KIF23*	Homo sapiens kinesin family member 23 (KIF23), transcript variant 1, mRNA [NM_138555]	-1.56
KIFC1*	Homo sapiens kinesin family member C1 (KIFC1), mRNA [NM_002263]	-1.26
MCM4	Homo sapiens minichromosome maintenance complex component 4 (MCM4), transcript variant 1, mRNA [NM_005914]	-1.09
MCM6	Homo sapiens minichromosome maintenance complex component 6 (MCM6), mRNA [NM_005915]	-1.10
MELK	Homo sapiens maternal embryonic leucine zipper kinase (MELK), mRNA [NM_014791]	-1.11
MOBKL2B*	Homo sapiens MOB1, Mps One Binder kinase activator-like 2B (yeast) (MOBKL2B), mRNA [NM_024761]	-1.11
MYBBP1A	Homo sapiens MYB binding protein (P160) 1a (MYBBP1A), transcript variant 2, mRNA [NM_014520]	-1.02
MYBL1	Homo sapiens v-myb myeloblastosis viral oncogene homolog (avian)-like 1 (MYBL1), mRNA [NM_001080416]	-1.84
MYC	Homo sapiens v-myc myelocytomatosis viral oncogene homolog (avian) (MYC), mRNA [NM_002467]	-1.48
MYH9*	Homo sapiens myosin, heavy chain 9, non-muscle (MYH9), mRNA [NM_002473]	-1.21
MYO5C*	Homo sapiens myosin VC (MYO5C), mRNA [NM_018728]	-1.39
NCAPD2*	Homo sapiens non-SMC condensin I complex, subunit D2 (NCAPD2), mRNA [NM_014865]	-1.12
NDC80	Homo sapiens NDC80 homolog, kinetochore complex component (S. cerevisiae) (NDC80), mRNA [NM_006101]	-1.01
NR2F2	Homo sapiens nuclear receptor subfamily 2, group F, member 2 (NR2F2), mRNA [NM_021005]	-1.11
NUF2*	Homo sapiens NUF2, NDC80 kinetochore complex component, homolog (S. cerevisiae) (NUF2), transcript variant 1, mRNA [NM_145697]	-1.17
OTUD4*	Homo sapiens OTU domain containing 4 (OTUD4), transcript variant 1, mRNA [NM_199324]	-1.47
PARP4	Homo sapiens poly (ADP-ribose) polymerase family, member 4 (PARP4), mRNA [NM_006437]	-1.18
PCNA	Homo sapiens proliferating cell nuclear antigen (PCNA), transcript variant 1, mRNA [NM_002592]	-1.37
PDGFA	Homo sapiens platelet-derived growth factor alpha polypeptide (PDGFA), transcript variant 1, mRNA [NM_002607]	-1.04
PLK2	Homo sapiens polo-like kinase 2 (Drosophila) (PLK2), mRNA [NM_006622]	-1.29
PMS1	Homo sapiens PMS1 postmeiotic segregation increased 1 (S. cerevisiae) (PMS1), transcript variant 1, mRNA [NM_000534]	-1.66
POLI*	Homo sapiens polymerase (DNA directed) iota (POLI), mRNA [NM_007195]	-1.58
PSKH1*	Homo sapiens protein serine kinase H1 (PSKH1), mRNA [NM_006742]	-1.28
PSRC1	Homo sapiens proline/serine-rich coiled-coil 1 (PSRC1), transcript variant 1, mRNA [NM_032636]	-1.61
PTPN13	Homo sapiens protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase) (PTPN13), transcript variant 4, mRNA [NM_080685]	-1.77
PTTG1*	Homo sapiens pituitary tumor-transforming 1 (PTTG1), mRNA [NM_004219]	-1.04
RAPGEF6*	Homo sapiens Rap guanine nucleotide exchange factor (GEF) 6 (RAPGEF6), mRNA [NM_016340]	-1.08
RBBP8	Homo sapiens retinoblastoma binding protein 8 (RBBP8), transcript variant 1, mRNA [NM_002894]	-1.31
ROR1	Tyrosine-protein kinase transmembrane receptor ROR1 Precursor (EC 2.7.10.1)(Neurotrophic tyrosine kinase, receptor-related 1) [Source:UniProtKB/Swiss-Prot;Acc:Q01973] [ENST00000371079]	-1.43
SASS6*	Homo sapiens spindle assembly 6 homolog (C. elegans) (SASS6), mRNA [NM_194292]	-1.07
SPTBN1	Homo sapiens spectrin, beta, non-erythrocytic 1 (SPTBN1), transcript variant 1, mRNA [NM_003128]	-1.21
SRGAP2	Homo sapiens SLIT-ROBO Rho GTPase activating protein 2 (SRGAP2), transcript variant 1, mRNA [NM_015326]	-1.17
TNS3	Homo sapiens tensin 3 (TNS3), mRNA [NM_022748]	-1.98
TOP2A*	Homo sapiens topoisomerase (DNA) II alpha 170kDa (TOP2A), mRNA [NM_001067]	-2.00
TPX2*	Homo sapiens TPX2, microtubule-associated, homolog (Xenopus laevis) (TPX2), mRNA [NM_012112]	-1.25
TRAF7	Homo sapiens TNF receptor-associated factor 7 (TRAF7), mRNA [NM_032271]	-1.01
TRIM14	Homo sapiens tripartite motif-containing 14 (TRIM14), transcript variant 1, mRNA	-1.87

	[NM_014788]	
TRIM59	Homo sapiens tripartite motif-containing 59 (TRIM59), mRNA [NM_173084]	-1.86
TRIM6	Homo sapiens tripartite motif-containing 6 (TRIM6), transcript variant 1, mRNA [NM_001003818]	-1.64
TUBA3D*	Homo sapiens tubulin, alpha 3d (TUBA3D), mRNA [NM_080386]	-1.20
TUBB2B*	Homo sapiens tubulin, beta 2B (TUBB2B), mRNA [NM_178012]	-1.07
TUBB6*	Homo sapiens tubulin, beta 6 (TUBB6), mRNA [NM_032525]	-1.21
UBE2T*	Homo sapiens ubiquitin-conjugating enzyme E2T (putative) (UBE2T), mRNA [NM_014176]	-1.14
UHRF1	Homo sapiens ubiquitin-like with PHD and ring finger domains 1 (UHRF1), transcript variant 2, mRNA [NM_013282]	-1.37
UTRN	Homo sapiens utrophin (UTRN), mRNA [NM_007124]	-1.01
YWHAH	Homo sapiens tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide (YWHAH), mRNA [NM_003405]	-1.34
ZFP36L1*	Homo sapiens zinc finger protein 36, C3H type-like 1 (ZFP36L1), mRNA [NM_004926]	-1.45
ZFP36L2*	Homo sapiens zinc finger protein 36, C3H type-like 2 (ZFP36L2), mRNA [NM_006887]	-1.22

Table S4. List of genes downregulated by CuO-NPs classified into the GO category of "cytokinesis". Fold-change is represented by logarithmic ratio (log_2 ratio) to expression level in control.

Gene name	Description	Fold-change (log_2 ratio)
ACTR3B	Homo sapiens ARP3 actin-related protein 3 homolog B (yeast) (ACTR3B), transcript variant 2, mRNA [NM_001040135]	-1.069
AURKA	Homo sapiens aurora kinase A (AURKA), transcript variant 1, mRNA [NM_198433]	-1.206
AURKB	Homo sapiens aurora kinase B (AURKB), mRNA [NM_004217]	-1.129
CIT	Homo sapiens citron (rho-interacting, serine/threonine kinase 21) (CIT), mRNA [NM_007174]	-1.821
GPSM2	Homo sapiens G-protein signaling modulator 2 (AGS3-like, C. elegans) (GPSM2), mRNA [NM_013296]	-1.185
KIF18A	Homo sapiens kinesin family member 18A (KIF18A), mRNA [NM_031217]	-1.165
KIF18B	Homo sapiens hypothetical protein LOC146909, mRNA (cDNA clone IMAGE:4418755), partial cds. [BC048263]	-2.068
KIF20A	Homo sapiens kinesin family member 20A (KIF20A), mRNA [NM_005733]	-2.049
KIF20B	Homo sapiens kinesin family member 20B (KIF20B), mRNA [NM_016195]	-1.253
KIF23	Homo sapiens kinesin family member 23 (KIF23), transcript variant 1, mRNA [NM_138555]	-1.562
KIFC1	Homo sapiens kinesin family member C1 (KIFC1), mRNA [NM_002263]	-1.264
MOBKL2B	Homo sapiens MOB1, Mps One Binder kinase activator-like 2B (yeast) (MOBKL2B), mRNA [NM_024761]	-1.112
MYH9	Homo sapiens myosin, heavy chain 9, non-muscle (MYH9), mRNA [NM_002473]	-1.213
MYO5C	Homo sapiens myosin VC (MYO5C), mRNA [NM_018728]	-1.393
PSKH1	Homo sapiens protein serine kinase H1 (PSKH1), mRNA [NM_006742]	-1.281
PTPN13	Homo sapiens protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase) (PTPN13), transcript variant 4, mRNA [NM_080685]	-1.774

Table S5. List of genes downregulated by CuO-NPs classified into the GO category of "chromosome segregation". Fold-change is represented by logarithmic ratio (log_2 ratio) to expression level in control.

Gene name	Description	Fold-change (log_2 ratio)
KIF18A	Homo sapiens kinesin family member 18A (KIF18A), mRNA [NM_031217]	-1.17
KIF18B	Homo sapiens hypothetical protein LOC146909, mRNA (cDNA clone IMAGE:4418755), partial cds. [BC048263]	-2.07
KIF20A	Homo sapiens kinesin family member 20A (KIF20A), mRNA [NM_005733]	-2.05
KIF20B	Homo sapiens kinesin family member 20B (KIF20B), mRNA [NM_016195]	-1.25
KIF23	Homo sapiens kinesin family member 23 (KIF23), transcript variant 1, mRNA [NM_138555]	-1.56
KIFC1	Homo sapiens kinesin family member C1 (KIFC1), mRNA [NM_002263]	-1.26
NUF2	Homo sapiens NUF2, NDC80 kinetochore complex component, homolog (S. cerevisiae) (NUF2), transcript variant 1, mRNA [NM_145697]	-1.17
OTUD4	Homo sapiens OTU domain containing 4 (OTUD4), transcript variant 1, mRNA [NM_199324]	-1.47
POLI	Homo sapiens polymerase (DNA directed) iota (POLI), mRNA [NM_007195]	-1.58
PTTG1	Homo sapiens pituitary tumor-transforming 1 (PTTG1), mRNA [NM_004219]	-1.04
SASS6	Homo sapiens spindle assembly 6 homolog (C. elegans) (SASS6), mRNA [NM_194292]	-1.07
TOP2A	Homo sapiens topoisomerase (DNA) II alpha 170kDa (TOP2A), mRNA [NM_001067]	-2.00
TPX2	Homo sapiens TPX2, microtubule-associated, homolog (Xenopus laevis) (TPX2), mRNA [NM_012112]	-1.25
TUBA3D	Homo sapiens tubulin, alpha 3d (TUBA3D), mRNA [NM_080386]	-1.20
TUBB2B	Homo sapiens tubulin, beta 2B (TUBB2B), mRNA [NM_178012]	-1.07
TUBB6	Homo sapiens tubulin, beta 6 (TUBB6), mRNA [NM_032525]	-1.21
UBE2T	Homo sapiens ubiquitin-conjugating enzyme E2T (putative) (UBE2T), mRNA [NM_014176]	-1.14

Table S6. List of genes upregulated by CuO-NPs classified into the GO category of "cellular component organization". Fold-change is represented by logarithmic ratio (log_2 ratio) to expression level in control. *, Also classified into the category of "cellular component morphogenesis"

Gene name	Description	Fold-change (log_2 ratio)
ACTR3B*	Homo sapiens ARP3 actin-related protein 3 homolog B (yeast) (ACTR3B), transcript variant 2, mRNA [NM_001040135]	-1.07
ANGPTL4*	Homo sapiens angiopoietin-like 4 (ANGPTL4), transcript variant 1, mRNA [NM_139314]	-1.09
ATR	Homo sapiens ataxia telangiectasia and Rad3 related (ATR), mRNA [NM_001184]	-1.11
ATRX	Homo sapiens alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, S. cerevisiae) (ATRX), transcript variant 1, mRNA [NM_000489]	-1.04
CENPA	Homo sapiens centromere protein A (CENPA), transcript variant 1, mRNA [NM_001809]	-1.80
CIT*	Homo sapiens citron (rho-interacting, serine/threonine kinase 21) (CIT), mRNA [NM_007174]	-1.82
CKAP5*	Homo sapiens cytoskeleton associated protein 5 (CKAP5), transcript variant 1, mRNA [NM_001008938]	-1.07
CLDN23*	Homo sapiens claudin 23 (CLDN23), mRNA [NM_194284]	-1.11
COCH*	Homo sapiens coagulation factor C homolog, cochlin (Limulus polyphemus) (COCH), transcript variant 2, mRNA [NM_004086]	-1.05
COL4A1*	Homo sapiens collagen, type IV, alpha 1 (COL4A1), mRNA [NM_001845]	-1.01
COL5A1*	Homo sapiens collagen, type V, alpha 1 (COL5A1), mRNA [NM_000093]	-1.30
COL5A2*	Homo sapiens collagen, type V, alpha 2 (COL5A2), mRNA [NM_000393]	-1.03
DAPK1*	Homo sapiens death-associated protein kinase 1 (DAPK1), mRNA [NM_004938]	-1.44
DLC1*	Homo sapiens deleted in liver cancer 1 (DLC1), transcript variant 1, mRNA [NM_182643]	-1.09
DLG5*	Homo sapiens discs, large homolog 5 (Drosophila) (DLG5), mRNA [NM_004747]	-1.36
DST*	Homo sapiens cDNA: FLJ21489 fis, clone COL05450. [AK025142]	-1.03
EHMT1	Homo sapiens euchromatic histone-lysine N-methyltransferase 1 (EHMT1), mRNA [NM_024757]	-1.18
EVI5*	Homo sapiens ecotropic viral integration site 5 (EVI5), mRNA [NM_005665]	-1.08
FOXF2*	Homo sapiens forkhead box F2 (FOXF2), mRNA [NM_001452]	-1.14
GEMIN5	Homo sapiens gem (nuclear organelle) associated protein 5 (GEMIN5), mRNA [NM_015465]	-1.59
GTSE1*	Homo sapiens G-2 and S-phase expressed 1 (GTSE1), mRNA [NM_016426]	-1.42
H1FX	Homo sapiens H1 histone family, member X (H1FX), mRNA [NM_006026]	-1.52
H2AFX	Homo sapiens H2A histone family, member X (H2AFX), mRNA [NM_002105]	-1.16
HMGB2	Homo sapiens high-mobility group box 2 (HMGB2), transcript variant 1, mRNA [NM_002129]	-2.06
IPP*	Homo sapiens intracisternal A particle-promoted polypeptide (IPP), mRNA [NM_005897]	-1.05
JUB*	Homo sapiens jub, ajuba homolog (Xenopus laevis) (JUB), transcript variant 1, mRNA [NM_032876]	-1.41
KIAA1804*	Homo sapiens mixed lineage kinase 4 (KIAA1804), mRNA [NM_032435]	-1.37
KIF18A*	Homo sapiens kinesin family member 18A (KIF18A), mRNA [NM_031217]	-1.17
KIF18B*	Homo sapiens hypothetical protein LOC146909, mRNA (cDNA clone IMAGE:4418755), partial cds. [BC048263]	-2.07
KIF20A*	Homo sapiens kinesin family member 20A (KIF20A), mRNA [NM_005733]	-2.05
KIF20B	Homo sapiens kinesin family member 20B (KIF20B), mRNA [NM_016195]	-1.25
KIF23*	Homo sapiens kinesin family member 23 (KIF23), transcript variant 1, mRNA [NM_138555]	-1.56
KIFC1*	Homo sapiens kinesin family member C1 (KIFC1), mRNA [NM_002263]	-1.26
KLHDC5*	Homo sapiens kelch domain containing 5 (KLHDC5), mRNA [NM_020782]	-1.43
LIMK2*	Homo sapiens LIM domain kinase 2 (LIMK2), transcript variant 2b, mRNA [NM_016733]	-1.18
LMNB1*	Homo sapiens lamin B1 (LMNB1), mRNA [NM_005573]	-2.04
LMNB2*	Homo sapiens lamin B2 (LMNB2), mRNA [NM_032737]	-1.71
MELK*	Homo sapiens maternal embryonic leucine zipper kinase (MELK), mRNA [NM_014791]	-1.11
MESDC1*	Homo sapiens mesoderm development candidate 1 (MESDC1), mRNA [NM_022566]	-1.01
MYO1B*	Homo sapiens myosin IB (MYO1B), transcript variant 2, mRNA [NM_012223]	-1.30
MYO5C*	Homo sapiens myosin VC (MYO5C), mRNA [NM_018728]	-1.39

NCAPD2	Homo sapiens non-SMC condensin I complex, subunit D2 (NCAPD2), mRNA [NM_014865]	-1.12
OBSL1*	Homo sapiens obscurin-like 1 (OBSL1), mRNA [NM_015311]	-1.11
OLFML2A*	Homo sapiens olfactomedin-like 2A (OLFML2A), mRNA [NM_182487]	-1.15
PCDH9*	Homo sapiens protocadherin 9 (PCDH9), transcript variant 1, mRNA [NM_203487]	-1.43
PDGFA*	Homo sapiens platelet-derived growth factor alpha polypeptide (PDGFA), transcript variant 1, mRNA [NM_002607]	-1.04
PODXL*	Homo sapiens podocalyxin-like (PODXL), transcript variant 1, mRNA [NM_001018111]	-1.69
PSKH1*	Homo sapiens protein serine kinase H1 (PSKH1), mRNA [NM_006742]	-1.28
PSRC1*	Homo sapiens proline/serine-rich coiled-coil 1 (PSRC1), transcript variant 1, mRNA [NM_032636]	-1.61
SASS6	Homo sapiens spindle assembly 6 homolog (C. elegans) (SASS6), mRNA [NM_194292]	-1.07
SETBP1	Homo sapiens SET binding protein 1 (SETBP1), transcript variant 1, mRNA [NM_015559]	-1.04
SIM2	Homo sapiens single-minded homolog 2 (Drosophila) (SIM2), transcript variant SIM2, mRNA [NM_005069]	-1.41
SPTBN1*	Homo sapiens spectrin, beta, non-erythrocytic 1 (SPTBN1), transcript variant 1, mRNA [NM_003128]	-1.21
TBC1D9B*	Homo sapiens TBC1 domain family, member 9B (with GRAM domain) (TBC1D9B), transcript variant 1, mRNA [NM_198868]	-1.08
THSD7A*	Homo sapiens cDNA FLJ11022 fis, clone PLACE1003771. [AK001884]	-1.39
TPM1*	Homo sapiens tropomyosin 1 (alpha) (TPM1), transcript variant 5, mRNA [NM_000366]	-1.09
TRIOBP*	Homo sapiens TRIO and F-actin binding protein (TRIOBP), transcript variant 6, mRNA [NM_001039141]	-1.20
TUBA3D*	Homo sapiens tubulin, alpha 3d (TUBA3D), mRNA [NM_080386]	-1.20
TUBB2B*	Homo sapiens tubulin, beta 2B (TUBB2B), mRNA [NM_178012]	-1.07
TUBB6*	Homo sapiens tubulin, beta 6 (TUBB6), mRNA [NM_032525]	-1.21
UTRN*	Homo sapiens utrophin (UTRN), mRNA [NM_007124]	-1.01

Table S7. List of shared genes whose expressions were upregulated in cells exposed to both CuO-NPs and released Cu ions. Fold-change is represented by logarithmic ratio (log_2 ratio) to expression level in control.

Gene name	Description	Fold-change (log_2 ratio)	
		CuO-NPs	Cu ions
MT1F	Homo sapiens metallothionein 1F (MT1F), mRNA [NM_005949]	4.80	4.59
NR4A1	Homo sapiens nuclear receptor subfamily 4, group A, member 1 (NR4A1), transcript variant 1, mRNA [NM_002135]	5.28	2.71
LOC100129113	Homo sapiens cDNA FLJ37158 fis, clone BRACE2026293. [AK094477]	2.71	2.31
DHRS2	Homo sapiens dehydrogenase/reductase (SDR family) member 2 (DHRS2), transcript variant 1, mRNA [NM_182908]	2.45	2.24
CSTA	Homo sapiens cystatin A (stefin A) (CSTA), mRNA [NM_005213]	2.15	2.12
VCX3A	Homo sapiens variable charge, X-linked 3A (VCX3A), mRNA [NM_016379]	5.29	2.11
MT1G	Homo sapiens metallothionein 1G (MT1G), mRNA [NM_005950]	2.31	2.10
NUPR1	Homo sapiens nuclear protein 1 (NUPR1), transcript variant 1, mRNA [NM_001042483]	2.40	2.10
MT2A	Homo sapiens metallothionein 2A (MT2A), mRNA [NM_005953]	2.24	1.98
CDK5R2	Homo sapiens cyclin-dependent kinase 5, regulatory subunit 2 (p39) (CDK5R2), mRNA [NM_003936]	2.06	1.96
MT1E	Homo sapiens unknown mRNA. [AF495759]	2.16	1.95
HTRA3	Homo sapiens HtrA serine peptidase 3 (HTRA3), mRNA [NM_053044]	2.51	1.93
LOC133874	Homo sapiens hypothetical gene LOC133874 (LOC133874), mRNA [NM_001102609]	1.92	1.84
S100P	Homo sapiens S100 calcium binding protein P (S100P), mRNA [NM_005980]	2.15	1.84
MT1A	Homo sapiens metallothionein 1A (MT1A), mRNA [NM_005946]	2.19	1.75
MT1X	Homo sapiens metallothionein 1X (MT1X), mRNA [NM_005952]	1.98	1.71
SPANXD	Homo sapiens SPANX family, member D (SPANXD), mRNA [NM_032417]	2.54	1.68
GABARAPL1	Homo sapiens GABA(A) receptor-associated protein like 1 (GABARAPL1), mRNA [NM_031412]	4.44	1.62
MT1B	Homo sapiens metallothionein 1B (MT1B), mRNA [NM_005947]	2.24	1.60
INSIG1	Homo sapiens insulin induced gene 1 (INSIG1), transcript variant 2, mRNA [NM_198336]	2.42	1.58
MT1H	Homo sapiens metallothionein 1H (MT1H), mRNA [NM_005951]	1.98	1.57
SPANXA1	Homo sapiens sperm protein associated with the nucleus, X-linked, family member A1 (SPANXA1), mRNA [NM_013453]	2.18	1.55
MT1L	Homo sapiens metallothionein 1L (gene/pseudogene) (MT1L), non-coding RNA [NR_001447]	1.79	1.52
BEX2	Homo sapiens brain expressed X-linked 2 (BEX2), mRNA [NM_032621]	3.29	1.49
SPANXB2	Homo sapiens SPANX family, member B2 (SPANXB2), mRNA [NM_145664]	2.20	1.48
SNX8	Homo sapiens sorting nexin 8 (SNX8), mRNA [NM_013321]	1.80	1.45
KIAA0430	Homo sapiens KIAA0430 (KIAA0430), mRNA [NM_014647]	1.47	1.44
IGF2	Homo sapiens insulin-like growth factor 2 (somatomedin A) (IGF2), transcript variant 1, mRNA [NM_000612]	1.36	1.47
CLCN6	Homo sapiens chloride channel 6 (CLCN6), transcript variant ClC-6a, mRNA [NM_001286]	1.73	1.32
ASNS	Homo sapiens asparagine synthetase, mRNA (cDNA clone IMAGE:5266877), **** WARNING: chimeric clone ****. [BC030024]	1.58	1.32
PNPLA8	Homo sapiens patatin-like phospholipase domain containing 8 (PNPLA8), mRNA [NM_015723]	2.06	1.27
TAF8	Homo sapiens TAF8 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 43kDa, mRNA (cDNA clone IMAGE:5166848), with apparent retained intron. [BC033728]	1.29	1.24
TRIB3	Homo sapiens tribbles homolog 3 (Drosophila) (TRIB3), mRNA [NM_021158]	1.57	1.20
SEC61A2	Homo sapiens Sec61 alpha 2 subunit (S. cerevisiae) (SEC61A2), transcript variant 1, mRNA [NM_018144]	1.72	1.15
OR5L2	Homo sapiens olfactory receptor, family 5, subfamily L, member 2 (OR5L2), mRNA [NM_001004739]	1.36	1.14

FXC1	Homo sapiens fracture callus 1 homolog (rat) (FXC1), nuclear gene encoding mitochondrial protein, mRNA [NM_012192]	1.13	1.14
BNIP3L	Homo sapiens BCL2/adenovirus E1B 19kDa interacting protein 3-like (BNIP3L), mRNA [NM_004331]	2.17	1.11
PPP1R3B	Homo sapiens protein phosphatase 1, regulatory (inhibitor) subunit 3B (PPP1R3B), mRNA [NM_024607]	1.14	1.10
SIRT6	Homo sapiens sirtuin (silent mating type information regulation 2 homolog) 6 (S. cerevisiae) (SIRT6), mRNA [NM_016539]	1.68	1.10
CYB5R2	Homo sapiens cytochrome b5 reductase 2 (CYB5R2), mRNA [NM_016229]	1.08	1.11
GDF15	Homo sapiens growth differentiation factor 15 (GDF15), mRNA [NM_004864]	1.08	1.95
CTSL2	Homo sapiens cathepsin L2 (CTSL2), mRNA [NM_001333]	1.44	1.07
SOD2	Homo sapiens superoxide dismutase 2, mitochondrial (SOD2), nuclear gene encoding mitochondrial protein, transcript variant 2, mRNA [NM_001024465]	1.28	1.07
C1S	Homo sapiens complement component 1, s subcomponent (C1S), transcript variant 1, mRNA [NM_001734]	1.06	1.21
MSI2	Homo sapiens musashi homolog 2 (Drosophila) (MSI2), transcript variant 2, mRNA [NM_170721]	1.52	1.06
SREBF2	Homo sapiens sterol regulatory element binding transcription factor 2 (SREBF2), mRNA [NM_004599]	1.32	1.06
LARP4	Homo sapiens La ribonucleoprotein domain family, member 4 (LARP4), transcript variant 2, mRNA [NM_199188]	1.76	1.05
MVD	Homo sapiens mevalonate (diphospho) decarboxylase (MVD), mRNA [NM_002461]	1.04	1.61
SEC14L1	Homo sapiens SEC14-like 1 (S. cerevisiae) (SEC14L1), transcript variant 1, mRNA [NM_003003]	1.72	1.03
NFATC1	Homo sapiens nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1 (NFATC1), transcript variant 1, mRNA [NM_172390]	1.83	1.03
TBC1D15	Homo sapiens TBC1 domain family, member 15 (TBC1D15), mRNA [NM_022771]	1.98	1.02
SAT1	Homo sapiens spermidine/spermine N1-acetyltransferase 1 (SAT1), mRNA [NM_002970]	1.53	1.02
C10orf35	Homo sapiens chromosome 10 open reading frame 35 (C10orf35), mRNA [NM_145306]	1.37	1.02
RNF12	Homo sapiens ring finger protein 12 (RNF12), transcript variant 1, mRNA [NM_016120]	1.68	1.01

Table S8. List of shared genes whose expressions were downregulated in cells exposed to both CuO-NPs and released Cu ions. Fold-change is represented by logarithmic ratio (log_2 ratio) to expression level in control.

Gene name	Description	Fold-change (log_2 ratio)	
		CuO-NPs	Cu ions
FAM83D	Homo sapiens family with sequence similarity 83, member D (FAM83D), mRNA [NM_030919]	-2.40	-2.25
HMGB2	Homo sapiens high-mobility group box 2 (HMGB2), transcript variant 1, mRNA [NM_002129]	-2.06	-1.83
IGFBP3	Homo sapiens insulin-like growth factor binding protein 3 (IGFBP3), transcript variant 1, mRNA [NM_001013398]	-2.40	-1.81
CENPA	Homo sapiens centromere protein A (CENPA), transcript variant 1, mRNA [NM_001809]	-1.80	-2.04
TRIM59	Homo sapiens tripartite motif-containing 59 (TRIM59), mRNA [NM_173084]	-1.86	-1.59
KIF20A	Homo sapiens kinesin family member 20A (KIF20A), mRNA [NM_005733]	-2.05	-1.53
MYBL1	Homo sapiens v-myb myeloblastosis viral oncogene homolog (avian)-like 1 (MYBL1), mRNA [NM_001080416]	-1.84	-1.48
CKAP2	Homo sapiens cytoskeleton associated protein 2 (CKAP2), transcript variant 1, mRNA [NM_018204]	-1.76	-1.44
LOC338620	Homo sapiens hypothetical protein LOC338620, mRNA (cDNA clone IMAGE:6023208), partial cds. [BC043009]	-2.01	-1.42
TOP2A	Homo sapiens topoisomerase (DNA) II alpha 170kDa (TOP2A), mRNA [NM_001067]	-2.00	-1.41
CCDC80	Homo sapiens coiled-coil domain containing 80 (CCDC80), transcript variant 1, mRNA [NM_199511]	-1.40	-1.41
BARD1	Homo sapiens BRCA1 associated RING domain 1 (BARD1), mRNA [NM_000465]	-1.35	-1.55
CCNB1	Homo sapiens cyclin B1 (CCNB1), mRNA [NM_031966]	-1.74	-1.33
G0S2	Homo sapiens G0/G1switch 2 (G0S2), mRNA [NM_015714]	-1.33	-1.56
NFE2L3	Homo sapiens nuclear factor (erythroid-derived 2)-like 3 (NFE2L3), mRNA [NM_004289]	-1.99	-1.32
ZNF185	Homo sapiens zinc finger protein 185 (LIM domain) (ZNF185), mRNA [NM_007150]	-1.28	-1.77
ALPK2	Homo sapiens alpha-kinase 2 (ALPK2), mRNA [NM_052947]	-1.58	-1.28
PABPC3	Homo sapiens poly(A) binding protein, cytoplasmic 3 (PABPC3), mRNA [NM_030979]	-1.71	-1.26
SLC27A2	Homo sapiens solute carrier family 27 (fatty acid transporter), member 2 (SLC27A2), mRNA [NM_003645]	-1.26	-1.57
HN1	Homo sapiens hematological and neurological expressed 1 (HN1), transcript variant 3, mRNA [NM_001002033]	-1.25	-1.32
C15orf23	Homo sapiens chromosome 15 open reading frame 23 (C15orf23), transcript variant 2, mRNA [NM_001142761]	-1.63	-1.24
CDC20	Homo sapiens cell division cycle 20 homolog (S. cerevisiae) (CDC20), mRNA [NM_001255]	-1.23	-1.42
EFEMP1	Homo sapiens EGF-containing fibulin-like extracellular matrix protein 1 (EFEMP1), transcript variant 1, mRNA [NM_004105]	-1.78	-1.22
RACGAP1	Homo sapiens Rac GTPase activating protein 1 (RACGAP1), transcript variant 1, mRNA [NM_013277]	-1.56	-1.22
KIF23	Homo sapiens kinesin family member 23 (KIF23), transcript variant 1, mRNA [NM_138555]	-1.56	-1.21
TUBA3D	Homo sapiens tubulin, alpha 3d (TUBA3D), mRNA [NM_080386]	-1.20	-1.32
LOC100128974	PREDICTED: Homo sapiens misc_RNA (LOC100128974), miscRNA [XR_037045]	-1.31	-1.19
LMNB2	Homo sapiens lamin B2 (LMNB2), mRNA [NM_032737]	-1.71	-1.19
PLAGL1	Homo sapiens pleiomorphic adenoma gene-like 1 (PLAGL1), transcript variant 2, mRNA [NM_006718]	-1.18	-1.50
CCNA2	Homo sapiens cyclin A2 (CCNA2), mRNA [NM_001237]	-1.39	-1.18
PRSS23	Homo sapiens protease, serine, 23 (PRSS23), mRNA [NM_007173]	-1.80	-1.17
TPX2	Homo sapiens TPX2, microtubule-associated, homolog (Xenopus laevis) (TPX2), mRNA [NM_012112]	-1.25	-1.17
PIF1	Homo sapiens PIF1 5'-to-3' DNA helicase homolog (S. cerevisiae) (PIF1), mRNA [NM_025049]	-1.65	-1.16

CDC2	Homo sapiens cell division cycle 2, G1 to S and G2 to M (CDC2), transcript variant 1, mRNA [NM_001786]	-1.41	-1.16
RRM2	Homo sapiens ribonucleotide reductase M2 polypeptide (RRM2), mRNA [NM_001034]	-1.38	-1.15
SFRP1	Homo sapiens secreted frizzled-related protein 1 (SFRP1), mRNA [NM_003012]	-1.14	-1.16
TMEM171	Homo sapiens transmembrane protein 171 (TMEM171), mRNA [NM_173490]	-1.15	-1.14
GPSM2	Homo sapiens G-protein signaling modulator 2 (AGS3-like, C. elegans) (GPSM2), mRNA [NM_013296]	-1.19	-1.14
TRIM14	Homo sapiens tripartite motif-containing 14 (TRIM14), transcript variant 1, mRNA [NM_014788]	-1.87	-1.13
TRAM1	Homo sapiens translocation associated membrane protein 1 (TRAM1), mRNA [NM_014294]	-1.11	-1.40
LOC389842	PREDICTED: Homo sapiens similar to RanBP1 (LOC389842), mRNA [XM_372200]	-1.44	-1.11
TPM1	Homo sapiens tropomyosin 1 (alpha) (TPM1), transcript variant 5, mRNA [NM_000366]	-1.09	-1.55
HYLS1	Homo sapiens hydrolethalus syndrome 1 (HYLS1), transcript variant 1, mRNA [NM_145014]	-1.25	-1.09
TMSB4X	Homo sapiens thymosin beta 4, X-linked (TMSB4X), mRNA [NM_021109]	-1.13	-1.09
IRS1	Homo sapiens insulin receptor substrate 1 (IRS1), mRNA [NM_005544]	-2.21	-1.08
ABCB10	Homo sapiens ATP-binding cassette, sub-family B (MDR/TAP), member 10 (ABCB10), nuclear gene encoding mitochondrial protein, mRNA [NM_012089]	-1.70	-1.07
KIF18B	Homo sapiens hypothetical protein LOC146909, mRNA (cDNA clone IMAGE:4418755), partial cds. [BC048263]	-2.07	-1.07
LOC100132658	PREDICTED: Homo sapiens misc_RNA (LOC100132658), miscRNA [XR_038952]	-1.15	-1.07
FBXO5	Homo sapiens F-box protein 5 (FBXO5), transcript variant 1, mRNA [NM_012177]	-1.17	-1.06
TGFB2	Homo sapiens transforming growth factor, beta 2 (TGFB2), transcript variant 1, mRNA [NM_001135599]	-2.11	-1.05
CDCA3	Homo sapiens cell division cycle associated 3 (CDCA3), mRNA [NM_031299]	-1.34	-1.04
AREG	Homo sapiens amphiregulin (AREG), mRNA [NM_001657]	-1.50	-1.02
ZFP36L1	Homo sapiens zinc finger protein 36, C3H type-like 1 (ZFP36L1), mRNA [NM_004926]	-1.45	-1.02
DLGAP5	Homo sapiens discs, large (Drosophila) homolog-associated protein 5 (DLGAP5), mRNA [NM_014750]	-1.34	-1.01
BCAR3	Homo sapiens breast cancer anti-estrogen resistance 3 (BCAR3), mRNA [NM_003567]	-1.10	-1.00

Table S9. Shared downregulated genes by CuO-NPs and released Cu ions, which fall into the categories of "mitosis", chromosome segregation", and "cell cycle".

GO category	Gene name	Description
mitosis	CCNA2	Homo sapiens cyclin A2 (CCNA2), mRNA [NM_001237]
	CCNB1	Homo sapiens cyclin B1 (CCNB1), mRNA [NM_031966]
	CDC2	Homo sapiens cell division cycle 2, G1 to S and G2 to M (CDC2), transcript variant 1, mRNA [NM_001786]
	GPSM2	Homo sapiens G-protein signaling modulator 2 (AGS3-like, C. elegans) (GPSM2), mRNA [NM_013296]
	KIF18B	Homo sapiens hypothetical protein LOC146909, mRNA (cDNA clone IMAGE:4418755), partial cds. [BC048263]
	KIF20A	Homo sapiens kinesin family member 20A (KIF20A), mRNA [NM_005733]
	KIF23	Homo sapiens kinesin family member 20A (KIF20A), mRNA [NM_005733]
	TOP2A	Homo sapiens topoisomerase (DNA) II alpha 170kDa (TOP2A), mRNA [NM_001067]
	TPX2	Homo sapiens TPX2, microtubule-associated, homolog (Xenopus laevis) (TPX2), mRNA [NM_012112]
	TUBA3D	Homo sapiens tubulin, alpha 3d (TUBA3D), mRNA [NM_080386]
	ZFP36L1	Homo sapiens zinc finger protein 36, C3H type-like 1 (ZFP36L1), mRNA [NM_004926]
chromosome segregation	KIF18B	Homo sapiens hypothetical protein LOC146909, mRNA (cDNA clone IMAGE:4418755), partial cds. [BC048263]
	KIF20A	Homo sapiens kinesin family member 20A (KIF20A), mRNA [NM_005733]
	KIF23	Homo sapiens kinesin family member 20A (KIF20A), mRNA [NM_005733]
	TOP2A	Homo sapiens topoisomerase (DNA) II alpha 170kDa (TOP2A), mRNA [NM_001067]
	TPX2	Homo sapiens TPX2, microtubule-associated, homolog (Xenopus laevis) (TPX2), mRNA [NM_012112]
	TUBA3D	Homo sapiens tubulin, alpha 3d (TUBA3D), mRNA [NM_080386]
cell cycle	CCNA2	Homo sapiens cyclin A2 (CCNA2), mRNA [NM_001237]
	CCNB1	Homo sapiens cyclin B1 (CCNB1), mRNA [NM_031966]
	CDC2	Homo sapiens cell division cycle 2, G1 to S and G2 to M (CDC2), transcript variant 1, mRNA [NM_001786]
	CDC20	Homo sapiens cell division cycle 20 homolog (S. cerevisiae) (CDC20), mRNA [NM_001255]
	GPSM2	Homo sapiens G-protein signaling modulator 2 (AGS3-like, C. elegans) (GPSM2), mRNA [NM_013296]
	KIF18B	Homo sapiens hypothetical protein LOC146909, mRNA (cDNA clone IMAGE:4418755), partial cds.

		[BC048263]
	KIF20A	Homo sapiens kinesin family member 20A (KIF20A), mRNA [NM_005733]
	KIF23	Homo sapiens kinesin family member 20A (KIF20A), mRNA [NM_005733]
	MYBL1	Homo sapiens v-myb myeloblastosis viral oncogene homolog (avian)-like 1 (MYBL1), mRNA [NM_001080416]
	TOP2A	Homo sapiens topoisomerase (DNA) II alpha 170kDa (TOP2A), mRNA [NM_001067]
cell cycle	TPX2	Homo sapiens TPX2, microtubule-associated, homolog (Xenopus laevis) (TPX2), mRNA [NM_012112]
	TRIM14	Homo sapiens tripartite motif-containing 14 (TRIM14), transcript variant 1, mRNA [NM_014788]
	TRIM59	Homo sapiens tripartite motif-containing 59 (TRIM59), mRNA [NM_173084]
	TUBA3D	Homo sapiens tubulin, alpha 3d (TUBA3D), mRNA [NM_080386]
	ZFP36L1	Homo sapiens zinc finger protein 36, C3H type-like 1 (ZFP36L1), mRNA [NM_004926]

Table S10. Primer sequences for qPCR

Gene Name	Forward sequence (5'→3')	Reverse sequence (5'→3')
GADD45A	ctgaacggtgatggcatctg	ccccttggcatcagtttctg
GADD45B	taccgttggtttccgcaact	gccagagagcccaaaacctt
GADD45G	gtgctgagctctggctgtca	gctgtgctttccggaactgt
PCNA	ggtgttggaggcactcaagg	ccaaagagacgtgggacgag
CDC2	ttcagagctttgggcactcc	gggatgctaggcttcctggt
CCNB1	actgcaggccaaaatgccta	aggttctggctctggcactg
CDKN1A	tcctctagctgtgggggtga	aaggtcgctggacgatttga
FOS	cctcgtactccaaccgcatc	tgggtaggagcacggtcact
FOSB	caagaggtacagcggcatcc	caacgtcccgttccaacaat
ATF3	tgggtccagaagacctgcat	aaaccctggtgatgccacag
JDP2	tgaaggaggcaggacagagg	tcatggcttttcctggctgt
ATR	gctctggtccaagggtgatg	accctcaggtggggtttcat
TP53	cgtcccaagcaatggatgat	tggcattctgggagcttcat
NR4A1	gcaccttcatggacggctac	ctgaggagcatggctggact
NR4A2	tgtaccaaatgcccctgtcc	gagtgcggcatcatctcctc
NR4A3	agccttcctgcgtgtaccaa	aatggatggctgctgatgct
AURKA	tcagcgggtcttgtgtcctt	aaccggcttgtgactggaga
AURKB	ccccatctgcacttgtcctc	tgtgaagtgccgcgttaaga
TPX2	ccccctcggatttcatcaat	ttggccttctcctcaaacca

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Induction of glandular stomach cancers in *Helicobacter pylori*-infected Mongolian Gerbils by 1-nitrosoindole-3-acetonitrile

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***Helicobacter pylori* (*H. pylori*) infection and high intake of various traditional salt-preserved foods are regarded as risk factors for human gastric cancer. We previously reported that Chinese cabbage contains indole compounds, such as indole-3-acetonitrile, a mutagen precursor. 1-Nitrosoindole-3-acetonitrile (NIAN), formed by the treatment of indole-3-acetonitrile with nitrite under acidic conditions, shows direct-acting mutagenicity. In the present study, NIAN administration by gavage to Mongolian gerbils (MGs) at the dose of 100 mg/kg two times a week resulted in three adduct spots (1.6 adducts/10^8 nucleotides in total), detected in DNA samples from the glandular stomach by ^{32}P-postlabeling methods. Treatment with six consecutive doses of 100 mg/kg of NIAN, two times a week for 3 weeks, induced well—and moderately—differentiated glandular stomach adenocarcinomas in the MGs at the incidence of 31% under *H. pylori* infection at 54–104 weeks. Such lesions were not induced in MGs given broth alone, broth + NIAN or infection with *H. pylori* alone. Thus, endogenous carcinogens formed from nitrosation of indole compounds could be critical risk factors for human gastric cancer development under the influence of *H. pylori* infection.**

Gastric cancer is the second most frequent cause of cancer death worldwide.[1] Although gastric cancer has become a relatively rare cancer in North America and most Northern and Western European countries, it remains common in East Asia, Eastern Europe, Russia, and selected areas of Central and South America.[2] *Helicobacter pylori* (*H. pylori*) is a well-established major risk factor for gastric cancer,[3–5] and the prevalence of *H. pylori* infection in East Asia countries, including Japan and Korea is reported to be relatively high.[6,7] In addition, the risk of gastric cancer is increased with a high intake of various traditional salt-preserved foods.[3] In fact, pickled vegetable consumption is reported to increase gastric cancer risk in Japan and Korea.[8–10] In Korea, kimchi, commonly prepared with Chinese cabbage or radish, is a traditional and popular food, which contains high levels of nitrate (median 1550 mg/kg).[11] Furthermore, Chinese cabbage is well known as a pickled vegetable commonly consumed in Japan. Moreover, ingestion of nitrate, mainly from food, is suggested to correlate with mortality from gastric cancer.[12–14] Ingested nitrate is mainly converted to nitrite by bacteria in the oral cavity after secretion into saliva.[15] Carcinogenic *N*-nitroso compounds can be formed from nitrite and secondary amines under acidic conditions. Furthermore, direct-acting *N*-nitroso compounds, such as *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG)[16] and *N*-methyl-*N*-nitrosourea (MNU),[17] are known to induce cancer in the glandular stomach of experimental animals. Thus, it is suggested that *N*-nitroso compounds that are formed in the stomach under acidic conditions could be positively associated with the risk of gastric cancer. Nitric oxide, formed by nitric oxide synthase, is also reported to contribute to production of *N*-nitroso compounds.[18]

We have previously reported that treatments of various foodstuffs with nitrite under acidic conditions produce direct-acting mutagens towards *Salmonella* tester strains.[19,20] Among those foodstuffs, Chinese cabbage is shown to contain three indole compounds, indole-3-acetonitrile, 4-methoxyindole-3-acetonitrile and 4-methoxyindole-3-aldehyde as mutagen precursors. 1-Nitrosoindole-3-acetonitrile (NIAN), an *N*-nitroso-substituted compound formed by treatment of indole-3-

Key words: gastric cancer, *Helicobacter pylori*, Mongolian gerbil 1-nitrosoindole-3-acetonitrile, indole-3-acetonitrile

Abbreviations: DMSO: dimethyl sulfoxide; H&E: hematoxylin and eosin; *H. pylori*: *Helicobacter pylori*; MG: Mongolian gerbil; MNNG: *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; MNU: *N*-methyl-*N*-nitrosourea; NIAN: 1-nitrosoindole-3-acetonitrile.

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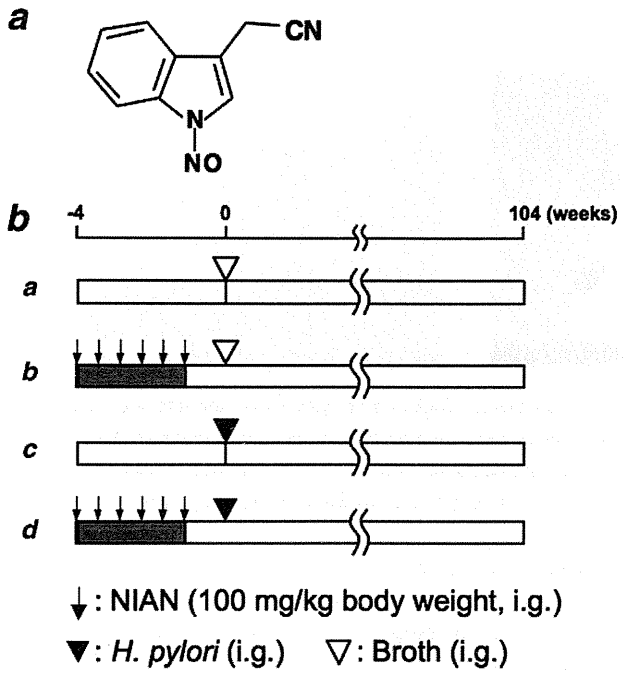

Figure 1. Chemical structure of NIAN and experimental protocol for the carcinogenicity study. (*a*) Chemical structure of NIAN. (*b*) Male 6-week-old MGs were orally administered NIAN (100 mg/kg) in 50% DMSO (groups B and D) or 50% DMSO alone (groups A and C) two times a week for 3 weeks. One week after the final administration, the animals were inoculated with *H. pylori* (ATCC 43504) (groups C and D) or sterilized broth (groups A and B).

acetonitrile with nitrite under acidic conditions, is a direct-acting mutagen in *S. typhimurium* and Chinese hamster lung cells,[20–22] and it is confirmed to form DNA adducts and to induce DNA single-strand scission in the rat glandular stomach.[23,24] Therefore, NIAN could play some role in gastric cancer development, as in the case of the well-known direct-acting mutagens, MNNG and MNU, in animal experiments.[16,17,25]

The Mongolian gerbil (MG) is reported to be susceptible to colonization by *H. pylori*, and *H. pylori* infection greatly enhances MNNG or MNU-induced gastric carcinogenesis in MGs.[26,27] Therefore, the MG is considered to be a useful animal model for evaluating the gastric cancer risk of direct-acting *N*-nitroso compounds, with or without *H. pylori* infection.

Chinese cabbage, containing nitrate and indole compounds, is commonly consumed in East Asian countries, including Japan, Korea and China, in which gastric cancer mortality is very high. In the present study, DNA adducts were detected with NIAN treatment in the glandular stomach of MGs, and the carcinogenicity of NIAN for gastric cancer *in vivo* was examined. The results clearly demonstrated that gastric cancer developed with a combination of NIAN administration and *H. pylori* infection in MGs. Possible involvement of indole compounds and nitrate derived from various foodstuffs, including Chinese cabbage, in gastric cancer development in humans is discussed.

Material and Methods

Materials

Indole-3-acetonitrile was purchased from Tokyo Food Techno (Tokyo, Japan), sodium nitrite from Wako Pure Chemical Industries (Osaka, Japan) and ammonium sulfamate from Kanto Chemical (Tokyo, Japan). Brucella broth was obtained from Becton Dickinson (Cockeysville, MD) and horse serum from Nippon Bio-Supply (Tokyo, Japan).

Preparation of NIAN

The chemical structure of NIAN is shown in Figure 1*a*. Indole-3-acetonitrile in 27 mM citrate-phosphate buffer (pH 3.0) was treated with 50 mM sodium nitrite for 1 hr at room temperature in the dark, as previously reported.[21] Nitrosation was stopped by addition of ammonium sulfamate at a final concentration of 50 mM. The reaction solution was filtered and the residue was washed with deionized water, then with *n*-hexane. The residual paste was dried and stored at −80°C until use. The preparation was >93% pure as judged by its UV absorbance on HPLC.

Bacterial culture

H. pylori (ATCC 43504; American Type Culture Collection, Manassas, VA) was cultured in brucella broth supplemented with 10% heat-inactivated horse serum for 24 hr at 37°C under microaerobic conditions (5% O_2, 10% CO_2 and 85% N_2), as previously described.[28]

Animal treatment

Specific pathogen-free male, 6-week-old MGs (MGS/Sea, Kyudo, Fukuoka, Japan) were housed in a biohazard room, air-conditioned at 24°C ± 2°C and 55% humidity, on a 12 hr light–dark cycle and were allowed free access to commercial diet (CE-2; CLEA Japan, Tokyo, Japan) and water.

To analyze the formation of DNA adducts in the glandular stomach of MGs by NIAN treatment, NIAN was dissolved in 50% dimethyl sulfoxide (DMSO), and administered to three MGs by gavage of 0.5 ml solution, two times a week at a level of 100 mg/kg body weight. Two further MGs served as a control group receiving the solvent alone (0.5 ml). At 8 hr after administration of NIAN, both groups of animals were sacrificed under ether anesthesia, and their stomachs were resected and stored at −80°C until use. DNA was extracted by a standard procedure with enzymatic digestion of protein and RNA followed by extraction with phenol and chloroform/isoamyl alcohol (24:1, v/v).

The protocol for long-term gastric carcinogenicity in MGs treated with NIAN + *H. pylori* infection is illustrated in Figure 1*b*. The animals were randomly divided into four groups (groups A–D). Groups A and C were given 50% DMSO without NIAN (0.5 ml) whereas groups B and D were orally administrated NIAN (0.5 ml, 100 mg/kg body weight) dissolved in 50% DMSO by gavage, two times a week for 3 weeks. At one week after the last administration, the

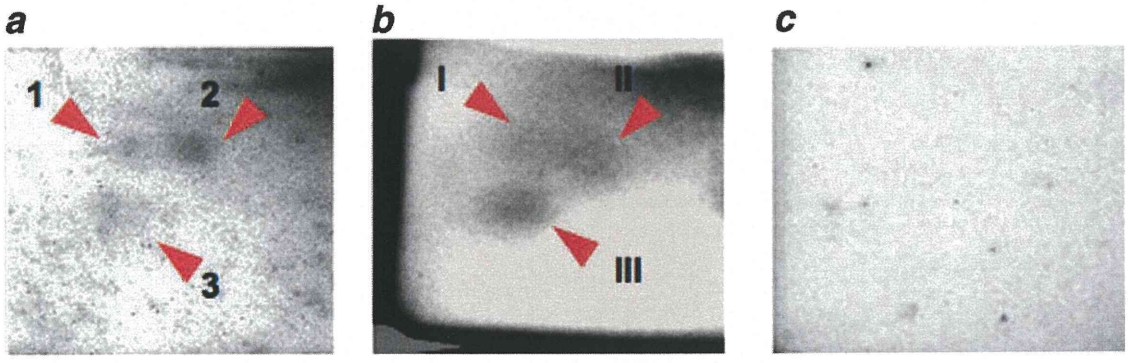

Figure 2. Autoradiograms of NIAN-DNA adducts in glandular stomach of MGs or calf thymus DNA treated with NIAN. Adducts were analyzed by ^{32}P-postlabeling method, as described in the Material and Methods. DNA samples were isolated from glandular stomach of MGs (*a*) or calf thymus DNA (*b*) after treatment with NIAN. DNA samples were also prepared from glandular stomach of MGs without NIAN treatment (*c*). Arrowheads indicate adducts. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

animals of groups C and D were given an intragastric inoculation of *H. pylori* broth culture (0.5 ml, 0.9×10^8 CFU/animal) whereas animals of groups A and B were given sterilized broth alone (0.5 ml).[28]

During the experiments, animals which became moribund or emaciated (<80 g body weight) were sacrificed. At 104 weeks after *H. pylori* infection, all surviving animals were sacrificed under ether anesthesia. At performance of necropsy, all tissues were carefully checked macroscopically and the stomachs and major organs were removed and assessed for macroscopic lesion development. Effective numbers of animals were defined as those surviving until week 54 of the study, when gastric tumors were observed for the first time. In addition, in the *H. pylori*-infected groups, the animals developing gastritis observed on histological examination were regarded as effective. The percentages of gastritis-bearing animals by the single inoculation of *H. pylori* were 62% for group C and 76% for group D, being similar to those previously reported.[27] All animal experiments were performed according to the "Guidelines for Animal Experiments in the National Cancer Center" and were approved by the Institutional Ethics Review Committee for Animal Experimentation in the National Cancer Center.

Detection of DNA adducts by ^{32}P-postlabeling method

Calf thymus DNA (0.5 mg, Sigma, St. Louis, MO) treated with NIAN (3 mg) for 12 hr under neutral conditions was used for authentic NIAN-DNA adducts.[23] DNA samples from the glandular stomach of MGs and calf thymus DNA samples were digested with micrococcal nuclease and phosphodiesterase II, and subjected to ^{32}P-postlabeling analysis using the same procedure as described previously[23] except with solvent systems for two-dimensional development. The solvent system consisted of buffer A (4.0 M lithium formate, 7.7 M urea, pH 3.5) from bottom to top, and buffer B (0.90 M lithium chloride, 0.45 M Tris-HCl, 7.7 M urea, pH 8.0) from left to right, followed by 1.7 M sodium phosphate buffer, pH 6.0, from left to right, with 3.5 cm filter paper. Adducts were detected with a Bio-Image Analyzer (BAS 3000; Fuji Photo Film, Tokyo, Japan) after exposing the TLC sheets to Fuji imaging plates. Relative adduct labeling was determined by the methods of Reddy *et al.*,[29] and values were calculated as averages using data from three assays.

Histological examination

All excised stomachs were opened along the greater curvature and washed twice with saline, then fixed in 10% neutral-buffered formalin. The fixed stomachs were sliced along the longitudinal axis into 9–12 strips of equal width, and routinely processed to sections stained with hematoxylin and eosin (H&E). The degree of chronic active gastritis was graded according to criteria modified from the Updated Sydney System,[30] by scoring the infiltration of neutrophils and mononuclear cells. Other organs, in which macroscopic lesions were observed, were also fixed in 10% neutral-buffered formalin and routinely processed to sections stained with H&E for histological examination.

Statistical analysis

The significance of differences in quantitative data for gastric inflammation, gastric adenocarcinoma and tumors of other organs was analyzed by Fisher's exact test. Data for stomach wet weight and inflammation score were examined using Tukey's multiple comparison test. Significance was concluded at $p < 0.05$.

Results

DNA adduct formation by NIAN administration in the glandular stomach of MGs

To confirm the formation of NIAN-DNA adducts in the glandular stomach of MGs, NIAN was injected two times a week at a dose of 100 mg/kg by gavage, and then analyzed by ^{32}P-postlabeling method. Three adduct spots were observed in DNA samples derived from NIAN-treated animals (Fig. 2*a*). The adduct levels were 0.3 for adduct 1, 1.1 for adduct 2, 0.2 for adduct 3 and 1.6 adducts/10^8 nucleotides

Table 1. *H. pylori* infection induced-gastritis in MGs

Group	Treatment	Effective No.	Stomach wet weight (g)	Inflammation score
A	Broth	15	0.647 ± 0.097	0
B	NIAN + Broth	22	0.631 ± 0.094	0
C	*H. pylori*	18	1.432 ± 0.445*	2.22 ± 0.43*
D	NIAN + *H. pylori*	26	1.483 ± 0.445*	2.38 ± 0.64*

*$p < 0.01$ versus group A and B.
Values for results are expressed as averages ± SD.

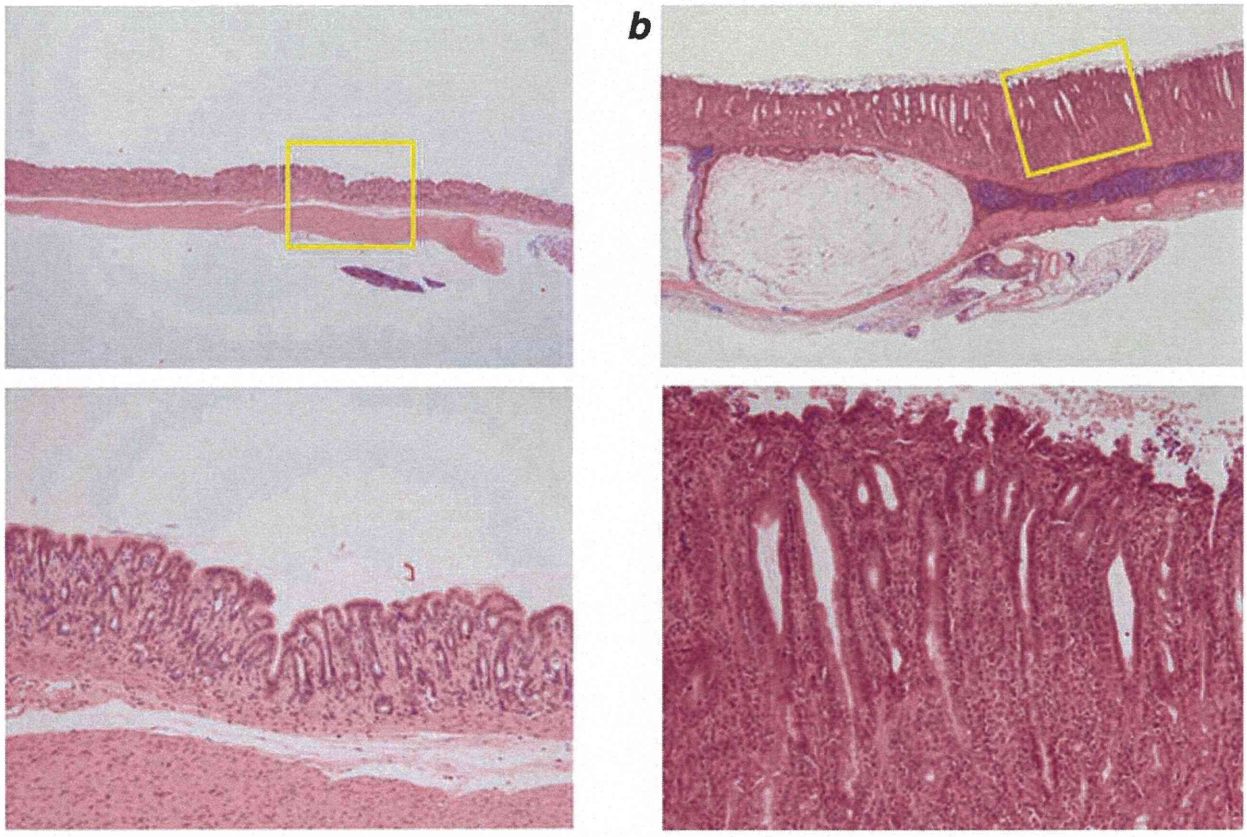

Figure 3. Macroscopic and microscopic views of gastritis in MGs infected or uninfected with *H. pylori*. (*a*) Normal gastric mucosa in group A. (*b*) Severe infiltration of many inflammatory cells with development of heterophilic proliferative glands in group C; H&E staining, ×40. Yellow boxes are shown at greater magnification below, ×200.

in total. This TLC pattern was similar to that in the *in vitro* reaction of calf thymus DNA with NIAN (total adduct level of 4.8 adducts/10^7 nucleotides, Fig. 2*b*). In the case of DNA samples derived from control animals, no adduct spots were seen on the TLC sheets (Fig. 2*c*).

Macroscopical and microscopical observation of *H. pylori*-induced gastritis in MGs

MGs were sacrificed until 104 weeks after *H. pylori* infection, and gastric disorders were analyzed. Stomach wet weights and gastric inflammation scores are shown in Table 1. Macroscopically, edematous thickening with hemorrhagic spots was apparent in the gastric mucosa in *H. pylori*-infected MGs (groups C and D), but not in animals uninfected with *H. pylori* (groups A and B). The stomach wet weight, reflecting edematous thickening, in animals infected with *H. pylori* (groups C and D) was significantly increased compared with that of animals not infected with *H. pylori* (groups A and B) ($p < 0.01$). No significant differences of stomach wet weight were detected between groups A and B and also between groups C and D.

Microscopically, gastritis, featuring infiltration of many inflammatory cells, and hyperplastic change of glandular epithelium, and erosion were observed in the pyloric regions of

Table 2. Incidence of glandular stomach adenocarcinoma in MGs

Group	Treatment	Effective No.	No. of animals with glandular stomach adenocarcinoma (%)		
			Total	Well dif.	Moderately dif.
A	Broth	15	0 (0)	0 (0)	0 (0)
B	NIAN + Broth	22	0 (0)	0 (0)	0 (0)
C	*H. pylori*	18	0 (0)	0 (0)	0 (0)
D	NIAN + *H. pylori*	26	8 (31)*	7 (27)	1 (4)

Well dif., well differentiated adenocarcinoma; Moderately dif., moderately differentiated adenocarcinoma.
*$p < 0.05$ versus group A and C and $p < 0.01$ versus group B.

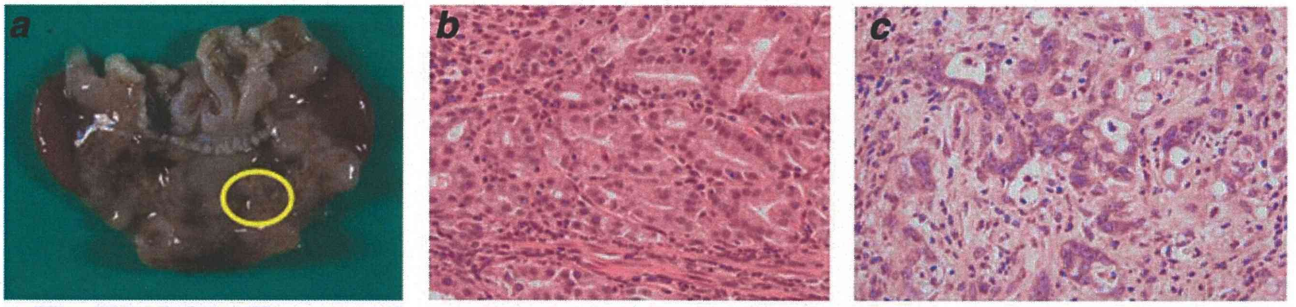

Figure 4. Histological findings of gastric adenocarcinoma in the animals treated with both NIAN and *H. pylori*. (*a*) Typical macrograph of a stomach. The yellow circle shows the suspected lesion of gastric cancer. (*b*) Well differentiated adenocarcinoma. (*c*) Moderately differentiated adenocarcinoma. (*b* and *c*) H&E staining, ×400.

the animals infected with *H. pylori* (groups C and D) (Fig. 3). Heterotopic proliferative glands, whose development is related to severe gastritis in *H. pylori*-infected MGs, were sometimes observed in *H. pylori*-infected groups (groups C and D). No gastritis was found in animals not infected with *H. pylori* (groups A and B). The gastric inflammation score in *H. pylori*-infected animals was significantly increased compared with that of animals uninfected with *H. pylori* ($p < 0.01$). There were no significant differences of gastric inflammation score between groups C and D.

Development of glandular stomach adenocarcinomas in MGs treated with both NIAN and *H. pylori*

The observed incidences of glandular stomach adenocarcinomas are shown in Table 2. Glandular stomach adenocarcinomas, histologically featuring tubular structures with cellular atypia infiltrating into the muscle layer, were found in eight animals treated with both NIAN and *H. pylori* (8/26 = 31%) at 54–104 weeks. All adenocarcinomas were observed in the pyloric mucosa and located in the lesser curvature of the stomach, where macroscopically severe edematous thickening was also seen (Fig. 4*a*). The observed adenocarcinomas in seven animals were of well differentiated (Fig. 4*b*), and a moderately differentiated lesion was observed in one animal (Fig. 4*c*). In the animals treated with broth alone, broth + NIAN and *H. pylori* alone (groups A, B and C), no glandular stomach adenocarcinomas were observed. The incidence of glandular stomach adenocarcinomas in group D was significantly higher than that in groups A, B and C ($p < 0.05$, $p < 0.01$ and $p < 0.05$, respectively).

Irrespective of NIAN treatment and *H. pylori* infection, skin tumors, which histologically were well to poor differentiated squamous cell carcinomas, sebaceous carcinomas and melanomas, were found in one animal (1/15 = 7%) in group A, three animals (3/22 = 14%) in group B, two animals (2/18 = 11%) in group C and five animals (5/26 = 19%) in group D. A hemangioma was also observed in a kidney of one animal in group D (1/26 = 4%). No significant differences were apparent in these tumor incidences among groups A–D.

Discussion

In the present study, NIAN was found to induce glandular stomach adenocarcinomas in MGs in combination with *H. pylori* infection. NIAN-DNA adducts were also detected in the glandular stomach of MGs after treatment with NIAN, although clarification of their chemical structure(s) has yet to be performed. DNA adducts observed in the glandular stomachs of NIAN-treated MGs probably contain an indole-3-acetonitrile moiety. However, it is further likely that NIAN would act as an NO donor under aqueous conditions, thereby causing DNA modifications.[31–33] In fact, Lucas *et al.* demonstrated that NIAN can efficiently transfer nitroso groups to nucleophilic targets in purine nucleotides, causing *N*-nitrosation, deamination and the formation of a novel guanine analog, oxanine.[33]

Glandular stomach adenocarcinomas induced by NIAN treatment plus *H. pylori* infection were located in the pyloric region, similar to MNNG or MNU treatment plus *H. pylori* infection-induced glandular stomach adenocarcinomas in MGs.[26,27] Meanwhile, no glandular stomach cancers were observed in the groups of *H. pylori*-infected MGs without NIAN treatment, which is consistent with previous studies,[26,27] nor in the group treated with only NIAN. These findings indicated that *H. pylori* is a strong promoter of gastric carcinogenesis. Histological examination revealed that the tumors developed by NIAN + *H. pylori* were of well or moderately differentiated adenocarcinomas. Well or poorly differentiated adenocarcinomas and signet ring cell carcinomas were observed in *H. pylori*-infected MGs treated with MNNG or MNU.[26,27] Further studies are required to clarify the histological variety of stomach adenocarcinomas induced by NIAN, MNNG or MNU, since the type of cancer might depend on the genotoxic action of chemical carcinogens, rather than the effects of *H. pylori* infection.[27] In addition, tumors were observed in skin and kidney, which were suspected to spontaneously develop. The MGs have been reported to develop spontaneous skin tumors such as sebaceous and squamous cell carcinoma.[34]

Epidemiological studies have indicated that nitrate intake increases gastric cancer risk, and major sources are vegetables including Chinese cabbage, spinach and parsley.[14] Indole-3-acetonitrile, a precursor of NIAN, is distributed widely in cruciferous vegetables including Chinese cabbage and sprouts.[35] Furthermore, fava beans (*Vicia faba*), which are commonly consumed in Colombia, give rise to a potent mutagen in the presence of nitrite under acidic conditions.[36] The nitrosatable precursor of the mutagen in fava beans and the major product of nitrosation are reported to be an indole compound, 4-chloro-6-methoxyindole and an *N*-nitroso compound, 4-chloro-2-hydroxy-N^1-nitroso-indolin-3-one oxime, respectively.[37] Other indole compounds are also reported to produce direct-acting mutagens after nitrite treatment under acidic conditions.[38,39] In general, conversion of indole derivatives to nitrosated forms *in vitro* is known to be rapid and efficient at physiologically feasible nitrite concentrations with the low pH of the human stomach.[37] Thus, it is conceivable that nitrosation of indole compounds such as indole-3-acetonitrile probably occurs in human stomach. On the other hand, nitric oxide is suggested to be produced by activated macrophages in inflamed organs with *H. pylori* infection.[18] Therefore, nitrosation of indole compounds could be mediated by both acid catalysis and inflammatory responses in the human stomach.[18,20,37–40] On the basis of the conversion rate of NIAN from indole-3-acetonitrile under physiological conditions, the dose of NIAN used in the present study appears about 500–1000 fold the expected human exposure to NIAN *via* fresh or pickled Chinese cabbage. However, humans continually consume various kinds of foods containing indole compounds and nitrate during ordinary life. Thus, it is probable that the total amount of nitroso-indole compounds would be much closer to the dose of NIAN used in the present study. Moreover, it has been reported that low doses of chemical carcinogens, such as MNNG and MNU, could induce glandular stomach cancers in rodents under inflammation conditions including NaCl treatment and *H. pylori* infection, but hardly induce glandular stomach cancer without NaCl treatment and *H. pylori* infection. Therefore, the continuous intake of indole compounds and nitrate may play an important role for gastric carcinogenesis in East Asian countries still with a high salt consumption and *H. pylori* infection rate.

Gastric cancer is tending to decline in most countries.[41–43] One of the explanations for this tendency is the reduced prevalence of *H. pylori* infection.[42] Changes in dietary habits, mainly being lower salt consumption, could be also related to reduced gastric cancer incidence. However, the gastric cancer prevalence in East Asian countries, such as Japan and Korea, is still high.[2] At present, we have not succeeded in detecting NIAN in human bodies nor the exposure levels of the precursor, indole compounds for humans. Thus, it is necessary to estimate the human exposure levels to nitroso-indole compounds including NIAN, and to study further animal experiments and epidemiological analyses for clarification of contribution of nitroso-indole compounds under *H. pylori* infection in humans gastric carcinogenesis.

In conclusion, the present study demonstrated that NIAN can induce gastric cancer in *H. pylori*-infected MGs. It is noteworthy that nitrosatable precursors widely exist in foods. Thus, it is suggested that *N*-nitroso indole compounds including NIAN might contribute to the frequent development of gastric cancer in East Asian countries such as Japan and Korea in which the prevalence of *H. pylori* infection is relatively high. Further studies of interaction with other dietary elements appear warranted to promote the prevention of human gastric cancer.

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References

1. Boyle P, Ferlay J. Cancer incidence and mortality in Europe, 2004. *Ann Oncol* 2005;16:481–8.
2. Bertuccio P, Chatenoud L, Levi F, Praud D, Ferlay J, Negri E, Malvezzi M, La Vecchia C. Recent patterns in gastric cancer: a global overview. *Int J Cancer* 2009;125:666–73.
3. Tsugane S, Sasazuki S. Diet and the risk of gastric cancer: review of epidemiological evidence. *Gastric Cancer* 2007;10:75–83.
4. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med* 1991;325:1127–31.

5. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ. Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001;345:784–9.
6. Parkin DM. The global health burden of infection-associated cancers in the year 2002. *Int J Cancer* 2006;118:3030–44.
7. Yim JY, Kim N, Choi SH, Kim YS, Cho KR, Kim SS, Seo GS, Kim HU, Baik GH, Sin CS, Cho SH, Oh BH. Seroprevalence of Helicobacter pylori in South Korea. *Helicobacter* 2007;12:333–40.
8. Tajima K, Tominaga S. Dietary habits and gastro-intestinal cancers: a comparative case-control study of stomach and large intestinal cancers in Nagoya, Japan. *Jpn J Cancer Res* 1985;76:705–16.
9. Kim HJ, Chang WK, Kim MK, Lee SS, Choi BY. Dietary factors and gastric cancer in Korea: a case-control study. *Int J Cancer* 2002;97:531–5.
10. Nan HM, Park JW, Song YJ, Yun HY, Park JS, Hyun T, Youn SJ, Kim YD, Kang JW, Kim H. Kimchi and soybean pastes are risk factors of gastric cancer. *World J Gastroenterol* 2005;11:3175–81.
11. Seel DJ, Kawabata T, Nakamura M, Ishibashi T, Hamano M, Mashimo M, Shin SH, Sakamoto K, Jhee EC, Watanabe S. N-Nitroso compounds in two nitrosated food products in southwest Korea. *Food Chem Toxicol* 1994;32:1117–23.
12. Jakszyn P, Bingham S, Pera G, Agudo A, Luben R, Welch A, Boeing H, Del Giudice G, Palli D, Saieva C, Krogh V, Sacerdote C, *et al.* Endogenous *versus* exogenous exposure to N-nitroso compounds and gastric cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST) study. *Carcinogenesis* 2006;27:1497–501.
13. Joossens JV, Hill MJ, Elliott P, Stamler R, Lesaffre E, Dyer A, Nichols R, Kesteloot H. Dietary salt, nitrate and stomach cancer mortality in 24 countries. European Cancer Prevention (ECP) and the INTERSALT Cooperative Research Group. *Int J Epidemiol* 1996;25:494–504.
14. van Velzen AG, Sips AJ, Schothorst RC, Lambers AC, Meulenbelt J. The oral bioavailability of nitrate from nitrate-rich vegetables in humans. *Toxicol Lett* 2008; 181:177–81.
15. Spiegelhalder B, Eisenbrand G, Preussmann R. Influence of dietary nitrate on nitrite content of human saliva: possible relevance to *in vivo* formation of N-nitroso compounds. *Food Cosmet Toxicol* 1976;14: 545–8.
16. Sugimura T, Fujimura S. Tumour production in glandular stomach of rat by N-methyl-N′-nitro-N-nitrosoguanidine. *Nature* 1967;216:943–4.
17. Hirota N, Aonuma T, Yamada S, Kawai T, Saito K, Yokoyama T. Selective induction of glandular stomach carcinoma in F344 rats by N-methyl-N-nitrosourea. *Jpn J Cancer Res* 1987;78:634–8.
18. Ohshima H, Bartsch H. Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. *Mutat Res* 1994;305:253–64.
19. Wakabayashi K, Nagao M, Ochiai M, Tahira T, Yamaizumi Z, Sugimura T. A mutagen precursor in Chinese cabbage, indole-3-acetonitrile, which becomes mutagenic on nitrite treatment. *Mutat Res* 1985;143:17–21.
20. Wakabayashi K, Nagao M, Ochiai M, Fujita Y, Tahira T, Nakayasu M, Ohgaki H, Takayama S, Sugimura T. Recently identified nitrite-reactive compounds in food: occurrence and biological properties of the nitrosated products. *IARC Sci Publ* 1987:287–91.
21. Wakabayashi K, Nagao M, Tahira T, Saito H, Katayama M, Marumo S, Sugimura T. 1-Nitrosoindole-3-acetonitrile, a mutagen produced by nitrite treatment of indole-3-acetonitrile. *Proc Jpn Acad Ser* 1985;B61: 190–92.
22. Tiedink HG, Davies JA, Visser NA, Jongen WM, van Broekhoven LW. The stability of the nitrosated products of indole, indole-3-acetonitrile, indole-3-carbinol and 4-chloroindole. *Food Chem Toxicol* 1989; 27:723–30.
23. Yamashita K, Wakabayashi K, Kitagawa Y, Nagao M, Sugimura T. ^{32}P-postlabeling analysis of DNA adducts in rat stomach with 1-nitrosoindole-3-acetonitrile, a direct-acting mutagenic indole compound formed by nitrosation. *Carcinogenesis* 1988; 9:1905–7.
24. Furihata C, Ikui E, Matsushima T. DNA single-strand scission in the pyloric mucosa of rat stomach induced by four glandular stomach carcinogens and three other chemicals. *Mutat Res* 1996; 368:1–6.
25. Tatematsu M, Yamamoto M, Iwata H, Fukami H, Yuasa H, Tezuka N, Masui T, Nakanishi H. Induction of glandular stomach cancers in C3H mice treated with N-methyl-N-nitrosourea in the drinking water. *Jpn J Cancer Res* 1993;84: 1258–64.
26. Sugiyama A, Maruta F, Ikeno T, Ishida K, Kawasaki S, Katsuyama T, Shimizu N, Tatematsu M. Helicobacter pylori infection enhances N-methyl-N-nitrosourea-induced stomach carcinogenesis in the Mongolian gerbil. *Cancer Res* 1998;58:2067–9.
27. Shimizu N, Inada K, Nakanishi H, Tsukamoto T, Ikehara Y, Kaminishi M, Kuramoto S, Sugiyama A, Katsuyama T, Tatematsu M. Helicobacter pylori infection enhances glandular stomach carcinogenesis in Mongolian gerbils treated with chemical carcinogens. *Carcinogenesis* 1999;20: 669–76.
28. Matsubara S, Shibata H, Ishikawa F, Yokokura T, Takahashi M, Sugimura T, Wakabayashi K. Suppression of Helicobacter pylori-induced gastritis by green tea extract in Mongolian gerbils. *Biochem Biophys Res Commun* 2003;310: 715–9.
29. Reddy MV, Randerath K. Nuclease P1-mediated enhancement of sensitivity of ^{32}P-postlabeling test for structurally diverse DNA adducts. *Carcinogenesis* 1986;7: 1543–51.
30. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996;20:1161–81.
31. Lucas LT, Gatehouse D, Shuker DE. Efficient nitroso group transfer from N-nitrosoindoles to nucleotides and 2′-deoxyguanosine at physiological pH. A new pathway for N-nitrosocompounds to exert genotoxicity. *J Biol Chem* 1999;274: 18319–26.
32. Burney S, Caulfield JL, Niles JC, Wishnok JS, Tannenbaum SR. The chemistry of DNA damage from nitric oxide and peroxynitrite. *Mutat Res* 1999;424:37–49.
33. Lucas LT, Gatehouse D, Jones GD, Shuker DE. Characterization of DNA damage at purine residues in oligonucleotides and calf thymus DNA induced by the mutagen 1-nitrosoindole-3-acetonitrile. *Chem Res Toxicol* 2001;14:158–64
34. Vincent AL, Ash LR. Further observations on spontaneous neoplasms in the Mongolian gerbil, Meriones unguiculatus. *Lab Anim Sci* 1978;28:297–300.
35. Okamoto T, Isogai Y, Koizumi T, Fujishiro H, Sato Y. Studies on plant growth regulators, III. Isolation of indole-3-acetonitrile and methyl indole-3-acetate from the neutral fraction of the Moyashi extract. *Chem Pharm Bull* 1967;15: 163–68.
36. Piacek-Llanes BG, Tannenbaum SR. Formation of an activated N-nitroso compound in nitrite-treated fava beans (Vicia faba). *Carcinogenesis* 1982;3: 1379–84.
37. Yang D, Tannenbaum SR, Buchi G, Lee GC. 4-Chloro-6-methoxyindole is the precursor of a potent mutagen (4-chloro-6-methoxy-2-hydroxy-1-nitroso-indolin-3-one oxime) that forms during nitrosation of the fava bean (Vicia faba). *Carcinogenesis* 1984;5:1219–24.
38. Ochiai M, Wakabayashi K, Sugimura T, Nagao M. Mutagenicities of indole and 30 derivatives after nitrite treatment. *Mutat Res* 1986;172:189–97.