.60 ± 0.22$^{**,\#\#}$	3.97 ± 0.15$^{**,\#\#}$	3.90±0.22$^{**,\#\#}$	4.30 ± 0.18$^{**,\#\#}$	5.14 ± 0.53**
Average food intake (g/kg body weight/day)	14.81 ± 1.12	14.48 ± 1.20	13.81 ± 1.10	14.11±1.43	14.17 ± 1.58	13.50 ± 2.04
Average BNF intake (g/kg body weight/day)	-	-	-	0.08±0.01	0.17 ± 0.01	0.09 ± 0.01
GST-P positive foci (≥0.2 mm)						
Numbers (number/cm2)	3.18 ± 1.93	4.47 ± 1.65$^{\#\#}$	5.78 ± 2.18$^{*,\#\#}$	7.05±1.45$^{**,\#\#}$	7.30 ± 2.45$^{**,\#\#}$	10.66 ± 2.76**
Areas (mm2/cm2)	0.20 ± 0.12	0.37 ± 0.27$^{\#\#}$	0.42 ± 0.20$^{*,\#\#}$	0.57±0.20**	0.58 ± 0.31**	0.82 ± 0.34**
PCNA-positive cells (%)	0.29 ± 0.18	0.25 ± 0.19$^{\#}$	0.41 ± 0.18	0.36±0.18	0.39 ± 0.22	0.64 ± 0.41*
No. of animals examined	5	5	5	5	5	5
ROS production (%)						
+NADPH	100.00 ± 16.72	75.19 ± 5.91**	71.77 ± 10.81**	91.12±6.52	76.22 ± 15.32**	68.32 ± 7.55**
+NADPH+SKF-525A	48.55 ± 7.32	42.74 ± 5.43	45.23 ± 8.35	43.77±5.24	47.25 ± 6.76	43.17 ± 4.94
-NADPH	21.36 ± 2.54	21.36 ± 1.10	21.24 ± 1.08	21.91±2.05	17.00 ± 9.59	20.96 ± 3.14
No. of animals examined	6	6	6	6	6	6
TBARS (nmol MDA/mg protein)	0.97 ± 0.08	0.99 ± 0.06	1.00 ± 0.05	1.00±0.04	1.01 ± 0.12	0.93 ± 0.07

DEN: *N*-diethylnitrosamine, Low OPZ: 138 mg/kg Omeprazole, High OPZ: 276 mg/kg Omeprazole, Low BNF: 0.125% β-naphthoflavone, High BNF: 0.25% β-naphthoflavone, and OPZ+BNF: 138 mg/kg Omeprazole + 0.125% β-naphthoflavone. The data represent mean ± S.D.

*, ** significantly different from the DEN-alone group at $p < 0.05$ or $p < 0.01$, respectively.

$^{\#,\#\#}$ significantly different from the OPZ+BNF group at $p < 0.05$ or $p < 0.01$, respectively.

Table 10.

Real-time RT-PCR analysis of the liver tissues from male F344 rats given OPZ and/or BNF for 6 weeks after DEN treatment

Group	DEN-alone	Low OPZ	High OPZ	Low BNF	High BNF	OPZ+BNF
AhR-regulated genes						
Cyp1a1	1.19 ±0.67	158.19 ± 197.79$^{**,\#\#}$	138.19 ± 120.20$^{**,\#\#}$	2842.87 ± 824.82**	3357.84 ± 831.00**	3138.85 ± 579.83**
Cyp1a2	1.01 ±0.18	6.50 ± 1.95$^{**,\#\#}$	6.93 ± 2.83$^{**,\#\#}$	27.79 ± 4.53$^{**,\#\#}$	42.91 ± 6.37**	52.03 ± 11.37**
Cyp1b1	1.06 ±0.38	4.04 ± 2.15$^{**,\#\#}$	3.28 ± 1.15$^{*,\#\#}$	188.10 ± 50.95$^{**,\#\#}$	706.52 ± 297.60**	1117.09 ± 263.51**
Cyp2b2	1.05 ±0.34	5.17 ± 1.26$^{**,\#\#}$	6.66 ± 2.57$^{**,\#\#}$	0.66 ± 0.13	0.53 ± 0.17*	0.92 ± 0.40
Aldh1a1	1.08 ±0.52	3.50 ± 0.92**	3.67 ± 1.45*	1.25 ± 0.12$^{\#\#}$	1.98 ± 0.39*	2.30 ± 0.64*
Aldh3a1	1.23 ±0.80	1.80 ± 1.02$^{\#\#}$	1.32 ± 0.54$^{\#\#}$	2.56 ± 0.89$^{\#\#}$	90.70 ± 66.77$^{**,\#\#}$	860.74 ± 361.47**
Nqo1	1.03 ±0.29	2.54 ± 0.88$^{**,\#\#}$	2.81 ± 0.75$^{**,\#\#}$	5.40 ± 0.58$^{**,\#\#}$	10.10 ± 2.85$^{**,\#}$	16.86 ± 3.37**
Ugt1a6	1.03 ±0.25	1.86 ± 0.35$^{**,\#}$	1.71 ± 0.50$^{*,\#\#}$	1.62 ± 0.18$^{*,\#\#}$	2.45 ± 0.54$^{**,\#}$	3.85 ± 0.86**
Ahrr	2.62 ±4.31	7.52 ± 3.70$^{\#\#}$	8.62 ± 4.86$^{\#\#}$	55.52 ± 20.05$^{*,\#}$	138.07 ± 52.11*	196.60 ± 71.27*
Nrf2-regulated genes						
Akr7a3	1.01 ±0.17	3.28 ± 1.03**	4.78 ± 2.18**	1.88 ± 0.71$^{**,\#\#}$	3.25 ± 1.20**	5.75 ± 3.77**
Gpx2	1.04 ±0.29	4.63 ± 1.5$^{**,\#}$	6.18 ± 1.51**	2.90 ± 0.80$^{**,\#}$	6.89 ± 2.74**	9.18 ± 3.10**
Yc2	1.00 ±0.10	1.73 ± 0.36$^{**,\#}$	1.73 ± 0.27**	1.26 ± 0.18$^{*,\#\#}$	1.60 ± 0.25$^{**,\#}$	2.42 ± 0.60**
Me1	1.08 ±0.39	2.35 ± 0.77**	2.57 ± 0.62**	1.94 ± 0.51$^{*,\#}$	1.93 ± 0.88$^{*,\#}$	4.14 ± 2.20**
Immflamation						
Cox2	1.07 ±0.36	1.52 ± 0.35	1.30 ± 0.22	2.89 ± 0.99**	3.31 ± 1.31**	3.61 ± 1.87*
Serpine1	1.02 ±0.24	0.95 ± 0.24$^{\#}$	0.99 ± 0.46$^{\#}$	1.59 ± 0.37$^{*,\#}$	2.23 ± 1.18$^{*,\#}$	3.16 ± 0.42**
Mmp12	1.01 ±0.14	1.01 ± 0.53$^{\#}$	0.90 ± 0.27$^{\#}$	1.26 ± 0.18	2.14 ± 0.65*	2.28 ± 1.24**
Il-6	1.19 ±0.88	2.12 ± 0.64	1.28 ± 0.80	1.37 ± 0.62	1.37 ± 0.41	2.72 ± 1.98
Fgf21	1.08 ±0.54	0.94 ± 0.46$^{\#}$	0.94 ± 0.45$^{\#}$	1.68 ± 0.52	1.97 ± 0.80	3.35 ± 2.29*
Nfkb1	1.01 ±0.16	1.04 ± 0.14	0.94 ± 0.17	0.96 ± 0.07	1.12 ± 0.16	1.00 ± 0.16
Nfkbia	1.1 ±0.47	1.05 ± 0.39	0.9 ± 0.32	0.6 ± 0.27	0.65 ± 0.17	0.98 ± 0.56
Tnf	1.02 ±0.22	1 ± 0.2	0.91 ± 0.15	1.23 ± 0.32	1.59 ± 0.26*	1.28 ± 0.46
Cell cycle related genes						
Chek1	1.01 ±0.17	1.29 ± 0.31	1.41 ± 0.28**	1.14 ± 0.17$^{\#\#}$	1.27 ± 0.34$^{\#}$	1.69 ± 0.34**
Wee1	1.01 ±0.15	1.36 ± 0.33	1.43 ± 0.34	1.43 ± 0.19	1.60 ± 0.57*	1.86 ± 0.28**
MAP kinase pathway family related genes						
Myc	1.03 ±0.26	2.59 ± 1.15*	3.00 ± 1.18*	1.10 ± 0.43$^{\#\#}$	1.50 ± 0.52$^{\#}$	3.15 ± 1.62**

DEN: *N*-diethylnitrosamine, Low OPZ: 138 mg/kg Omeprazole, High OPZ: 276 mg/kg Omeprazole, Low BNF: 0.125% β-naphthoflavone, High BNF: 0.25% β-naphthoflavone, and OPZ+BNF: 138 mg/kg Omeprazole + 0.125% β-naphthoflavone. The data represent mean ± S.D.

*, ** significantly different from the DEN-alone group at $p < 0.05$ or $p < 0.01$, respectively.

$^{\#, \#\#}$ significantly different from the OPZ+BNF group at $p < 0.05$ or $p < 0.01$, respectively.

Table 11.

Final body and liver weights, number/area of GST-P positive foci, distribution of GST-P positive foci and number of PCNA positive cells in the liver of male F344 rats given OPZ and/or BNF for 9 days before MeIQx treatment

Group	MeIQx-alone	Low OPZ	High OPZ	Low BNF	High BNF	OPZ+BNF
No. of animals	12	10	12	11	11	11
Final body weight (g)	257.8 ± 9.8	259.2 ± 9.0	254.9 ± 10.6	261.6 ± 13.5	259.8 ± 6.1	256.6 ± 12.9
Absolute liver weight (g)	9.14 ± 0.53	9.37 ± 0.52	9.26 ± 0.62	9.73 ± 0.84	9.87 ± 0.27**	9.59 ± 0.69
Relative liver weight (g% body weight)	3.58 ± 0.13	3.59 ± 0.10$^{\#}$	3.61 ± 0.12	3.68 ± 0.19	3.79 ± 0.13**	3.73 ± 0.11*
GST-P positive foci (≧0.2 mm)						
Number (number/cm^2)	0.38 ± 0.60	0.18 ± 0.25	0.83 ± 1.24	1.41 ± 1.29*	1.44 ± 1.47	0.78 ± 1.21
Area (mm^2/cm^2)	0.02 ± 0.03	0.01 ± 0.02	0.06 ± 0.09	0.10 ± 0.09*	0.14 ± 0.22	0.05 ± 0.11
% of GST P-positive foci for each zone						
Zone 1	0	20.8 ± 25.0	12.5 ± 35.4	13.0 ± 14.5	17.8 ± 21.1	33.4 ± 43.8
Zone 2	68.0 ± 29.5	37.5 ± 47.9	76.0 ± 37.1	51.6 ± 19.3	57.5 ± 29.0	56.7 ± 42.1
Zone 3	32.0 ± 29.5	41.7 ± 44.1	11.5 ± 21.3	39.1 ± 20.6	24.7 ± 20.7	9.9 ± 17.7
PCNA positive hepatocyte (%)	0.51 ± 0.54	0.40 ± 0.25	0.40 ± 0.48	0.39 ± 0.28	0.50 ± 0.97	0.43 ± 0.36

MeIQx: 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline, Low OPZ: 138 mg/kg Omeprazole, High OPZ: 276 mg/kg Omeprazole, Low BNF: 0.03% β-naphthoflavone, High BNF: 0.06% β-naphthoflavone, OPZ+BNF: 138 mg/kg Omeprazole+0.125% β-naphthoflavone.

The data represent mean ± S.D.

*, ** significantly different from the MeIQx-alone group at $p < 0.05$ or $p < 0.01$, respectively.

Table 12.

Final body and liver weights, food intake, BNF intake, number/area of GST-P positive foci, Ki67-positive cells, ROS production and TBARS in the liver of male F344 rats given BNF and/or PBO for 6 weeks after DEN treatment

Final body weight (g)	DEN-alone	Low BNF	High BNF	Low PBO	High PBO	BNF+PBO
No. of animals	11	12	12	9	12	11
Final body weight (g)	273.91 ± 11.54	265.27 ± 9.27$^{\#}$	258.63 ± 9.66**	278.15 ± 8.55$^{\#\#}$	268.65 ± 13.31$^{\#\#}$	254.9 ± 9.97**
Absolute liver weight (g)	8.54 ± 0.51	9.91 ± 0.35$^{**,\#\#}$	11.14 ± 0.63**	9.33 ± 0.55$^{**,\#\#}$	9.49 ± 0.70$^{**,\#\#}$	10.98 ± 0.65**
Relative liver weight (g% body weight)	3.12 ± 0.13	3.74 ± 0.10$^{**,\#\#}$	4.31 ± 0.15**	3.35 ± 0.14$^{**,\#\#}$	3.53 ± 0.14$^{**,\#\#}$	4.31 ± 0.16**
Food intake (g/rat/day)	14.36 ± 1.95	14.23 ± 0.78	12.88 ± 0.72	14.53 ± 1.31	13.57 ± 1.27	12.63 ± 1.16
BNF intake (g/rat/day)	-	0.10 ± 0.04	0.18 ± 0.09	-	-	0.07 ± 0.02
PBO intake (g/rat/day)	-	-	-	0.08 ± 0.02	0.15 ± 0.02	0.07 ± 0.02
GST-P positive foci (≧0.2mm)						
Numbers ((number/cm2)	2.97 ± 0.99	5.31 ± 1.37$^{**,\#}$	6.44 ± 2.52**	4.75 ± 1.69$^{*,\#}$	4.75 ± 6.20*	7.43 ± 2.64**
Areas (mm2/cm2)	0.22 ± 0.07	0.51 ± 0.26**	0.54 ± 0.26**	0.38 ± 0.16	0.52 ± 0.27*	0.63 ± 0.38*
Ki67-positive cells (%)	0.93 ± 0.56	0.99 ± 0.44	0.97 ± 0.73	0.87 ± 0.35	1.03 ± 0.42	0.94 ± 0.56
No. of animals	5	5	5	5	5	5
ROS production (%)						
+NADPH	100.00 ± 9.15	89.88 ± 15.84	85.84 ± 10.47	101.81 ± 3.46	114.20 ± 8.82	102.66 ± 13.81
+NADPH+SKF525A	50.65 ± 7.71	48.27 ± 7.71	46.59 ± 2.94	63.80 ± 18.70	65.60 ± 5.84*	66.92 ± 32.59
-NADPH	28.37 ± 2.20	28.40 ± 2.47	26.74 ± 0.95	28.47 ± 4.49	27.54 ± 1.95	28.09 ± 2.26
No. of animals	6	6	6	6	6	6
TBARS (nmol MDA/mg protein)	1.26 ± 0.25	1.16 ± 0.12	1.05 ± 0.25	1.38 ± 0.25	1.65 ± 0.21$^{**,\#\#}$	1.13 ± 0.13

DEN: *N*-diethylnitrosamine, Low BNF: 0.125% β-naphthoflavone, High BNF: 0.25% β-naphthoflavone, Low PBO: 0.125% piperonylbutoxide, High PBO: 0.25% piperonylbutoxide and OPZ+BNF: 0.125% β-naphthoflavone + 0.125% piperonylbutoxide. The data represent mean ± S.D.

*, ** significantly different from the DEN-alone group at $p < 0.05$ or $p < 0.01$, respectively.

$^{\#,\#\#}$ significantly different from the BNF+PBO group at $p < 0.05$ or $p < 0.01$, respectively.

Table 13.

Real-time RT-PCR analysis of the liver tissues from male F344 rats given BNF and/or PBO for 6 weeks after DEN treatment

Group	DEN-alone	Low BNF	High BNF	Low PBO	High PBO	BNF+PBO
Ahr-regulated genes						
Cyp1a1	1.06±0.42	3505.32±573.71$^{**,\#\#}$	4995.48±2861.44**	6.61±2.48$^{**,\#\#}$	11.23±0.96$^{**,\#\#}$	1644.03±261.63**
Cyp1a2	1.00±0.11	13.53±1.51$^{**,\#}$	23.44±7.33**	0.88±0.15$^{\#\#}$	0.81±0.14$^{\#\#}$	19.09±3.03**
Cyp1b1	1.05±0.38	226.72±81.27$^{**,\#}$	1138.84±518.87**	2.85±0.90$^{**,\#\#}$	2.45±0.69$^{*,\#\#}$	491.64±131.94**
Cyp2b2	1.05±0.34	0.80±0.38	0.49±0.17	6.22±2.17$^{**,\#\#}$	15.90±1.21$^{**,\#\#}$	2.01±0.61*
Nqo1	1.04±0.30	4.12±0.97**	6.99±3.49**	1.55±0.40$^{\#\#}$	2.25±0.27$^{**,\#\#}$	6.41±2.15**
Ugt1a6	1.02±0.19	1.49±0.13$^{**,\#\#}$	2.29±0.60**	1.08±0.21$^{\#\#}$	1.56±0.27$^{**,\#\#}$	2.37±0.21**
Nrf2-regulated genes						
Akr7a3	1.04±0.30	2.37±1.26*	2.37±0.90**	1.80±0.39*	3.95±2.27**	2.63±1.27*
Gpx2	1.04±0.33	2.68±1.14**	4.41±2.90**	2.23±1.11*	4.96±1.50**	3.77±1.32**
Yc2	1.03±0.27	1.12±0.14$^{\#\#}$	1.25±0.20$^{\#}$	1.32±0.13	1.94±0.30**	1.62±0.34**

DEN: *N*-diethylnitrosamine, Low BNF: 0.125% β-naphthoflavone, High BNF: 0.25% β-naphthoflavone, Low PBO: 0.125% piperonylbutoxide, High PBO: 0.25% piperonylbutoxide and OPZ+BNF, 0.125% β-naphthoflavone + 0.125% piperonylbutoxide. The data represent mean ± S.D.

*, ** significantly different from the DEN-alone group at $p < 0.05$ or $p < 0.01$, respectively.

$^{\#,\#\#}$ significantly different from the BNF+PBO group at $p < 0.05$ or $p < 0.01$, respectively.

Table 14.

Final body and liver weights of male F344 rats given PB and/or I3C for 6 weeks after DEN treatment

Group	DEN-alone	Low PB	High PB	Low I3C	High I3C	PB+I3C
Number of rats	12	12	10	10	9	11
Final body weight(g)	280.0 ± 13.2**	280.7 ± 8.5**	278.0 ± 20.1*	273.3 ± 13.1	253.9 ± 16.5	264.5 ± 18.7
Absolute liver weight(g)	9.1 ± 0.4$^{\#\#}$	9.4 ± 0.5$^{\#\#}$	10.6 ± 0.6$^{*,\#\#}$	12.0 ± 0.8**	11.9 ± 1.1$^{**,\#}$	11.4 ± 0.9**
Relative liver weight(% BW)	3.2 ± 0.1$^{\#\#}$	3.3 ± 0.1$^{\#\#}$	3.5 ± 0.2$^{*,\#\#}$	4.3 ± 0.7**	5.0 ± 0.3$^{**,\#\#}$	4.3 ± 0.4**

*,** significantly different from the DEN-alone group at $p < 0.05$ or $p < 0.01$, respectively.

$^{\#,\#\#}$ significantly different from the PB+I3C group at $p < 0.05$ or $p < 0.01$, respectively.

Table 15.

Number/area of GST-P positive foci and Ki67-positive cells of male F344 rats given PB and/or I3C for 6 weeks after DEN treatment

Group	DEN-alone	Low PB	High PB	Low I3C	High I3C	PB+I3C
number of rats	12	12	10	10	9	11
GST-P positive foci						
Numbers(No./cm^2)	6.977 ± 1.849$^{\#\#}$	12.544 ± 3.783$^{**,\#}$	12.881 ± 2.004**	19.298 ± 2.330**	22.649 ± 3.996**	18.499 ± 5.834**
Areas(mm^2/cm^2)	0.346 ± 0.148$^{\#\#}$	0.504 ± 0.222$^{\#\#}$	0.440 ± 0.094$^{\#\#}$	0.685 ± 0.114**	0.840 ± 0.159**	0.783 ± 0.206**
ki-67 positive cell (%)	1.734 ± 0.604$^{\#\#}$	1.637 ± 0.415$^{\#\#}$	2.148 ± 0.601$^{\#\#}$	2.407 ± 0.508$^{\#}$	3.306 ± 0.854**	3.216 ± 0.621**

*,** significantly different from the DEN-alone group at $p < 0.05$ or $p < 0.01$, respectively.

$^{\#,\#\#}$ significantly different from the PB+I3C group at $p < 0.05$ or $p < 0.01$, respectively.

Table 16.

Real-time RT-PCR

Gene name	Group					
	DEN-alone	Low PB	High PB	Low I3C	High I3C	PB+I3C
Cyp1a1	1.09 ± 0.49$^{\#}$	2.17 ± 1.00$^{\#}$	2.12 ± 0.97$^{\#}$	5415.06 ± 563.85*	7453.79 ± 1167.65*	5915.88 ± 803.55*
Cyp2b1/2	1.18 ± 0.84$^{\#\#}$	17.55 ± 2.94$^{**,\#}$	23.29 ± 2.12**	20.82 ± 2.22**	28.20 ± 3.76$^{**,\#\#}$	22.03 ± 2.30**
Cyp3a1/2	1.02 ± 0.23$^{\#}$	1.75 ± 0.24$^{*,\#}$	4.58 ± 1.99*	4.4 ± 1.42*	6.24 ± 1.23*	5.91 ± 1.06*
Nqo1	1.01 ± 0.143$^{\#}$	1.36 ± 0.22$^{\#}$	3.19 ± 1.93$^{*,\#}$	10.74 ± 4.43*	11.14 ± 2.94*	16.41 ± 7.33*
Gstm3	1.07 ± 0.36$^{\#}$	4.58 ± 1.55$^{*,\#}$	9.2 ± 4.19*	12.48 ± 5.69*	26.97 ± 5.36*	19.39 ± 7.15*
Jun	1.06 ± 0.41$^{\#}$	1.00 ± 0.21$^{\#}$	1.18 ± 0.39$^{\#}$	1.74 ± 0.71	2.22 ± 0.66**	1.95 ± 0.46*
Nfkbia	1.06 ± 0.37	1.10 ± 0.25	1.06 ± 0.52	1.30 ± 0.87	1.01 ± 0.47	1.59 ± 0.35

*,** significantly different from the DEN-alone group at $p < 0.05$ or $p < 0.01$, respectively.

$^{\#,\#\#}$ significantly different from the PB+I3C group at $p < 0.05$ or $p < 0.01$, respectively.

Table 17.

ROS production and TBARS in the liver of male F344 rats given BNF and/or PBO for 6 weeks after DEN treatment

Group	DEN-alone	Low PB	High PB	Low I3C	High I3C	PB+I3C
number of rats	6	6	6	6	6	6
TBARS(nmol MDA/mg protin)	0.882 ± 0.055$^{\#}$	0.931 ± 0.045	0.967 ± 0.085	1.024 ± 0.077*	1.041 ± 0.048**	1.018 ± 0.125*
ROS production(%)						
−NADPH	108.33 ± 8.47	97.79 ± 8.11	99.82 ± 11.17	75.00 ± 8.47	71.15 ± 7.22	74.38 ± 8.04
+NADPH	371.67 ± 50.00$^{\#\#}$	518.49 ± 57.94**	641.41 ± 82.38**	600.69 ± 54.90**	612.26 ± 43.90**	601.26 ± 105.67**
+NADPH+ SKF-525A	141.72 ± 25.60	189.00 ± 29.29	217.82 ± 67.08	167.49 ± 27.46	179.28 ± 40.74	200.64 ± 36.31

*,** significantly different from the DEN-alone group at $p < 0.05$ or $p < 0.01$, respectively.

$^{\#,\#\#}$ significantly different from the PB+I3C group at $p < 0.05$ or $p < 0.01$, respectively.

Table 18.

Body weight, liver weights and PCNA-positive hepatocyte ratio of rats given PB and/or ORPH for 6 weeks after DEN initiation

Groups	DEN-alone	Low PB	High PB	Low ORPH	High ORPH	PB+ORPH
Number of rats	10	12	12	11	12	12
Final body weight (g)	257.0 ± 19.3[a]	271.0 ± 16.8	264.7 ± 18.2$^{\#}$	241.7 ± 13.0	205.1 ± 23.3$^{**,\#\#}$	251.4 ± 11.5
Absolute liver weight (g)	8.1 ± 0.6$^{\#\#}$	9.5 ± 0.8**	9.6 ± 0.9**	8.0 ± 0.7$^{\#\#}$	8.1 ± 1.2$^{\#\#}$	10.1 ± 0.8**
Relative liver weight (% BW)	3.1 ± 0.0$^{\#\#}$	3.5 ± 0.2$^{**,\#\#}$	3.6 ± 0.2$^{**,\#\#}$	3.3 ± 0.2$^{\#\#}$	3.9 ± 0.2**	4.0 ± 0.2**
PCNA positive hepatocyte ratio (%)	0.12 ± 0.05$^{\#}$	0.19 ± 0.21	0.29 ± 0.07$^{*,\#}$	0.23 ± 0.08$^{\#}$	0.42 ± 0.13*	0.51 ± 0.17*

[a] Values are expressed as the mean ± SD.

[b] Calculated from the last monitoring data.

*, ** significantly different from the DEN-alone group at $p < 0.05$ or $p < 0.01$, respectively. (Dunnett's test or Steel test)

$^{\#}$, $^{\#\#}$ significantly different from the PB+ORPH group at $p < 0.05$ or $p < 0.01$, respectively. (Dunnett's test or Steel test)

Table 19.

mRNA expression in the livers of rats given PB and/or ORPH for 6 weeks after DEN initiation

Groups	DEN-alone	Low PB	High PB	Low ORPH	High ORPH	PB+ORPH
Number of rats examined	6	6	6	6	6	6
Gene name						
Cyp1a1	1.06 ± 0.35$^{\#}$	2.27 ± 1.02	2.57 ± 2.04	2.83 ± 1.37*	16.64 ± 4.68$^{*,\#}$	4.71 ± 2.74*
Cyp2b1/2	1.04 ± 0.31$^{\#}$	38.94 ± 11.56$^{*,\#}$	63.78 ± 13.24*	14.88 ± 13.07$^{*,\#}$	75.71 ± 14.61*	78.96 ± 11.54*
Gstm3	1.02 ± 0.21$^{\#}$	3.94 ± 1.31$^{*,\#}$	6.44 ± 2.37*	1.81 ± 0.44$^{\#}$	8.61 ± 2.63*	10.32 ± 2.22*
Gpx2	1.02 ± 0.22$^{\#}$	1.83 ± 0.54*	2.49 ± 0.59*	1.02 ± 0.19$^{\#}$	3.02 ± 0.91*	2.63 ± 0.76*

[a] Values of mRNA expression levels (normalized by *actb*) are expressed as the mean ± SD.

* significantly different from the DEN-alone group at $p < 0.05$ (Steel test).

$^{\#}$ significantly different from the PB+ORPH group at $p < 0.05$ (Steel test).

Table 20.

TBARS level and microsomal ROS production of rats given PB and/or ORPH for 6 weeks after DEN initiation

Groups	DEN-alone	Low PB	High PB	Low ORPH	High ORPH	PB+ORPH
Number of rats	6	6	6	6	6	6
Microsomal ROS production (%)						
-NADPH	28.70 ± 1.85	28.45 ± 2.63	26.62 ± 1.31	26.62 ± 1.31	26.08 ± 1.77	24.99 ± 1.87
+NADPH	100.00 ± 8.09$^{\#\#}$	144.06 ± 16.69$^{**,\#\#}$	181.08 ± 31.49$^{**,\#}$	166.78 ± 25.64$^{**,\#\#}$	234.86 ± 14.17**	217.91 ± 26.76**
+NADPH+SKF-525A	39.68 ± 2.20†	52.37 ± 8.24†	63.22 ± 6.30†	49.01 ± 11.30†	83.21 ± 6.94†	69.66 ± 8.86†
TBARS level (nmol MDA/mg protein)	1.25 ± 0.18$^{\#\#,a}$	1.15 ± 0.16$^{\#\#}$	1.31 ± 0.15$^{\#}$	1.29 ± 0.21$^{\#}$	1.69 ± 0.16*	1.70 ± 0.37**

[a] Values are expressed as the mean ± SD.

*, ** significantly different from the DEN-alone group at $p < 0.05$ or $p < 0.01$, respectively. (Dunnett's or Steel test).

$^{\#}$, $^{\#\#}$ significantly different from the PB+ORPH group at $p < 0.05$ or $p < 0.01$, respectively. (Dunnett's or Steel test).

† significantly different from the production with NADPH (*t*-test).

Table 21.
gpt MFs in the kidneys of male F344 *gpt* delta rats given NFT, AHD or NFA for 13 weeks.

Treatment	Animal no.	Cm^R colonies ($\times 10^{-5}$)	6-TG^R and Cm^R colonies	Mutant frequency ($\times 10^{-5}$)	Mean ± SD
Control	6	4.5	1	0.22	
	7	5.3	1	0.19	
	8	4.1	3	0.73	
	9	25.1	9	0.36	
	10	6.6	3	0.45	0.39 ± 0.22
NFT	17	1.3	5	3.83	
	18	2.4	2	0.84	
	19	4.2	8	1.89	
	20	7.7	10	1.31	1.97 ± 1.32 *
AHD	26	3.5	2	0.57	
	27	4.0	1	0.25	
	28	8.2	1	0.12	
	29	4.5	2	0.44	
	30	3.8	3	0.78	0.43 ± 0.26
NFA	36	4.1	5	1.23	
	37	3.6	2	0.56	
	38	14.6	19	1.30	
	39	3.2	3	0.95	
	40	3.4	3	0.88	0.99 ± 0.30 *

* Significantly different from the control group at $p<0.05$.

Table 22.
gpt MFs in the kidneys of female F344 *gpt* delta rats given NFT for 13 weeks.

Treatment	Animal no.	Cm^R colonies ($\times 10^{-5}$)	6-TG^R and Cm^R colonies	Mutant frequency ($\times 10^{-5}$)	Mean ± SD
Control	1	3.1	6	1.93	
	2	8.1	5	0.62	
	3	5.4	6	1.12	
	4	3.0	3	1.01	
	5	6.0	4	0.67	1.07 ± 0.53
NFT	8	2.9	22	7.64	
	9#	0	0	-	
	11	1.9	13	6.88	
	12	5.0	27	5.36	
	13	3.0	17	5.72	6.40 ± 1.05 **

No mutant colonies were detected on the plate, with those data being excluded from the calculation of MF.
** Significantly different from the control group at $p<0.01$.

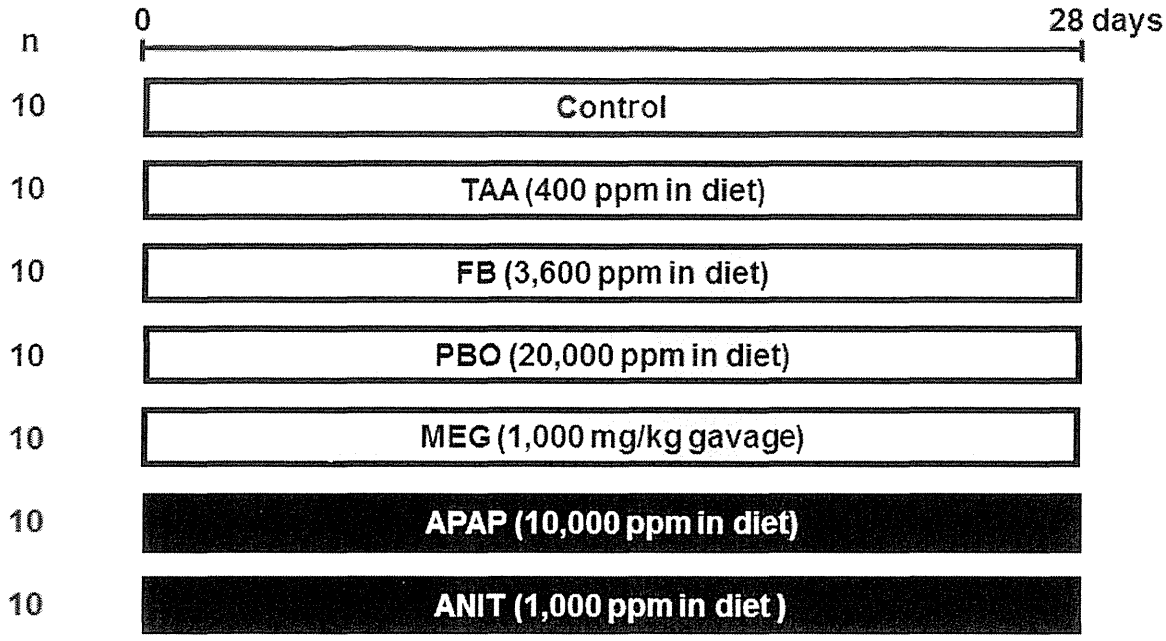

Fig. 1. Experimental design 1

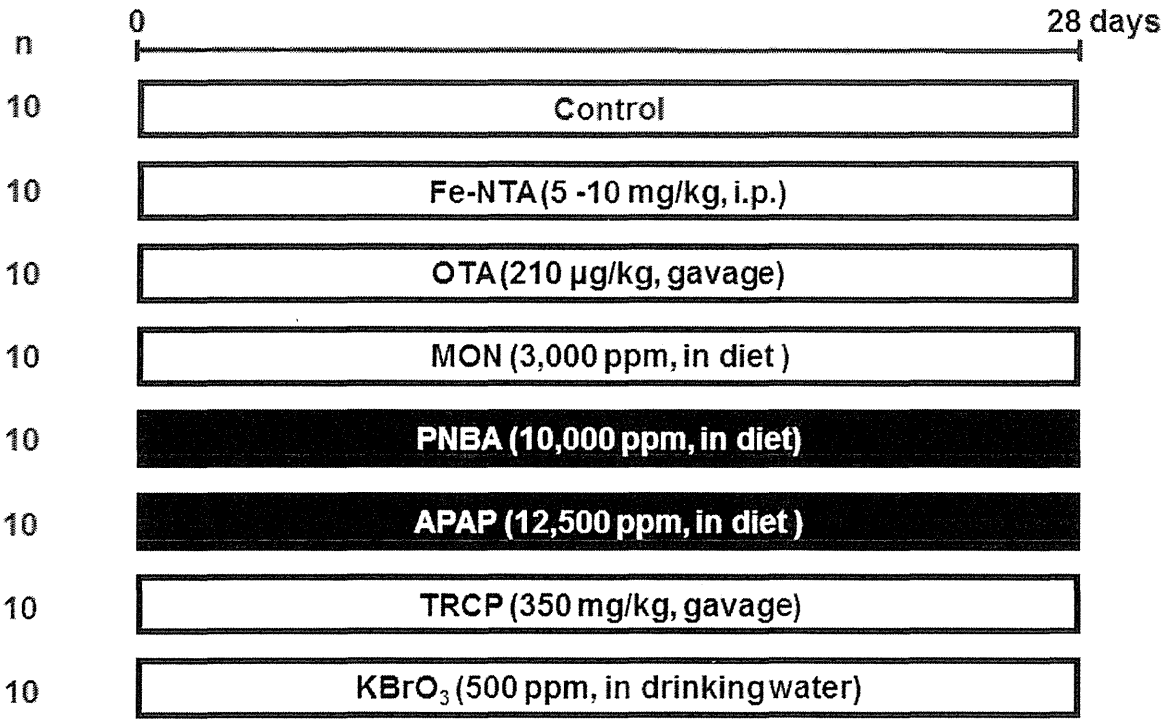

Fig. 2. Experimental design 2

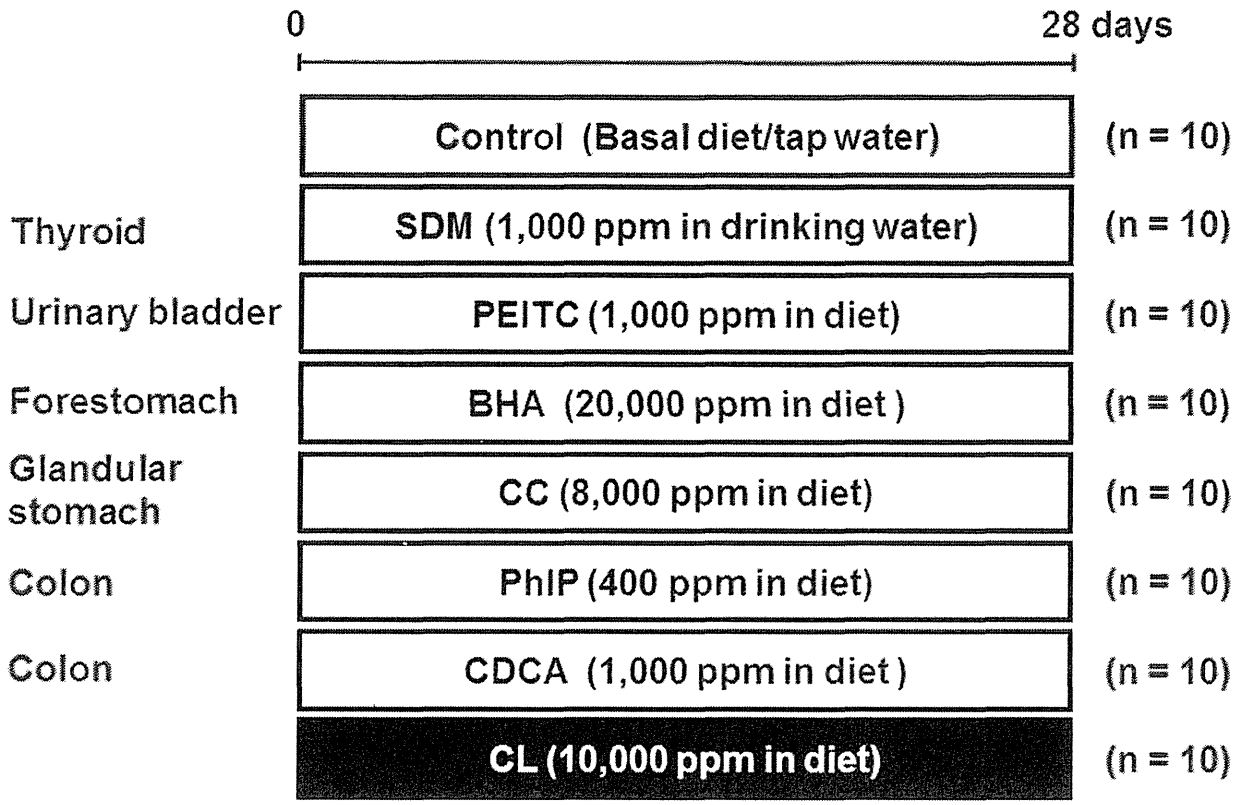

Fig. 3. Experimental design 3

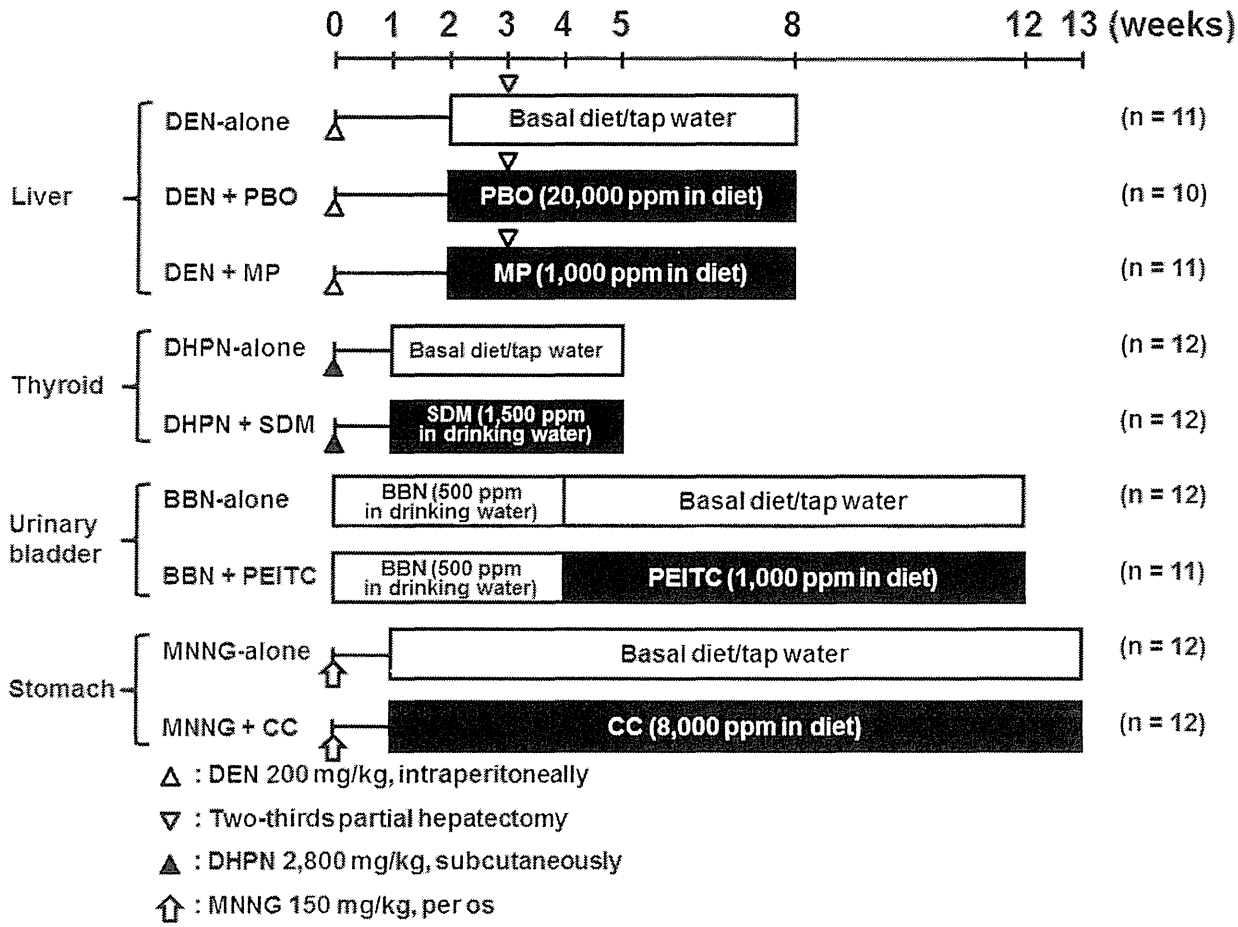

Fig. 4. Experimental design 4

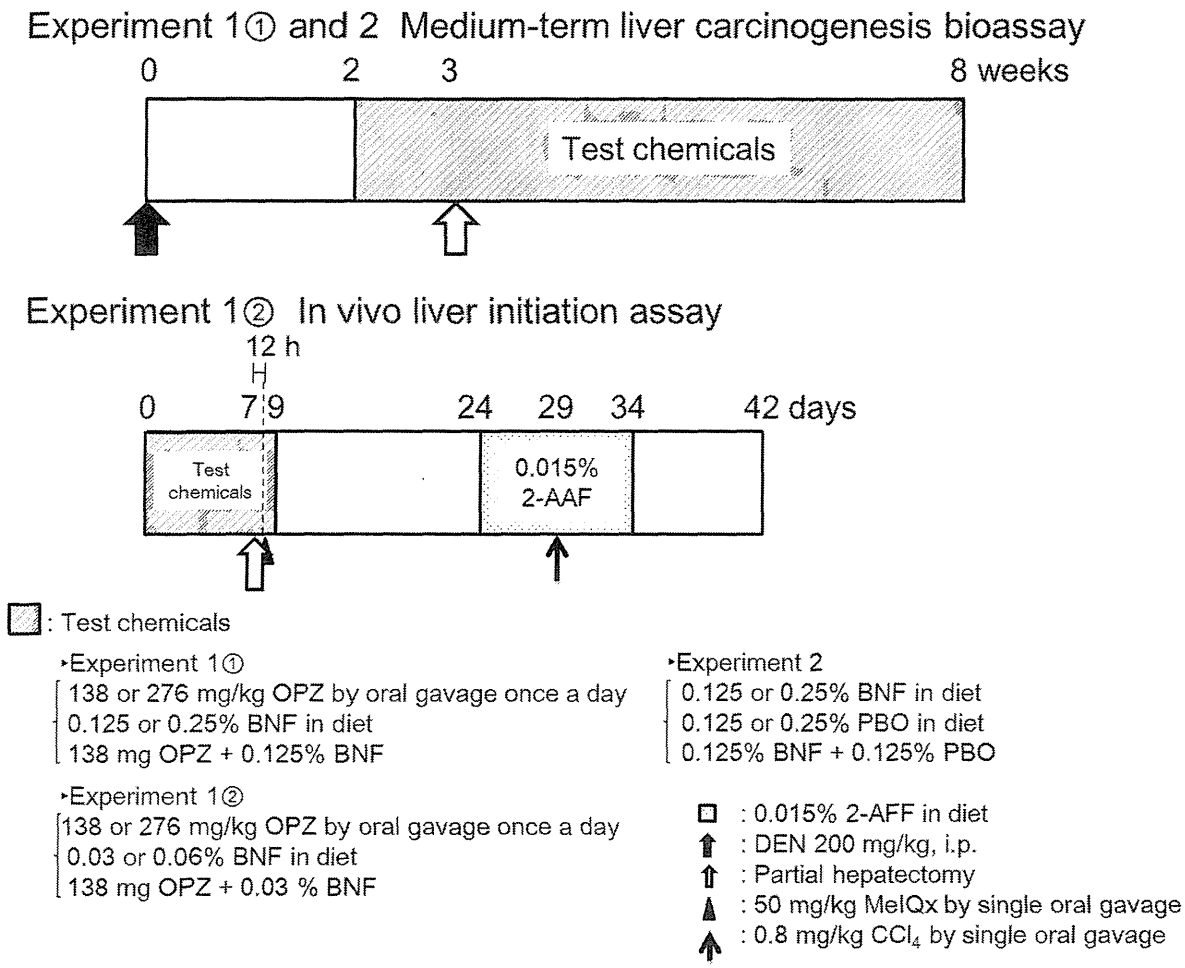

Fig. 5.

Experimental design of a medium-term liver carcinogenesis bioassay (Experiment 1) and an *in vivo* liver initiation assay (Experiment 2) in rats

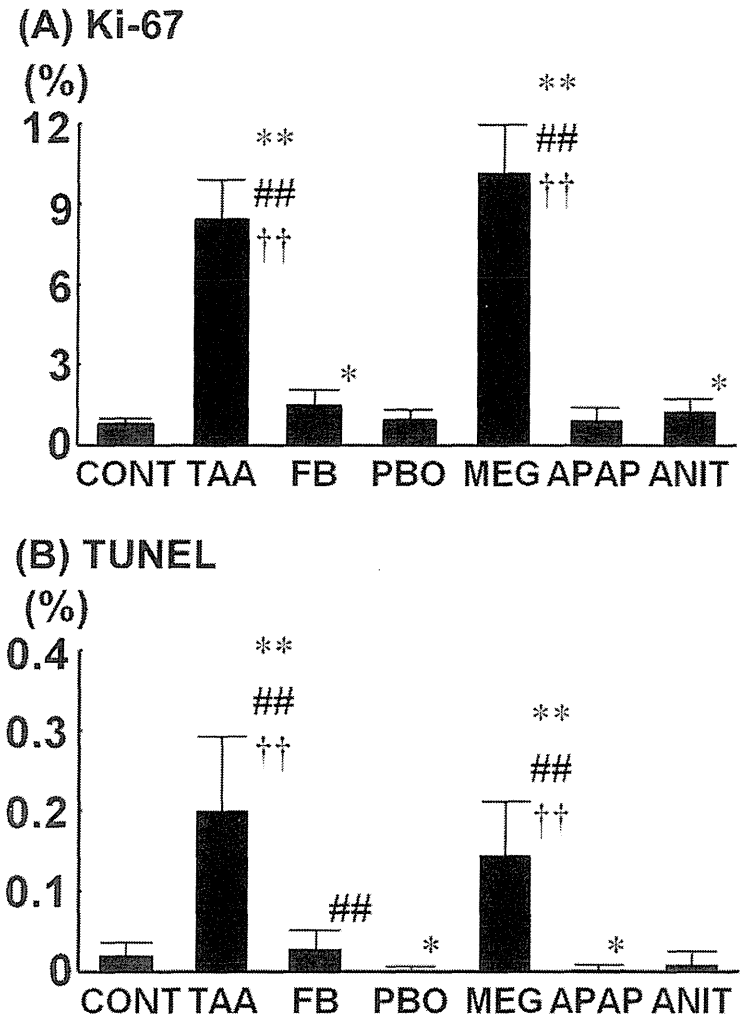

Fig. 6. Immunohistochemical cellular distribution of cell proliferation and TUNEL in liver cells after 28-day treatment with hepatocarcinogens or non-carcinogens in rats. The graphs show positive cell ratios (%) of liver cells per total cells counted using 10 animals in each group. Values are presented as mean + SD. (A) Ki-67 and (B) TUNEL. *,** $P < 0.05$, 0.01 vs. untreated controls (Dunnett's or Steel's test). $^{\#\#}$ $P <$ 0.01 vs. APAP (Dunnett's or Steel's test). †† $P < 0.01$ vs. ANIT (Dunnett's or Steel's test).

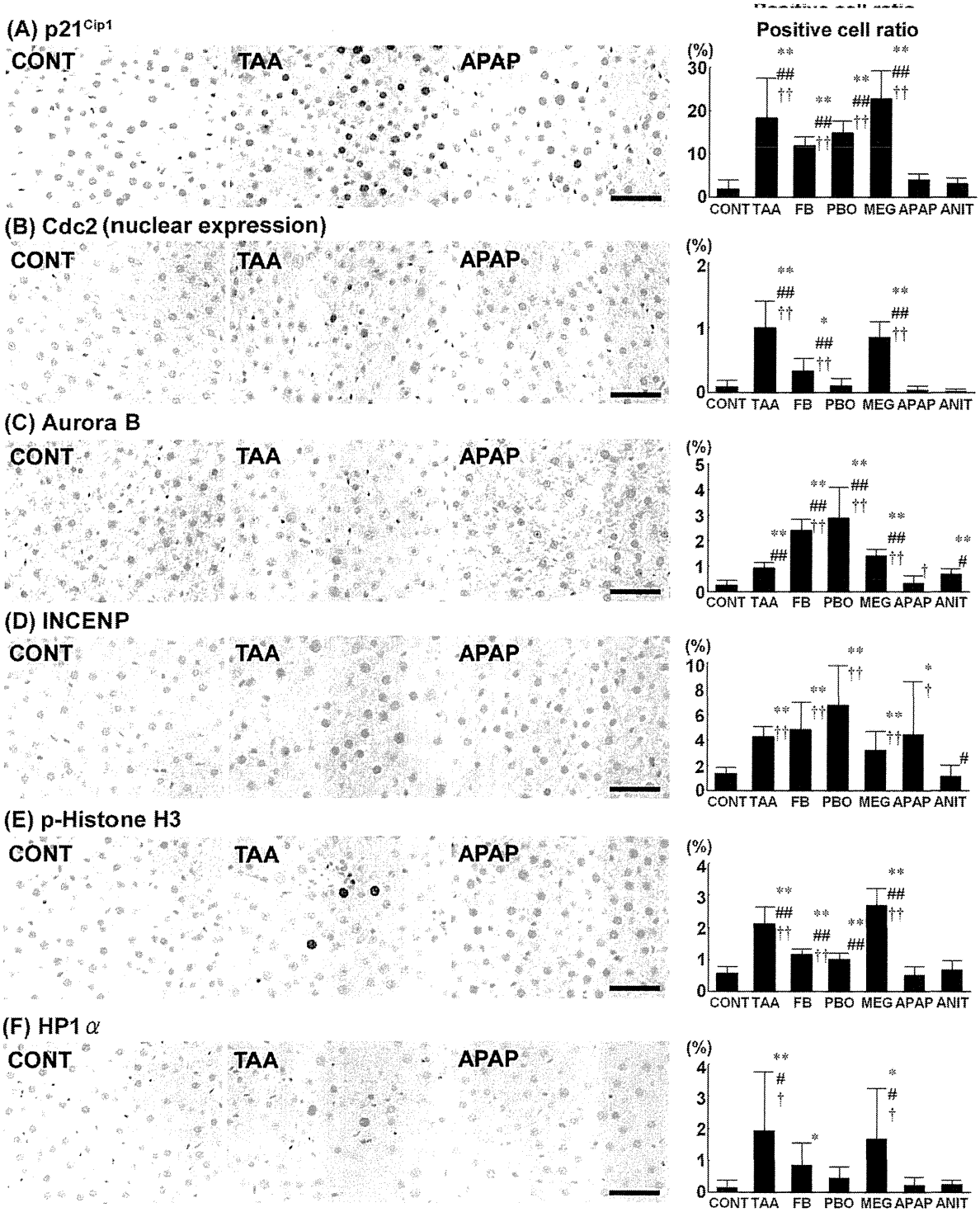

Fig. 7. Immunohistochemical cellular distribution of cell cycle proteins in liver cells after 28-day treatment with hepatocarcinogens or non-carcinogens in rats. Photomicrographs show distributions of $p21^{Cip1}$, nuclear Cdc2, Aurora B, Incenp, p-Histone H3, and HP1α immunoreactive cells in the livers of representative cases of an untreated control and animals treated with TAA or APAP. The graphs show positive cell ratios (%) of liver cells per total cells counted using 10 animals in each group. Values are presented as mean + SD (A) $p21^{Cip1}$, (B) nuclear Cdc2, (C) Aurora B, (D) Incenp, (E) p-Histone H3, and (F) HP1α. Magnification: ×400 (Bar = 50 μm). *, ** $P < 0.05$, 0.01 vs. untreated controls (Dunnett's or Steel's test). $^{\#,\#\#}$ $P < 0.05$, 0.01 vs. APAP (Dunnett's or Steel's test). †,†† $P < 0.05$, 0.01 vs. ANIT (Dunnett's or Steel's test).