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ORIGINAL PAPER

High-density oligonucleotide microarrays and functional network analysis reveal extended lung carcinogenesis pathway maps and multiple interacting genes in NNK [4-(methylnitrosamino)-1-(3-pyridyle)-1-butanone] induced CD1 mouse lung tumor

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Abstract
Purpose NNK [4-(methylnitrosamino)-1-(3-pyridyle)-1-butanone] is a nicotine-derived nitrosaminoketone contained in tobacco smoke used as a powerful chemical carcinogen for rodent experimental models of pulmonary carcinogenesis. To clarify its carcinogenetic mechanisms, we examined the expression status of 22,625 mouse genes.
Methods The affymetrix GeneChip mouse expression 430 A arrays have been used in CD1-induced mouse lung tumor. The affected genes were analyzed by Ingenuity pathway analysis to investigate functional network and gene ontology.
Results A total of 876 genes were found to be differentially expressed at least twofold between NNK-induced tumors and normal lung tissues, 390 up-regulated and 486 down-regulated in these lesions. The functions with the highest P values were related to cellular growth and proliferation ($P = 1.71 \times 10^{-4}$ to 4.10×10^{-2}). In addition, we identified canonical pathways for Wnt/β-cateNSnin signaling ($P = 0.0338$).
Conclusions These results suggest that application of gene expression profiling may provide an improved strategy for therapeutic targeting of tobacco smoking-induced lung cancer.

Keywords NNK · CD1 mouse · Lung tumor · Oligonucleotide microarrays · IPA

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Introduction

Some 80–90% of all human lung cancers are related to cigarette smoke (Minna et al. 2002), known to contain at least 55 carcinogens capable of causing tumors in laboratory animals and/or humans (Hoffmann and Hoffmann 1997; Hecht 1999) 4-(*N*-methyl-*N*-nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and polycyclic aromatic hydrocarbons (PAH) are among the most prominent pulmonary carcinogens. NNK is a potent tobacco carcinogen, and doses comparable to those received by smokers during their lifetime have been shown to be carcinogenic in rats, hamsters and mice (Hecht 1998). This is the only compound in cigarette smoke that has so far been found to induce lung tumors systemically in all three commonly used rodent models. NNK is known to be activator in the lung, with the production of methylating and pyridyloxobutylating agents that attack DNA and cause mutations. The A/J mouse is commonly used to study the carcinogen-induced pulmonary tumors (Nettesheim 1991), since it is among the most sensitive strains (Stoner 1991), but the more resistant CD1 mouse was employed in the present study to allow longer-term observation.

Gene expression profiling is useful for clarifying changes at the molecular level occurring in experimental

models. With lung carcinogenesis models it may help gain insights into basic lung tumor biology, assist in finding markers for early diagnosis, and finally, allow us to test and validate anti-lung cancer therapies. Several reports have stressed the importance of high-density oligonucleotide microarrays for rapid simultaneous analysis of expression levels of large numbers of genes (Lockhart et al. 1996), overcoming the limitations inherent in analysis of single genes (McPherson et al. 2001). In addition, studies of expression profiles of adenocarcinomas of the lung using different commercially available chips identified different classes of tumors with some overlap (Bhattacharjee et al. 2001).

In the present study, we investigated NNK chemical carcinogenesis in the CD1 mouse with high-density oligonucleotide microarrays and GeneSpring analysis. This was followed with mapping of gene expression data into a functional annotation and pathway database using Ingenuity pathway analysis (IPA) to clarify the changes occurring in this experimental model.

Materials and methods

Chemicals

NNK [4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone] was purchased from Toronto Research Chemicals Inc., North York, Canada. Its purity was higher than 98%.

Animals and diets

Female CD1 mice were purchased from Narc (Chiba, Japan) and housed three mice to a plastic cage, with paper chips for bedding. All had access to standard rodent food (CE-2, CLEA Japan, Tokyo, Japan) and water ad libitum and were housed under pathogen-free conditions in a temperature-controlled animal room with a 12-h light/dark illumination cycle. Animal use procedures were in accordance with the Guide for the Care and Use of Laboratory Animals and approved by the Committee on Animal Experimentation of the University of Toyama.

Animal treatment

NNK was dissolved in 0.9% sterile NaCl at a concentration of 2 mg/0.2 ml and administered by i.p. injection to two groups of 18 and 48 CD1 female mice at 2 mg (10 μmol) per mouse every week for 6 consecutive weeks. The first group was sacrificed after 28 weeks and the second group 32 weeks after the first injection. For each of the two groups, control groups of 14 mice injected i.p. with physiological saline were maintained.

Pathologic examination

After decapitation under sodium pentobarbital anesthesia, the lungs and major organs were fixed in 10% formalin. Lungs of all animals were carefully inspected grossly, and all abnormal lesions and other parts of the lung were routinely processed to 5 μm sections, stained with hematoxylin and eosin for histopathological examination. Lung lesions were independently examined and diagnosed by three pathologists (H.O.A., K. N and Y.T) in accordance with the International Classification of Rodent Tumors (Dungworth et al. 2001). We diagnosed the lung proliferative lesions as hyperplasia and tumors, and did not subclassify the tumors into adenomas and adenocarcinomas because of the difficulty in evaluating malignancy (Rehm et al. 1994). Other organs were also examined histopathologically.

RNA preparation

For preparation of RNA, lung tumors from treated mice and lung tissues from control mice were rapidly removed and stored at −80°C until assay. After homogenization of samples with TRIzol reagent (Invitrogen, Carlsbad, CA, USA), total RNA was extracted using an RNeasy Total RNA Extraction kit (Qiagen, Valencia, CA, USA) and treated with DNase I (RNase-free DNase kit, Qiagen) for 15 min at room temperature to remove residual genomic DNA.

Affymetrix GeneChip hybridization

For investigation of gene expression, the Affymetrix® mouse expression 430A array was applied. Sample preparation was carried out as described in the Affymetrix GeneChip® Expression Technical Manual.

Briefly, 5 μg of total RNA was used to synthesize double-strand cDNA with a GeneChip® Expression 3′-Amplification Reagents One-Cycle cDNA Synthesis Kit (Affymetrix, Santa Clara, CA, USA). Biotin-labeled cRNA was then synthesized using GeneChip® Expression 3′‘-Amplification Reagents for IVT Labeling (Affymetrix). After fragmentation, the biotinylated cRNA was hybridized to a GeneChip array at 45°C for 16 h. The chip was washed, stained with streptavidin-phycoerythrin, scanned with a GeneChip scanner 3000 (Affymetrix) and the results analyzed using GeneChip Analysis Suite Software (Affymetrix). Hybridization

intensity data were converted into presence/absence calls for each gene, and changes in gene expression between experiments were detected by comparison analysis. The data were further analyzed using GeneSpring version 7.3 (Silicon Genetics, Redwood City, CA, USA) to extract significant genes and determine the gene ontology including biological processes, cellular component, and molecular functions. A difference of twofold or more was applied to select up-regulated and down-regulated genes.

Network, gene ontology, and canonical pathway analysis

Genes identified by GeneSpring were used for network and gene ontology analyses. Gene accession numbers were imported into the Ingenuity Pathway Analysis version 3.1 (IPA) software (Ingenuity Systems, Mountain View, CA, USA) and the gene products were categorized based on location, cellular components, and reported or suggested biochemical, biologic, and molecular functions using the software. Mapping to genetic networks available in the Ingenuity database was also performed with ranking by score, the probability that a collection of genes equal to or greater than the number in a network could be achieved by chance alone. A score of three indicates that there is a 1/1,000 chance that the focus genes are in a network due to random chance. Therefore, scores of three or higher have a 99.9% confidence level of not being generated by random chance alone. This score was used as the cut-off for identifying gene networks.

Results

Incidence and multiplicity of mice lung tumors

Alveolar cell hyperplasia developed in 38.8 and 56.2% of NNK-treated mice after 28 and 32 weeks, respectively. Lung tumors (adenomas or adenocarcinoma) occurred in 33.3 and 52.1% (Table 1), some developing within hyperplasias. The tumors were single in 4 (60%) of the first group with a maximum of 3 lesions per mouse, whereas in the second group they were single in 11 mice (44%) and numbered 2–10 in the others.

Histopathology

Figure 1a shows an alveolar cell hyperplasia. Such lesions were small, less than 1 mm in diameter. Tumors were papillary (Fig. 1b) or solid (Fig. 1c) with cuboidal to columnar cells forming papillary projections indicating a well-differentiated growth pattern (Fig. 1d). There was no evidence of tumor formation in organs other than the lungs.

Affymetrix GeneChip analysis of NNK induced lung cancers

The expression of 22,625 mouse transcripts was detected using the Affymetrix® mouse expression 430A array. We found 425 probes with expression that was up-regulated more than twofold and 507 probes with expression that was down-regulated more than twofold in the NNK induced CD1 mouse lung tumors. Among them, we identified 390 genes that were overexpressed and 486 genes that were underexpressed in common.

Functional network and gene ontology analysis

To further refine the 876 genes, we next investigated biological interactions using the IPA tool and found 857 to map to genetic networks with functional relationships. Thirty-four networks were found in NNK-induced lung cancers. Fifteen networks were highly significant, in that, they had more of the identified genes present than would be expected by chance. Networks with high scores (>15), with more than half of the genes are affected, are listed in Table 2, being associated with cell growth and proliferation, cell movement, cell death, cell cycling and cell-to-cell signaling. High-scoring functions are illustrated in Fig. 2. The shaded genes are those identified by oligonucleotide microarry analysis, green for down-regulated and red for up-regulated (Table 2). Table 4 shows

Table 1 Incidences of lung alveolar cell hyperplasia and tumors in NNK-treated CD1 mice

Treatment	Number of mice	Duration of treatment (weeks)	Mice with alveolar cell hyperplasia	Mice with tumor
NNK	18	28	7 (38.8%)	6 (33.3%)
PBS	14	28	0	0
NNK	48	32	27 (56.2%)	25 (52.1%)
PBS	14	32	0	0

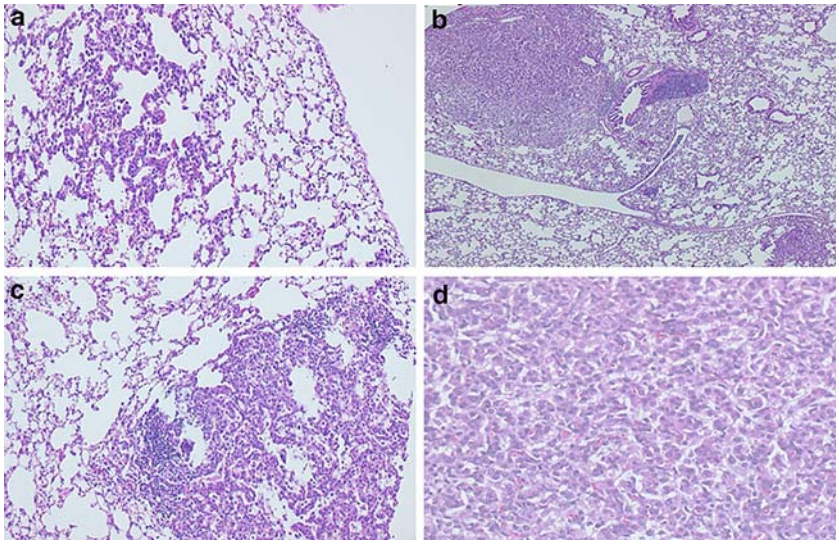


Fig. 1 Histopathology of lung lesions induced by NNK: **a** alveolar cell hyperplasia, **b** solid lung tumor, **c** papillary lung tumor, **d** higher magnification of the papillary tumor. Hematoxylin and eosin stain. Original magnification: **a**–**c** ×200; **d** ×400

the genes included in the high score network which have known relation with lung cancer.

We also performed gene ontology analysis using the IPA tool. Twenty-eight functions were identified as high level (Table 3). Of them, the top was the cellular growth and proliferation related function (Table 3, $P = 1.71 \times 10^{-4}$ to 4.1×10^{-2}). Gene ontology analysis of the highly significant network revealed top scoring functions for cellular movement, cellular growth and proliferation and cell to cell signaling ($P < 10^{-11}$) (Fig. 2). A considerable number of genes were revealed to have developmental functions, which partly overlapped with cancer related.

Canonical pathway analysis

Several pathways with known relations to cancer were found to have genes with altered expression in NNK induced mouse lung cancers; PDGF signaling, TGF-B signaling, cell cycle: G1/S checkpoint regulation, cyclic AMP mediated signaling pathway, ERK/MAPK signaling, p38 MAPK signaling, IGF-1 signaling, FGF signaling, VEGF signaling and PPAR signaling. However, the most highly significant was the Wnt/β-catenin signaling pathway ($P = 0.0338$), Fig. 3. Table 5 summarizes the related genes identified as upregulated or down regulated with the Affymytrix geneChip.

Discussion

With the high-density oligonucleotide microarry using affymetryx geneChip mouse expression 430A array we here identified a substantial number of genes with differential expression between NNK induced CD1 mouse lung tumors and normal lung tissue. Earlier studies in vivo focused on the effect of cigarette smoke on multigene expression (Izzotti et al. 2004). In one case, the expression of 2,031 genes was evaluated in the nasal epithelium and lungs of Sprague-Dawley rats exposed to mainstream cigarette smoke (Gebel et al. 2004). However, to our knowledge, gene expression profiling of NNK-induced lung cancers has not been previously reported. In the present study we analyzed over 800 altered genes regarding functional network and gene ontology using the IPA tool and detected ten cancer related networks. The function whose *P* value was the highest was a cell growth and proliferation related. Expression levels of all 35 genes (100%) in this network were altered significantly in the NNK-induced lung tumors in CD1 mice.

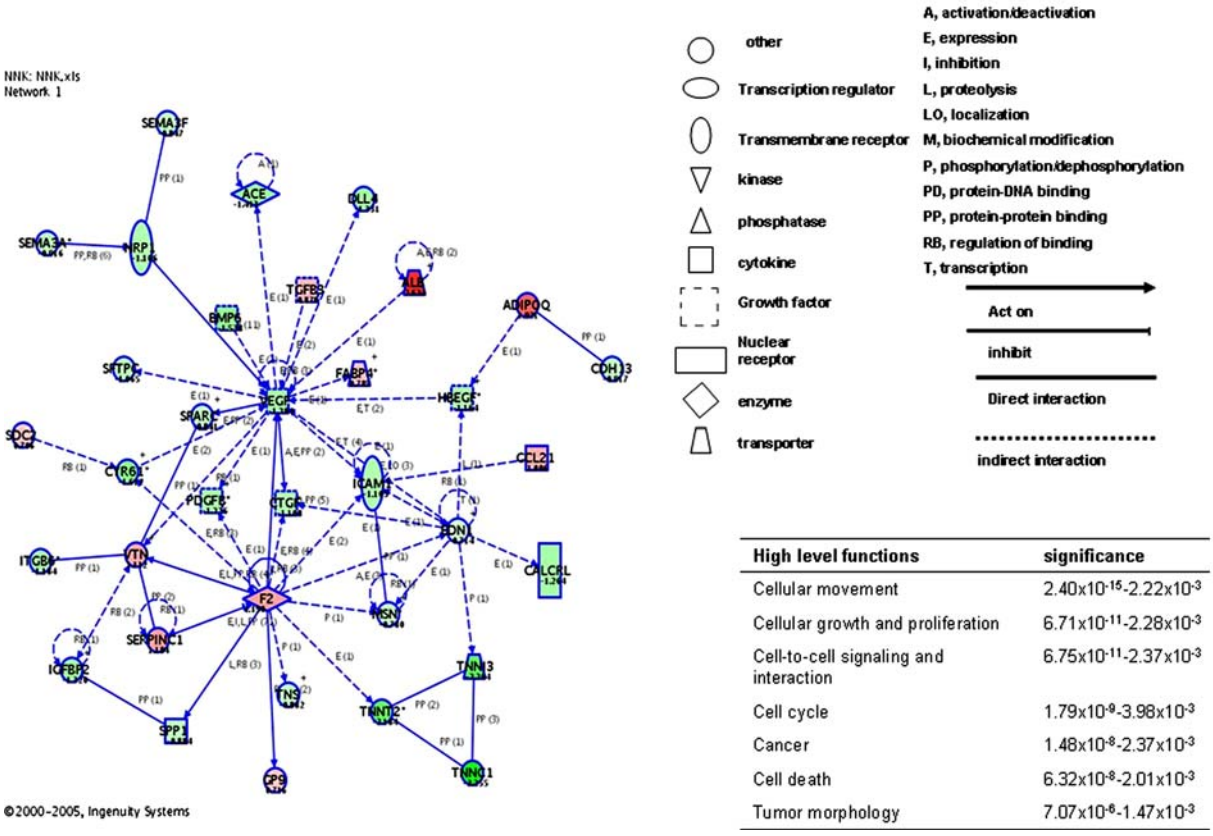
In addition the secreted wingless type Wnt/β-catenin signaling pathway was significantly affected. Wnt signaling has been implicated in many cancers, especially in the colon (Morin et al. 1997; Korinek et al. 1997; Katoh 2003). Furthermore, Mazieres et al. (2005) proposed that activation of the Wnt- mediated signal is critical for lung carcinogenesis, although activation may occur in a different manner from that in colorectal cancer. They added that the Wnt pathway appears to be activated upstream of β-catenin or in non-canonical pathway which is different from our finding. Constitutive activation of the Wnt signaling pathway has in fact been shown to be involved in the development of a subset of human lung cancers (Horii et al. 1992; Shigemitsu et al. 2001; Sunaga et al. 2001).

In particular, two genes were shown to be up-regulated in this pathway. That for the transforming growth factor β (TGF-β) has been reported to promote tumor

Table 2 Genetic networks with high scores (>15) in NNK-induced mouse lung tumors

network	Genetic in ingenuity networks [a]	score	Focus genes	Top functions
1	**ACE**, **ADIPOQ**, **ALB**, **BMP6**, **CALCRL**, **CCL21**, **CDH13**, **CTGF**, **CYR61**, **DLL4**, **EDN1**, **F2**, **FABP4**, **GP9**, **HBEGF**, **ICAM1**, **IGFBP2**, **ITGB6**, **MSN**, **NRP1**, **PDGFB**, **SDC2**, **SEMA3A**, **SEMA3F**, **SERPINC1**, **SFTPC**, **SPARC**, **SPP1**, **TGFB3**, **TNNC1**, **TNNI3**,**TNNT2**, **TNS**, **VEGF**, **VTN**	55	35	Cellular movement, cellular growth and proliferation, cell-to-cell signaling
2	**ANKRD1**, ATF3, BAG1, **C5**, CCL7, **CD19**, **COL14A1**, CTCF, **DNAJB1**, DYSF, **EFNB2**, GDF15, **HBEGF**, HDAC2, **HOP**, **HSPH1**, **IL18R1**, **LYZ**, **MB**, **MYL4**, **MYOM1**, NKX2-5, NQO1, PLK3, PPP1R15A, **PRDM1**, PRKRIR, RAD51, **RAD51AP1**, **SGCG**, SMARCB1, SPHK1, **STIP1**, **TMOD1**, TP53	20	19	Cell death, cancer.
3	ACTA1, ACTB, **APC**, BTRC, CCT3, **CLEC3B**, CLU, **COL14A1**, **CRYAB**, **FBLN5**, FLNA, **GJA1**, **HSPB7**, **IER3**, JUP, LMNA, **MSN**, MYC, **MYO1B**, **PDGFB**, **PHACTR1**, **PISD**, **PPID**, RSN, **SCEL**, SLC25A5, SUMO2, TCF1, TCP1, TYMS, VDAC2, VHL, **VLDLR**, **ZBP1**, **ZFP161**	18	18	Cellular growth and proliferation, cancer.
4	**ALB**, AVP, CCL7, CEBPD, **CLDN5**, **CYR61**, **DBP**, **EPN2**, **FOXP1**, GAS6, **HBEGF**, HP, IL1B, IL23A,IL3RA ITGAM, MMP2,**NPPA**, **NPR2**, **PDGFB**, PER1, PER2, **PER3**, PLA2G4A, PROCR, **PTPRE**, **S100A8**, S100A9, **SAA4**, SCUBE1, **SCUBE2**, **SLC25A4**, **SPARC**, STAT3, **TIMP4**	18	18	Cell- to- cell signaling and interaction, developmental functions.
5	ADM, **AGRN**, **BDNF**, BMP7, CALD1, CAP1, **CAP2**, **CAV2**, CKS1B, **CNN3**, **COL4A2**, COMP, CREB1, **CYR61**, E2F4, E2F5, **EDN1**, **ENO3**, **ETS1**,**FSTL3**, **HIST1H3D**, ID3, ILK, KDR, MAP3K7, **MATNA**, NCAM1, NCOA3, **NDST1**, **PLOD2**, RAD51, SMAD4, **SPTBN1**, TGFB1, **TPM1**	17	17	Cancer, cell death.
6	**AFP**, **AHNAK**, APOB, **ATBF1**,ATP2A1, **ATP2A2**, BMP10, CAM2D, **CASQ2**, **CDH16**, **CORIN**, CREM, **CYP7A1**, FGF10, GATA4, HAND1, HAND2, HEY2, LIPE, MEF2A, MEF2C, **MYH6**, **MYH7**, **NPPA**, NPPB, OXT, PIAS1, **PLN**, REN, **SLN**, **SMPX**, SUMO1, TBX5, **THRA**, **TM4SF3**	15	16	Cell signaling, developmental disorder.
7	**ABTB1**, **APOC1**, **ASPH**, **BDNF**, CAMK2D, **CNTF**, CNTFR, COX1, COX2, COX3, COX4I1, COX6A1, **COX6A2**, COX7A2, **COX8B**, FDFT1, **GJA1**, **GPC3**, IDE, IGF2, INS1, **MYCN**, **MYO10**, **NPY**, PARD3, **PARD6B**, PPYR1, PRKC1, PTEN, **RBBP6**, RHOQ, **RPL35**, SORD, **THRSP**, UCP3	15	16	Cell death, organismal development.

[a] Genes colored red or green are those identified by microarray analysis as upregulated and downregulated, respectively



High level functions	significance
Cellular movement	2.40×10^{-15}-2.22×10^{-3}
Cellular growth and proliferation	6.71×10^{-11}-2.28×10^{-3}
Cell-to-cell signaling and interaction	6.75×10^{-11}-2.37×10^{-3}
Cell cycle	1.79×10^{-9}-3.98×10^{-3}
Cancer	1.48×10^{-8}-2.37×10^{-3}
Cell death	6.32×10^{-8}-2.01×10^{-3}
Tumor morphology	7.07×10^{-6}-1.47×10^{-3}

Fig. 2 Top-scoring CD1 lung cancer networks composed of multiple genes, many of which have been previously implicated in tumorigenesis. *Nodes* represent genes, with *their shape* representing the functional class of the gene product, and *edges* indicate the biological relationships between the nodes, color coded according to their *d* score (*red*, overexpression; *green*, underexpression)

growth by suppression of tumor immunity (Salih et al. 2000) and is known to be highly expressed in lung cancer cell lines [21] (Teicher 2001). The other gene is the secreted frizzeled-related sequence protein 2 (SFRP1), a member of the SFRP family which are known as antagonists of the transmembrane frizzeled receptor preventing Wnt signaling by competing with Wnt ligands (Fukui et al. 2005). APC gene expression was here found to be downregulated in agreement with a previous report for mouse lung tumorigenesis (Oreffo et al. 1998). However, it was found that in human lung cancer APC gene mutation is rare (Mazieres et al. 2005). TGF-βR2 was also downregulated. Earlier, it was shown that repression of the type 2TGF-β receptor may act as a significant determinant of lung adenocarcinoma invasiveness (Borczuk et al. 2005). Cadherin 2 (CDH2) downregulation can be explained by the observation that reduced expression of E-cadherin is a key event in tumorigenicity (Charalabopoulos et al. 2004). SOX17 is a member of the SOX gene family of transcription factors. There is evidence that another example, SOX-4, is differentially expressed in a substantial fraction of SCLCs (Lee et al. 2002). Among the results in this study, the activation of Wnt/β-catenin as a major carcinogenic pathway in NNK induced lung tumors is the novel finding with higher significance. To our knowledge the canonical Wnt/β-catenin signaling pathway has not been studied thoroughly in human lung cancer cases and those genes which we have found in this pathway have not been stressed on in human lung cancer as regard to gene expression profiling. This significant activation of the canonical Wnt/β-catenin and the differential expression of APC gene and the other six genes might be of value as guide to future development of targeted gene therapies.

In the present study CD1 mice lung tumors detected 28 and 32 weeks after starting injections of NNK was 33.3 and 52.1%, lower incidences than with the A/J mouse (Castonguay and Rioux 1997; Kohnoa et al. 2001), consistent with the known variation among strains (Shimkin et al 1975). Because the tumor incidence is high within a relatively short time of treatment in A/J mouse, and since human lung cancer occurs after a relatively long time since starting of cigarette

Table 3 Ontology analysis of the genes affected in NNK induced mouse lung tumors

Related functions and diseases	Significance	Associated genes
Cancer genes		
Cellular growth and proliferation	1.71e−4 to 4.10e−2	39
Cell signaling	3.38e−4 to 4.10e−2	21
Cellular movement	4.06e−4 to 4.10e−2	61
Cell to cell signaling and interaction	8.76e−4 to 4.10e−2	57
Cancer	1.67e−3 to 4.10e−2	48
Cell death	1.68e−3 to 4.10e−2	17
Organismal survival	8.81e−3 to 4.10e−2	13
Tumor morphology	1.49e−2 to 4.10e−2	16
Cell cycle	2.25e−2 to 4.10e−2	5
Gene expression	2.25e−2 to 4.10e−2	7
Developmental genes		
Tissue development	2.65e−4 to 4.10e−2	42
Connective tissue development and function	2.65e−4 to 4.10e−2	21
Organismal development	6.42e−4 to 4.10e−2	16
Embryonic development	6.42e−4 to 410e−2	9
Developmental disorder	9.85e−4 to 4.10e−2	12
Immune and lymphatic system development and function	1.68e−3 to 4.10e−2	19
Organ development	1.54e−2 to 4.10e−2	13
Cellular development	2.25e−2 to 4.10e−2	10
Others		
Molecular transport	2.65e−4 to 4.10e−2	49
Small molecule biochemistry	3.40e−4 to 4.10e−2	54
Cellular function and maintenance	1.25e−3 to 4.10e−2	18
Cellular compromise	1.68e−3 to 4.10e−2	16
Organismal injury and abnormalities	1.68e−3 to 4.10e−2	26
Immune response	1.68e−3 to 4.10e−2	12
Metabolic diseases	4.77e−3 to 4.10e−2	7
Amino acid metabolism	9.51e−3 to 4.10e−2	6
Organismal functions	4.10e−2 to 4.10e−2	2

Table 4 Cancer related genes differentially expressed between NNK induced lung tumors and normal lung tissues

Affymetix probe set ID	Gene symbol	Gene name	Fold change
1451263_a_at	*FABP4*	Fatty acid binding protein 4, adipocyte	27.896
1418897_at	*F2*	Coagulation factor II	4.746
1449254_at	*SPP1*	Secreted phosphoprotein 1	3.246
1417455_at	*TGFB3*	Transforming growth factor, beta 3	2.649
1448545_at	*SDC2*	Syndecan 2	2.19
1448392_at	*SPARC*	Secreted acidic cysteine rich glycoprotein	0.495
1418670_s_at	*HBEGF*	Heparin-binding EGF-like growth factor	0.475
1416953_at	*CTGF*	Connective tissue growth factor	0.47
1448944_at	*NRP1*	Neuropilin	0.462
1420508_at	*SEMA3F*	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3 F	0.459
1451924_a_at	*EDN1*	Endothelin 1	0.457
1424067_at	*ICAM1*	Intercellular adhesion molecule	0.397
1449865_at	*SEMA3A*	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A	0.356
1418639_at	*SFTPC*	Surfactant associated protein C	0.342
1454159_a_at	*IGFBP2*	Insulin-like growth factor binding protein 2	0.334
1423551_at	*CDH13*	Cadherin 13	0.328
1416039_x_at	*CYR61*	Cysteine rich protein 61	0.326
1450414_at	*PDGFB*	Platelet derived growth factor, B polypeptide	0.321
1450759_at	*BMP6*	Bone morphogenetic protein 6	0.269
1420909_at	*VEGFA*	Vascular endothelial growth factor A	0.257
	ITGB6	Integrin beta 6	0.176

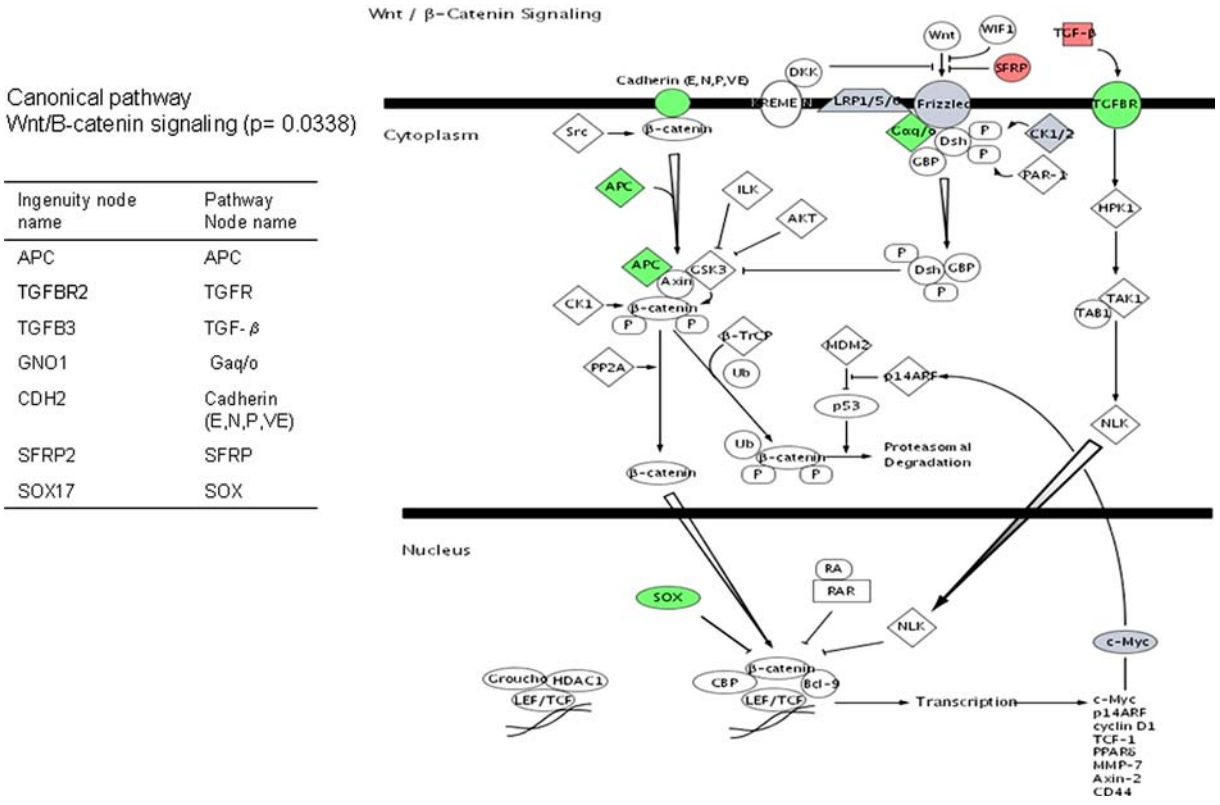


Fig. 3 IPA pathways integrating our expression data. The Wnt signaling pathway is the canonical pathway significant in the whole data set. *Red* indicates up-regulated and *green* indicates down-regulated genes. *Gray* indicates that the expression value did not meet the user-defined cutoff, and *white* indicates that the gene was not user specified, but incorporated into the network through relationships

Table 5 Genes in the Wnt signaling pathway differentially expressed between NNK induced lung tumors and normal lung tissues

Gene symbol	Gene name	PBS treated (control)	NNK treated (lung tumor)	Fold change
APC	Adenomatosis polyposis coli	0.74	0.339	0.458
TGFBR2	Transforming growth factor, beta receptor II	1.737	0.782	0.45
TGFB3	Transforming growth factor, beta 3	0.908	2.407	2.649
GNAO1	Guanine nucleotide binding protein, alpha o	1.763	0.491	0.278
CDH2	Cadherin 2	1.06	0.346	0.326
SFRP2	Secreted frizzled-related sequence protein 2	0.888	3.362	3.785
SOX17	SRY-box containing gene 17	0.914	0.431	0.472

smoking, we proposed that CD1 strain might be more useful for studies of the early phase of lung tumorigenesis. The histopathology of lung lesions induced by NNK was hyperplasia, adenomas and/or adenocarcinomas, with similar morphology to those in A/J mice (Belinsky et al. 1992).

In summary, our strategy of induction of lung tumor in CD1 mice and gene expression assessment allowed us to identify alteration in 857 genes whose products are active in cancer-related networks. Consequently, our data provide not only a new molecular basis for understanding the biologic properties of lung cancer but also useful resources for future development of therapeutic targets.

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