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Review Article

METHODS OF INDUCING LUNG CANCER IN ANIMAL MODELS FOR EXPERI-MENTAL STUDY -A REVIEW

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Abstract

In recent years, several new mouse models for lung cancer have been described. These include models for both non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). Several methods were followed for induction of tumor in animal model for experimentation study.This article has collated few methods popularly used to induce tumor especially in lung tumors.It gives awareness to researchers about tumor induction method at laboratory level during prototype development and supports the task ahead to determine which method are most effective.

Keywords: Lung Cancer, Tumor Induction, Animal model.

INTRODUCTION

Lung cancer is one of the most common malignant neoplasms all over the world. Smoking and a number of constituents of tobacco are responsible for development of lung tumors.

During the last decades lung cancer has become the leading cause of cancer deaths in the world, accounting for even more solid tumor deaths than breast, pancreatic, prostate, and colorectal combined

The deleterious effects of tobacco-derived carcinogen, nitrosamine 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (nicotine-derived nitrosamine ketone (NNK)) remain unmatched.

Lung cancer can be divided into two major histopathological groups: non-small-cell lung cancer (NSCLC) (Van Zandwijk et al. 1995) and small-cell lung cancer (SCLC) (Schiller 2001). About 80% of lung cancers are NSCLC, and they are subdivided into adenocarcinomas, squamous cell, bronchioalveolar, and large-cell carcinomas. Squamous cell carcinomas and adenocarcinomas are the most prominent. The remaining 20% of lung cancers show properties of neuroendocrine cells. These neuroendocrine lung tumors can be divided into four subgroups based upon their morphological characteristics (Wistuba et al. 2001). SCLC, which accounts for close to 18% of all lung tumors, and large-cell neuroendocrine carcinomas both have a very high proliferative and metastatic potential. The remaining neuroendocrine tumors consist of low- and intermediategrade typical and atypical carcinoids, respectively. The high-grade tumors have a significantly worse prognosis compared to the relative benign carcinoids. [4]

SCLC and NSCLC show major differences in histopathologic characteristics that can be explained by the distinct patterns of genetic lesions found in both tumor classes (Zochbauer-Muller et al. 2002). [4]

The overall 5-yr survival rate for lung cancer is ~14% (Travis et al. 1995); for SCLC alone it is even worse, ~5% (Worden and Kalemkerian 2000). [4]

Various study findings suggest that increased

oxidative stress can represent a risk factor for the development of chronic disease in early future.

Experiment Carried Out With Tumour Induction

Chu DJ et al assessed induction effects of Chlamydia pneumoniae (Cpn) on lung cancer in rats by developingcancer through repeated intratracheal injection of Cpn (TW-183) into the lungs of rats, with or without exposure to benzo(a)pyrene (Bp). And measured Cpn antibodies (Cpn-IgA, -IgG, and -IgM) in serum by microimmunofluorescence. Cpn-DNA or Cpn-Ag of rat lung cancer was detected through polymerase chain reaction or enzyme-linked immunosorbent assay.The prevalence of Cpn infection was 72.9% (35/48) in the Cpn group and 76.7% (33/43) in the Cpn plus benzo(a)pyrene (Bp) group, with incidences of lung carcinomas in the two groups of 14.6% (7/48) and 44.2% (19/43), respectively (P-values 0.001 and <0.001 compared with normal controls).From this study rat model of lung carcinomaa induced by Cpn infection was successfully established.[1]

Bhatnagar S et al reported the development of a novel rodent model by administering multiple doses of NNK to male Wistar rats and feeding them with high-fat and low-protein diet. Tumour cells in lungs were observed in approximately 98 % rats after 8 months of NNK treatment, as evident by histopathological analysis. This rodent model showed slow progression of lung tumors which has helped us to assess early indicators of oxidative damage in lungs by studying the levels of lipid peroxidation and antioxidant parameters. LPO was elevated by 46.94 %, SOD, CAT, GSH and GR activity was decreased by 48.67 %, 22.04 %, 21.46 % and 20.85 %, respectively in serum of NNK treated rats when compared with control. This new animal model is an attempt to greatly facilitate studies of the pathophysiology, biochemistry and therapy of lung cancer. [2]

Bo Ye et al developed effective chemo preventive agents against cigarette smoke-induced lung cancer by suitable laboratory animal models, such as animals treated with the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). They established a novel lung cancer model in Wistar rats treated with NNK and assessed the effects of two chemo preventive agents, aspirin and phenethyl isothiocyanate (PEITC), on tumor progression. NNK induced preneoplastic lesions in lungs, including 33.3% alveolar hyperplasia and 55.6% alveolar atypical dysplasia. COX-2 expression increased similarly in alveolar hyperplasia and alveolar atypical dysplasia, while PCNA expression increased more significantly in the latter than the former. A single dose of 25 mg/kg body weight NNK by intratracheal instillation was found to sufficient to induce preneoplastic lesions in Wistar rat lungs. COX-2 took part in NNK-induced tumorigenesis but is not involved in proliferation. NNK was dissolved in iodized oil at a stock concentration of 50 mg/ml and administered to Female Wister rats of 6–7 weeks and weighing 200 ± 10 g.The animals were housed in plastic cages under standard laboratory condition with food and water. The animals were instilled with NNK into the left lobe using the method previously established.after the animal was anesthetized, it was hung on a slanted surgical board and its vocal cord was exposed. Using a blunt ZY-type 12-gauge needle, NNK was instilled into the left lower lobe at a dose of 25 mg/kg body weight.To identify the origin of the proliferated cells in the group treated with NNK (group 1), small pieces of specimens from the left lung were fixed in 5% phosphate buffered glutaraldehyde for 2 hours, then postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol and acetone and embedded in epoxy resin 618. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Hitachi H-600 electron microscope.All animals that died during the study or were sacrificed under ether anesthesia received a complete necropsy and histopathological examination. Lungs were perfused intratracheally with 10% neutral buffered formalin (NBF) and immersed in 10% NFB for fixation. Major tissues were fixed and preserved in 10% NFB, processed and trimmed, embedded in paraffin, sectioned at a thickness of 3–4 μm, and stained with hematoxylin and eosin for microscopic examination. The following tissues were examined microscopically for gross lesions and tissue masses: bone and marrow, heart, large intestine, small intestine, kidney, liver, lung and associated lymph nodes, spleen, stomach, nasal cavity.[3]

Ralph Meuwissen and Anton Berns reviewed on Mouse models non-small-cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) for spontaneous

or chemically induced lung tumors. Induction of lung tumors with chemical carcinogens is very reproducible and almost invariably results in pulmonary adenoma and adenocarcinomas (Shimkin and Stoner 1975; Malkinson 1989). Very potent carcinogens are polycyclic aromatic hydrocarbons and nitrosamines derived from tobacco and ethyl carbamate (urethane). However, only two studies have so far reported on mouse models for pulmonary squamous cell carcinoma. In the first, intratracheal intubation of methyl carbamate (MC) was applied (Nettesheim and Hammons 1971), while in the second prolonged topical application of N-nitrosomethyl-bis-chloroethylurea (NMBCU) or N-nitroso-trischloroethylurea (NTCU) was used (Rehm et al. 1991).[4]

Baldomero Antonio Kato da Silva et al elaborated an experimental model of pulmonary carcinogenesis in Wistar rats. Male Rattus norvegicus albinus, Wistar lineage was carried through an intra-pulmonary instillation of the Benzo[a]pyrene (B[a]P) dilution in alcohol 70%, a polycyclic aromatic hydrocarbon widely known by its power of tumoral induction. Three experimental groups had been formed with 08 animals each: Control Group (Alcohol 70%); B[a]P Group 10 mg/kg; e B[a]P Group 20mg/kg, submitted to euthanasia 08, 10, 12 and 14 weeks after the experimental procedure. The pulmonary sections had been colored by hematoxilin-eosin (HE) and submitted to the morphometrical analysis to describe the tissue alterations. The presence of diffuse inflammatory alterations was observed in all groups, however, at the analysis of the pulmonary tissue of the experimental groups, it had been observed hyperplasic alterations (BALT hyperplasia), and in one of the animals of the experimental group 20mg/kg (12 weeks), it was noticed the presence of cellular epithelial tracheal pleomorphism, suggesting the adenocarcinoma formation in situ. The main secondary alterations to the intra-pulmonary instillation of B[a]P in Wistar rats were: cellular proliferation, inflammatory alterations of several degrees and nodular lymphoid hyperplasias. The association of an activator agent of the pulmonary metabolic reply is necessary to establish the ideal reply-dose to the development of the lung cancer. [5]

U. Saffiotti et al described for the experimental induction of lung cancer. Syrian golden hamsters, receiving repeated intratracheal administrations of benzo[a]pyrene in particulate form carried by an inert dust, develop a high incidence of bronchogenic carcinoma.The conditions of exposure to the carcinogen, involving penetration and retention of carcinogenparticles in lung tissues with the dust, are of prime importance in the determination of the results. Instillations of the same carcinogen in other media did not induce lung tumors. [6]

Leena et al studied development and efficacy testing of these radiation agents requires not only extensive in vitro testing, but also a set of reliable animal models to accurately recreate the complex situations of radiation-induced carcinogenesis. The laboratory mouse Mus musculus remains the most relevant animal model in cancer research due to the molecular and physiological similarities it shares with man, its small size and high rate of breeding in captivity, and its fully sequenced genome. They reviewed relevant M. musculusinbred and F1 hybrid animal models, as well as methods of induction of radiation-induced lung cancers. [7]

Fumio Hirao et al induced lung cancer in rabbits by the instillation of chemical carcinogens into the lower bronchus, with the use of a bronchoscope. Group 1 received a mixture of 3-methylcholanthrene (3-MCA) and 4-nitroquinoline 1-oxide in rabbit plasma, and Group 2 received 3-MCA alone in distilled water. Doses of 40 mg of 3-MCA and 0.4 mg of 4-nitroquinoline 1-oxide were given every 7 to 10 days.The total doses of carcinogens required for 50% incidence of lung cancer were 1000 Â±100 mg of 3-MCA and 10 Â±1 mg of 4-nitroquinoline 1-oxide in Group 1 and 1330 Â±100 mg of3-MCA in Group 2.Altogether, lung cancer was induced in 80 of 173 rabbits that received more than 4 doses of carcinogens and survived for more than 30 days. Histologically, the cancers were of various types (e.g., squamous cell carcinomas, adenocarcinomas, undifferentiated cell carcinoma, pleomorphic carcinomas, and mixed types).The main bronchus, in rabbits of another group, was swabbed every 1 to 2 days with cotton wool soaked in a 10% suspension of 3-MCA in Tween 60. Of the 65 rabbits that survived for more than 60 days, only 2 rabbits were observed lung cancer with metastasesor invasion of tissues adjacent to the lung.[8]

Tsujiuchi Tet al investigated characteristic of lung

adenocarcinoma growth behavior, by establishing rat lung cancer in Nara (RLCNR)from a tumor induced by N-nitrosobis(2-hydroxypropyl) amine (BHP) in a rat. This clone shows an epithelial cell morphology and grows as sheets in culture with an approximate doubling time of 19.2 h. Ultrastructurally, the RLCNR contains lamellar bodies in cytoplasm and the microvilli are present at the free cell surfaces. The line features well-developed desmosomes. The modal chromosome number of 42 is the same as for normal rat cells and its frequency was established to be 80.5%. To evaluate tumorigenicity, appropriate numbers of the cells were transplanted into syngeneic rats, but no tumor formation occurred. Genetic analyses revealed the RLCNR to have a GGT to GAT mutation at codon 12 of Ki-ras, but no p53 alteration. p16 gene expression was lost, associated with hypermethylation of CpG sites in the 5' upstream region of the gene. These results indicate that the present newly established cell line originated from an alveolar type II lesion of the lung. This is useful for further investigation of in vivo growth mechanisms, especially tumor progression, of lung adenocarcinomas, since it has low malignant potential. [9]

Yoshiyuki Tago et al studied to establish a new lung squamous cell carcinoma mouse model. In the first experiment, female A/J mice were painted topically on back skin twice weekly with 75 μL 0.013 M N-nitroso-tris-chloroethylurea for 2, 4, and 8 weeks (n = 15–20 per group) as initiation of lung lesions, and surviving mice were killed at 18 weeks. In the second experiment, mice were treated as above for 4 weeks and killed at 6, 12, or 18 weeks (n = 3 per group). Lung lobes were subjected to histopathological, immunohistochemical, immunoblotting, and ultrastructural analyses. In the case of treatment for 2, 4, and 8 weeks, incidences of lung squamous cell carcinoma were 25, 54, and 71%, respectively. Cytokeratin 5/6 and epidermal growth factor receptor were clearly expressed in dysplasia and squamous cell carcinoma. Desmosomes and tonofilaments developed in the squamous cell carcinoma. Considering the carcinogenesis model, we conclude that 2 or 4 weeks of N-nitroso-tris-chloroethylurea treatment may be suitable for investigating new chemicals for promotional or suppressive effects on lung squamous cell carcinoma. [10]

William H. Blair induced Lung carcinomas in rats with intratracheal instillations of benzo(a)pyrene in combination with ferric oxide suspended in sterile saline and did histologic observation of the lung and other involved tissues. Lung lesions were early squamous metaplasia without keratinization, followed by metaplasia with keratinization which developed into microtumors. The microtumors progressed to visible masses and appeared as highly differentiated squamous cell keratinizing tumors with limited local invasion and no distant metastases. With time the tumors appeared less well differentiated with the presence of local invasion and extension to the pleura. In the late stages of tumorigenesis the chest wall, diaphragm and liver were invaded by the induced epidermoid carcinoma. Metastases were seen in the liver, pancreas, kidney, adrenal and lymph nodes. In addition to the epidermoid carcinoma, adenocarcinoma, and small cell anaplastic (oat cell like) carcinoma were observed. The small cell tumors were found in approximately 20% of the rats and were associated with a sudden weight loss and death of animals.[11]

Amelia Kellar et al says the urethane-induced lung tumourigenesis model has several advantages. Intraperitoneal administration of urethane has been shown to be reliably reproducible and subsequent tumourigenesis develops in a time-dependent manner. Tumourigenesis progresses from hyperplasia to adenoma and eventual adenocarcinoma in response to sequential genetic changes that are characteristic of human lung cancer. Of these genetic changes, K-Ras and p53 are the most prominent mutations associated with the urethane-induced model. The benzo(a)pyrene-induced system also models adenoma in mice, however, it has been shown to result in extremely variable growth patterns in independent experiments. N-Nitrosobis-(2-chloroethyl) ureas such as N-nitroso-methyl-bischloroethylurea (NMBCU) and N-nitroso-trischloroethylurea (NTCU) have been shown to induce the growth of hyperplasia, dysplasia and metaplasia following topical administration in Cr:NIH(S) mice. 3-Methylcholanthrene, diethylnitrosamine, ethylnitrosourea, and dimethylhydrazine have all been shown to induce reproducible growth of adenoma in A/J mice. Although these models provide the distinct advantage of investigator control of tumourigenesis through carcinogen administration, there are also

multiple disadvantages associated with these models such as variability in administration technique leading to discrepancies in results.[12]

Toshifumi et al investigated in lung adenocarcinomas induced by N-nitrosobis92-hydroxypropyl) amine (BHP) in male wistar rats. Animals at 6 weeks of age were given 2000 p.p.m. of BHP in drinking water for 12 weeks, then maintained without further treatment until killed at the end of week 25. A total of 25 lung adenocarcinomas were observed and total RNAs were extracted and assessed for aberrant transcription and found in 15 adenocarcinomas. The results suggested that alteration of the FHIT of lungs carcinomas induced by BHP in rats. [13]

Table 1 NTP Chemicals Associated with Site-Specific Lung Tumor Induction in Rats and/or Mice

Chemical	Route	MR	FR	MM	FM	Primary lung tumor induced
Acrylonitrile	Gavage	x	x		SE	A/B adenoma and carcinoma
1-amino-2,4-dibromoanthraquinone	Dosed feed			CE	CE	A/B adenoma
AZT	In utero	x	x	CE		A/B adenoma and carcinoma
Benzene	Gavage			CE	CE	A/B adenoma and carcinoma
Benzofuran	Gavage			CE	CE	A/B adenoma and carcinoma
2,2-bis(bromomethyl)-1,3-propanediol	Dosed feed	CE		CE	CE	A/B adenoma and carcinoma
Bis(2-chloro-1-methylethyl) ether	Gavage	x	x	P	P	A/B adenoma
Bromoethane (ethyl bromide)	Inhalation	SE				A/B adenoma and carcinoma
1,3-butadiene	Inhalation	x	x	CE	CE	A/B adenoma and carcinoma
Chlorendic acid	Dosed feed	SE				A/B adenoma and carcinoma
Chloroprene	Inhalation	CE	SE	CE	CE	A/B adenoma and carcinoma
CI acid red 114	Dosed water	SE	CE	x	x	A/B adenoma and carcinoma
Cobalt sulfate heptahydrate	Inhalation	SE	CE	CE	CE	A/B adenoma and carcinoma, squamous cell carcinoma (FR)
Coumarin	Gavage			SE	CE	A/B adenoma and carcinoma
Dibromoacetic acid	Dosed water			CE	SE	A/B adenoma
1,2-dibromo-3-chloropropane	Inhalation			P	P	A/B adenoma and carcinoma
1,2-dibromoethane	Gavage			P	P	A/B adenoma
1,2-dibromoethane	Inhalation		P	P	P	A/B adenoma and carcinoma
2,3-dibromo-1-propanol	Topical application			CE	SE	A/B adenoma
1,2-dichloroethane	Gavage			P	P	A/B adenoma
1,3-dichloropropene (telone II)	Gavage			IS	CE	A/B adenoma
3,3′-dimethylbenzidine dihydrochloride	Dosed water	CE	CE	x	x	A/B adenoma and carcinoma
Dimethyl hydrogen phosphite	Gavage	CE				A/B adenoma and carcinoma, squamous cell carcinoma (MR)
1,2-epoxybutane	Inhalation	CE				A/B adenoma and carcinoma
Estradiol mustard	Gavage			P	P	A/B adenoma and carcinoma

Ethylbenzene	Inhalation	SE				A/B adenoma and carcinoma
Ethylene oxide	Inhalation	x	x	CE	CE	A/B adenoma and carcinoma
Gallium arsenide	Inhalation	CE				A/B adenoma and carcinoma
Glycidol	Gavage		CE			A/B adenoma and carcinoma
HC blue 1	Dosed feed	SE				A/B adenoma and carcinoma
Indium phosphide	Inhalation	CE	CE	CE	CE	A/B adenoma and carcinomas-quamous cell carcinoma (MR)
Isobutyl nitrite	Inhalation	CE	CE	CE	CE	A/B adenoma and carcinoma
8-methoxypsoralen	Gavage	SE		x	x	A/B adenoma
Methylene chloride	Inhalation			CE	CE	A/B adenoma and carcinoma
4-methylimidazole	Dosed feed			CE	CE	A/B adenoma and carcinoma
N-methylolacrylamide	Gavage			CE	CE	A/B adenoma and carcinoma
Molybdenum trioxide	Inhalation			SE	SE	A/B adenoma and carcinoma
Naphthalene	Inhalation	x	x		SE	A/B adenoma
1,5-naphthalenediamine	Dosed feed				P	A/B adenoma and carcinoma
Nickel (II) oxide	Inhalation	SE	SE			A/B adenoma and carcinoma, squamous cell carcinoma (MR)
Nickel subsulfide	Inhalation	CE	CE			A/B adenoma and carcinoma, squamous cell carcinoma (FR)
5-nitroacenaphthene	Dosed feed	P	P			A/B adenoma and carcinoma
Nitromethane	Inhalation			CE	CE	A/B adenoma and carcinoma
O-nitrotoluene	Dosed feed	CE				A/B adenoma and carcinoma
Oxymetholone	Gavage		CE	x	x	A/B adenoma and carcinoma
Ozone	Inhalation				SE	A/B adenoma and carcinoma
Phenesterin	Gavage			P	P	A/B carcinoma
Procarbazine hydrochloride	Intraperitoneal injection			P	P	A/B adenoma
Riddelliine	Gavage				CE	A/B adenoma and carcinoma
Selenium sulfide	Gavage				P	A/B adenoma and carcinoma
Sulfallate	Dosed feed			P		A/B adenoma and carcinoma
Talc	Inhalation		CE			A/B adenoma and carcinoma
PCB 126/PCB 153	Gavage	x	CE	x	x	Cystic keratinizing epithelioma
PECDF	Gavage	x	SE	x	x	Cystic keratinizing epithelioma
PCB 126/PCB 118	Gavage	x	CE	x	x	Cystic keratinizing epithelioma
TCDD	Gavage	x	CE	x	x	Cystic keratinizing epithelioma
Tetranitromethane	Inhalation	CE	CE	CE	CE	A/B adenoma and carcinoma, squamous cell carcinoma (MR/FR), sarcoma (MR/FR), mixed malignant tumors (FR)
Dioxin mixture	Gavage	x	CE	x	x	Cystic keratinizing epithelioma
PCB 126	Gavage	x	CE	x	x	Cystic keratinizing epithelioma
Trifluralin	Dosed feed				P	A/B adenoma
2,4,5-trimethylaniline	Dosed feed		P			A/B carcinoma

TRIS(2,3-dibromopropyl) phosphate	Dosed feed			P	P	A/B adenoma and carcinoma
Urethane	Dosed water	x	x	CE	CE	A/B adenoma and carcinoma
Vanadium pentoxide	Inhalation	SE	SE	CE	CE	A/B adenoma and carcinoma
4-vinyl-1-cyclohexene diepoxide	Topical application				SE	A/B adenoma and carcinoma

Abbreviations: A/B, alveolar/bronchiolar; AZT, 3′-azido-3′-deoxythymidine; CE, clear evidence; CI acid red, 114=8-((3,3′-DIMETHYL-4′-((4-(((4-methylphenyl)sulfonyl)oxy) phenyl)azo)(1,1′-biphenyl)-4-yl)azo)-7-hydroxy, disodium salt; HC blue, 2,2′((4-(methylamino)-3-nitrophenyl)imino)BIS(ethanol); IS, inadequate study; P, positive; PCB, polychlorinated biphenyl; PECDF, 2,3,4,7,8-pentachlorodibenzofuran; SE, some evidence; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; x, not studied.[14]

Takahiko Yoshimoto et al studied differential induction of squamous cell carcinomas, adenomas, and adenocarcinomas andobserved in the lungs of male C57BL/6 and C3H/He mice after repeated intratracheal instillation of benzo(a)pyrene (BP) and charcoal powder suspended in 0.9% NaCI solution. When a high dose of BP (1.0 mg BPand 0.5 mg charcoal powder) was instilled intratracheally oncea week for 8 weeks or when a low dose of BP (0.5 mg BP and 0.5 mg charcoal powder) was instilled once a week for 16weeks, squamous cell carcinomas were induced in high incidence (77 to 87%) in the early period of observation, whereas pulmonary adenomas and adenocarcinomas were induced in low incidence (0 to 48%) in the late period of observation inboth strains of mice. On the other hand, when a low dose of BP was instilled intratracheally once a week for 8 weeks, pulmonary adenomas and adenocarcinomas were induced in high incidence (76 to 91%), but squamous cell carcinomas were induced in low incidence (9 to 26%). These results show that a larger quantity of BP instilled intratracheally was needed for induction of squamous cell carcinomas than for induction of adenomas and adenocarcinomas in the lung of mice. Thus, when the carcinogen is administered to a single organ of a single mouse strain by the same route, different amounts of carcinogen have different effects on the incidences of various histological types of tumors. [15]

The incidence of lung adenocarcinoma has been remarkably increasing in recent years due to the introduction of filter cigarettes and secondary-hand smoking because the people are more exposed to higher amounts of nitrogen oxides, especially 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone(NNK), which is widely applied in animal model of lung tumors. In NNK-induced lung tumors, genetic mutation, chromosome instability, gene methylation, and activation of oncogenes have been found so as to disrupt the expression profiles of some proteins or enzymes in various cellular signal pathways. Transgenic animal with specific alteration of lung cancer-related molecules have also been introduced to clarify the molecular mechanisms of NNK in the pathogenesis and development of lung tumors. Based on these animal models, many antioxidant ingredients and antitumor chemotherapeutic agents have been proved to suppress the NNK-induced lung carcinogenesis. In the future, it is necessary to delineate the most potent biomarkers of NNK-induced lung tumorigenesis, and to develop efficient methods to fight against NNK-associated lung cancer using animal models.[16]

Squamous cell carcinomas induced in the rat lung by polycyclic hydrocarbons are seen grossly as solid masses containing central areas of friable material (keratin) (Shabad and Pylev 1970). Naturally occurring squamous cell carcinoma in man tends to arise in the hilar region and is thus more centrally located than other tumor types, which may arise in the periphery of the lung (WHO 1982). Induced squamous cell carcinomas of the rat lung often arise at the site of application of the carcinogen. A site predilection for the naturally occurring squamous cell carcinoma of the rat lung has not been reported. [17]

Pomegranate, a constituent of flavonoids, ellagitannins and ellagic acid is exposed to exert compelling anti-carcinogenic effects. In this study it is examined the anti-angiogenic and anti-tumorigenic potential of pomegranate juice on benzo(a)pyrene-induced mice lung carcinoma by analyzing the SOD and CAT anti-oxidants, MDA for oxidative

stress and the marker of angiogenesis (CD34). Oral administration of pomegranate (10% w/v) to Swiss albino mice found to suppress the development of lung carcinoma by disappointment MDA, histopathological lesions and the Micro-Vessels Density (MVD). The results obtained show that pomegranate inhibits the development of mice lung carcinogenesis through its ability to induce apoptosis and disappointment the formation of new vessels (angiogenesis). The protective benefits of pomegranate juice may be due in part to its potent antioxidant properties and ability to reduce oxidative stress, histopathological injuries by suppressing the formation of reactive oxygen species and protecting the anti-oxidant mechanism and inhibition of MVD. So, this study suggested that the pomegranate is anti-angiogenic effectiveness in the benzo(a)pyrene induced lung carcinoma in mice. [18]

WU Jian Jun et al studied the alteration of circulating microRNAs in 4-(methylnitrosamino)-1-(3-pyridyl) -1-butanone (NNK)-induced early stage lung carcinogenesis.Lung cancer model of male F344 rats was induced with systemic NNK and levels of 8 lung cancer-associated miRNAs in whole blood and serum of rats were measured by quantitative RT-PCR of each at weeks 1, 5, 10, and 20 following NNK treatment.[19]

Conclusion:

Although detailed knowledge of the tumors induction method might guide us to the most appealing use in the study, practice teaches that scheduling, dose, and the order in which tumor induced can be of critical importance for their effectiveness.With all this information at hand, tumor induction method could become a most valuable preclinical tool, and it will be interesting to see whether these methods would assist in the advanced research study in cancer for the betterment of the science research and technology.

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