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Original Article

A comparison between lung carcinoma and a subcutaneous malignant tumor induced in rats by a 3,4-benzopyrene injection

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Abstract: Lung cancer is one of the most common carcinomas worldwide. It is of value to know whether lung is more vulnerable to carcinogens than other tissues. In this study we compared the carcinogenic potential of 3,4-benzopyrene administered by intrapulmonary injection or subcutaneous injection. Ninety rats were randomly divided into three groups (n=30/group). Rats under deep anesthesia were treated with 3,4-benzopyrene by intrapulmonary injection or scapular subcutaneous injection, or with the vehicle by subcutaneous injection. The Rats were sacrificed when they developed advanced somatic sarcomas or severe dyspnea and the rats without severe phenotypes were sacrificed after 1 year. The tumors were isolated and examined with H&E staining. The expression of Bcl-2, CYP1A1, and NF-κB mRNA and protein in somatic sarcoma and lung carcinoma tissues was examined by in situ hybridization, immunohistochemistry, and Western blot. No tumor development was observed in the control rats. Fifteen of the 30 rats receiving an intrapulmonary injection of 3,4-benzopyrene developed lung carcinomas, whereas all 30 rats treated with subcutaneous injection developed a malignant neoplasm under the skin. Positive Bcl-2, CYP1A1, and NF-κB protein staining was observed in lung carcinoma and subcutaneous malignant neoplasm but Bcl-2 protein expression was much stronger in subcutaneous malignant neoplasms than in lung carcinoma. The expression pattern of *Bcl-2, CYP1A1*, and *NF-κB* mRNA in lung carcinoma and subcutaneous malignant neoplasms was consistent with its protein expression. Our results indicated that the lung is not more vulnerable to carcinogens than other tissues. The lung may acquire a protective mechanism against lung carcinogenesis through regulation of Bcl-2 expression.

Keywords: Lung carcinoma, subcutaneous malignant neoplasm, benzopyrene, Bcl-2

Introduction

There is a great difference in the incidence of lung cancer and subcutaneous malignant tumor; lung cancer is one of the most common carcinomas whereas primary subcutaneous malignant tumor is rare. Moreover, the mortality of lung cancer has been ranked the highest among all cancers [1-5]. The most common type of primary subcutaneous malignant tumor is liposarcoma, which usually occurs in deep soft tissues of the limb and the retroperitoneum [6].

The incidence of lung cancer is associated with many factors. Smoking and tobacco use are involved in nearly 90% of lung cancer cases [7]. Tobacco contains multiple types of recognized carcinogens including benzo(a)pyrenes, polycyclic aromatic hydrocarbons, and tobacco-specific nitrosamines [8]. These compounds play an important role in promoting the growth and metastasis of lung cancer. Other carcinogenic factors for lung cancer may include industrial air pollution and household air pollution [9], radon gas exposure [10], asbestos fiber exposure [11], and alcohol use. In recent years, the underlying causes of lung cancer development have been further investigated and the role of cancer stem cells in lung cancer initiation, progression, and resistance to treatment has been highlighted [12-16].

Cancer may be more likely to develop in a specific organ with more potentially malignant cells. Although lung cancer can theoretically derive from any part of the lung, over 90% of lung cancers arise from epithelial cells. Because lung cancer is the most common carcinoma, it is valuable to understand whether the lung is more vulnerable to multiple carcinogens compared with other tissues.

Bcl-2 is a regulator of apoptosis. We identified Bcl-2 overexpression in lung carcinogenesis in our previous study [17]. Cytochrome P450 CYP1A1 helps detoxify potential carcinogens in tobacco smoke [18], but occasionally converts compounds into potent genotoxins. CYP1A1 activates polyaromatic hydrocarbons, such as benzo[a]pyrene 7,8 dihydrodiol (BaP-DHD), rendering them genotoxic [19]. NF-κB activation is common in lung cancer, and NF-κB is thought to be involved in multiple steps of carcinogenesis [20]. Therefore, Bcl-2 may be linked to CYP1A1 and NF-κB in cancer development and progression [21]. In this study, we compared the carcinogenic potential of 3,4-benzopyrene administered by intrapulmonary injection or subcutaneous injection, and compared Bcl-2, CYP1A1, and NF-κB expression in tissues from lung carcinomas and subcutaneous malignant neoplasms.

Materials and methods

Animals

Female Sprague Dawley (SD) rats weighing 200 g were purchased from the Experimental Animal Department of Central South University. All rats were maintained in a 12 h/12 h dark-light cycle in a temperature-controlled room with free access to food and water. Ninety rats were randomly divided into three groups, with 30 rats in each group. After 2 weeks of adaptive breeding, the rats were anesthetized by intraperitoneal injection of pentobarbitone sodium (30 mg/kg body weight). Under deep anesthesia, the rats were treated twice at a 2-week intervals with 2 mg 3,4-benzopyrene (dissolved in 0.2 ml corn oil) through intrapulmonary injection through the skin or scapular subcutaneous injection, or with an equal volume of vehicle corn oil through subcutaneous injection, as previously described [17]. Following treatment all rats were intramuscularly injected with penicillin (40,000 units/day for 2 days) to prevent infection. All of the animals were treated humanely and in compliance with the Animal Welfare Act of America.

Tumor identification

All rats were regularly monitored after the first injection. Body weight and food consumption were measured weekly. The rats were sacrificed upon development of an advanced somatic tumor or severe dyspnea and the remaining rats without severe phenotypes were sacrificed at 1 year (52 weeks) after the first carcinogen treatment. Somatic tumors, the suspicious tumors, the brain, lung, liver, and the gastrointestinal tract were carefully dissected from each animal. The isolated tumors and lung tissues were fixed in 10% phosphate-buffered formalin for 24 hours at room temperature, embedded in paraffin, cut into 3-μm sections, and examined with hematoxylin-eosin (H&E) staining for malignant neoplasms. The tumors were diagnosed by two pathologists in a blinded manner.

Immunohistochemical staining

Serial sections of somatic sarcomas and lung carcinoma tissues were immunostained with antibodies against Bcl-2, CTP1A1, and NF-κB (NeoMarkers: 1:100 dilution). The negative control was treated similarly but without a primary antibody. Quantification of Bcl-2, CTP1A1, and NF-κB protein expression was based on the percentage of positive cells and staining intensity as previously described [22]. The scoring of the positively stained areas was determined as follows: score 0, <5% positive cells; score 1, 5-25% positive cells; score 2, 26%-50% positive cells; score 3, 51%-75% positive cells; and score 4, >75% positive cells [23]. The intensity of 3,3'-diaminobenzidine (DAB) staining was estimated as follows: score 1, light yellow; score 2, dark yellow or yellow-brown; and score 3, brown staining. For each slide, five vision fields were evaluated and the total score (percentage and intensity) of Bcl-2, CTP1A1, and NF-κB expression was used for statistical analysis. All slides were scored by two observers who were blinded to the study groups.

In situ hybridization

In situ hybridization was performed to evaluate *Bcl-2*, *CTP1A1*, and *NF-κB* mRNA expression in

Table 1. Incidence of malignant neoplasms induced by 3,4-benzopyrene in rats between intra-pulmonary injection group and subcutaneous injection group

Group	Rats	Neoplasm	Incidence (%)
Intra-pulmonary injection (lung carcinoma)	30	15	50
Subcutaneous injection (somatic sarcoma)	30	30	100*

*note: x^2=20.00, P=0.000.

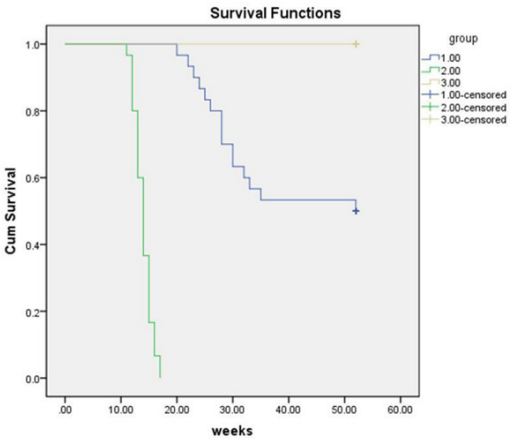

Figure 1. Survival plot of experimental rats. (1.00) Survival time of rats in the intrapulmonary injection group. (2.00) Survival time of rats in the subcutaneous injection group. (3.00) Survival time of rats in the control group. Survival data among groups were statistically compared by Kaplan-Meier analysis. Tests of equality of survival distribution in the different groups was carried out by the log rank test (Mantel-Cox): x^2=128.715, P=0.000.

somatic sarcoma and lung carcinoma tissues using digoxigenin-labeled oligonucleotide probes at 1:100 dilution according to the digoxigenin Labeled Probes Detection Kit (Haoyang Corp., Tianjin). The sequences of gene-specific oligonucleotide probes were as follows: Bcl-2, 5'-CUUCAGAGACAGCCAGGAGAAAUCAAACAGAGG-3'; CTP1A1, 5'-AGGCCGGAACTCGTTTGGATCACC-3'; NF-κB, 5'-CGGCCCTCGCACTTGTAACG GAA-3'. Oligonucleotide probes corresponding to β-actin were used as a positive control. Omission of gene-specific oligonucleotide probe was used as a negative control. The scoring of hybridization signals were evaluated following standard pathology techniques [24] by two observers who were blinded to the study.

Western blot analysis

The collected lung carcinoma tissues and subcutaneous malignant neoplasm tissues were cut into small pieces and washed with DPBS three times before suspension in RIPA buffer with protein inhibitor. The tissues were placed on an ice bath for 30 min and then centrifuged for 15 min. The supernatant was collected into a clean tube and stored at -80°C. For Western blot analysis, a 16-μL loading volume containing 150 μg of protein, 1.6 μL of 10×SDS buffer, and 4 μL of 4×SDS buffer was subjected to electrophoresis. Electrophoresis was stopped when the protein ladder had migrated to the end of the gel. The gel was blocked with 5% fat-free milk for 1 hour at room temperature and treated with specific antibodies against Bcl-2, CTP1A1, and NF-κB at 4°C overnight. The gel was washed for 10 min with TBS-T buffer three times and then incubated with secondary antibody for 2 hours at room temperature. After further washing with TBS-T buffer and development, the gel was scanned and recorded using the molecular imager ChemiDocTM XRS+ (Bio-Rad).

Statistical analysis

Data were statistically analyzed with SPSS 13.0 software. The frequency of carcinogenesis was determined in all of the rats treated with 3,4-benzopyrene. The incidence of neoplasm among the groups was evaluated by an χ^2 test. Differences in Bcl-2, CYP1A1, and NF-κB mRNA expression between intrapulmonary injection and subcutaneous injection were compared using a rank sum test. Scores of immunohistochemical staining were expressed as means ± SD and were compared by t-test. A *P* value <0.05 was considered statistically significant.

Results

3,4-benzopyrene-induced carcinogenesis

All animals recovered from the injection 12 hours after treatment. No rats died as a result of an injection or an infection caused by the treatment. At 20 weeks after intrapulmonary injection rats began to develop lung carcinomas, as characterized by dyspnea and rapid emaciation. A total of 15 of 30 rats in the intrapulmonary injection group developed lung car-

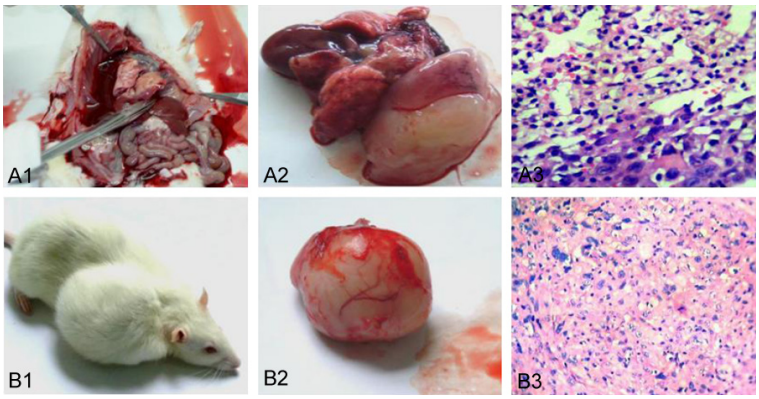

Figure 2. Carcinogenesis induced by 3,4-benzopyrene injection. A1, A2. Tumor formation in the right lung of rats after intrapulmonary injection of 3,4-benzopyrene. A3. Histology of lung carcinoma (poorly differentiated squamous cell carcinoma) in rats by H&E staining. B1, B2. Tumor formation after subcutaneous injection of 3,4-benzopyrene in rats. B3. Histology of malignant neoplasms under skin (poorly differentiated fibrosarcoma) in rats by H&E staining.

cinomas after 52 weeks, whereas all 30 rats in the scapular subcutaneous injection group developed advanced malignant neoplasm under the skin from 11 to 17 weeks after the 3,4-benzopyrene administration. Because the lung carcinomas and malignant neoplasms under the skin were poorly differentiated, we identified the subtypes based on immunohistochemical staining (data not shown). The subtype of the lung cancer was poorly differentiated squamous cell carcinoma but the sarcoma was poorly differentiated fibrosarcoma. No tumors occurred in rats subcutaneously injected with the vehicle alone. The incidence of advanced malignant neoplasms under the skin in the scapular subcutaneous injection group was significantly higher than the incidence of lung carcinomas in the intrapulmonary injection group ($P<0.01$) (**Table 1**). Moreover, the survival of rats with subcutaneous malignant tumors was shorter than that of rats with lung carcinomas ($P<0.01$) (**Figure 1**). Only 15 of 30 rats with lung carcinomas induced by intrapulmonary injection of 3,4-benzopyrene died, compared with all of the rats with malignant tumor induced by subcutaneous injection of 3,4-benzopyrene. These results suggest that the lung may have certain preventive properties against carcinogenesis. Pathologically, the characteristics of lung carcinomas in rats injected with 3,4-benzopyrene were largely similar to those of human lung cancer while the histology of advanced malignant neoplasm under the skin in rats with subcutaneous injection was similar to that of sarcomas (**Figure 2**).

Bcl-2 expression in advanced malignant skin neoplasm and lung carcinoma

Neoplasm tissues were immunostained with anti-Bcl-2 antibody and the percentage of positive cells and the intensity of immunoreactivity were quantified. Bcl-2 protein was mainly expressed in the cytoplasm of neoplasm cells. Compared with lung carcinoma tissues, the percentage of Bcl-2-positive cells and the intensity of Bcl-2 staining in subcutaneous malignant neoplasm tissues were significantly higher ($P<0.01$) (**Figure 3**) (**Table 2**).

In situ hybridization revealed positive expression of *Bcl-2* mRNA in neoplasm tissue cells. Consistent with its protein expression, the percentage of Bcl-2-positive cells and the intensity of Bcl-2 signals in subcutaneous malignant neoplasm tissues was significantly higher than that in lung carcinomas ($P<0.01$) (**Figure 3**) (**Table 3**).

Western blot analysis demonstrated that Bcl-2 protein was overexpressed in both lung carcinoma tissues and in subcutaneous malignant neoplasm tissues; however, Bcl-2 protein expression in subcutaneous malignant neoplasm tissues was significantly higher than it was in lung carcinomas (**Figure 3**).

CYP1A1 expression in advanced malignant skin neoplasm and lung carcinoma

Immunohistochemical staining showed that CYP1A1 protein was mainly expressed in the cytoplasm of neoplasm cells and all samples had positive CYP1A1 expression. Compared with lung carcinoma tissues, subcutaneous malignant neoplasm tissues exhibited a similar percentage of CYP1A1-positive cells and intensity of CYP1A1 signals. There was no statistical difference between the two groups ($P>0.05$) (**Figure 4**) (**Table 4**).

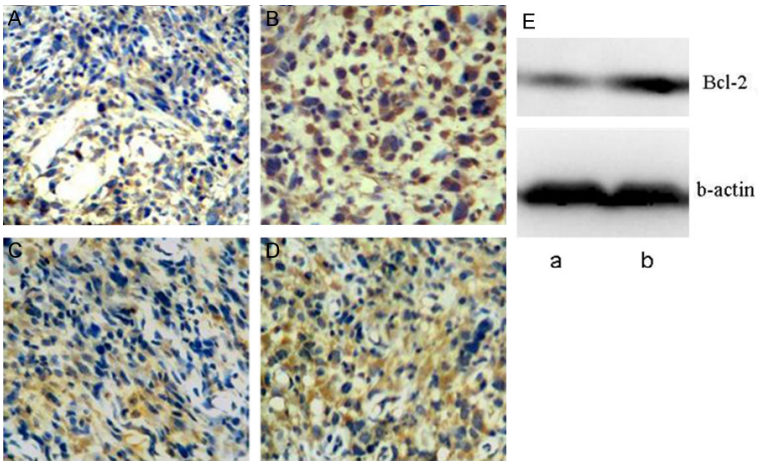

Figure 3. Bcl-2 expression in rat malignant neoplasms. A. Immunohistochemical staining of the Bcl-2 protein in rat lung carcinomas (poorly differentiated squamous cell carcinoma). The percentage of positive cells was score 2 and the intensity of positive cells was evaluated as score 2. B. Immunohistochemical staining of Bcl-2 in rat malignant neoplasms under skin (poorly differentiated fibrosarcoma). The percentage of positive cells was score 3 and the intensity of positive cells was evaluated as score 3. C. In situ hybridization of Bcl-2 mRNA expression in lung carcinoma. Positive staining of *Bcl-2* mRNA was observed in rat lung carcinomas (poorly differentiated squamous cell carcinoma). D. In situ hybridization illustration of *Bcl-2* mRNA in skin neoplasm. Very strong positive staining of *Bcl-2* mRNA was observed in rat malignant neoplasms under the skin (poorly differentiated fibrosarcoma); E. Western blot analysis of Bcl-2 expression in (a) lung carcinomas and (b) malignant neoplasms under skin. Expression of Bcl-2 protein in rat malignant neoplasms under skin was much stronger than that in rat lung carcinomas.

Table 2. Expression of Bcl-2 protein in lung carcinoma and somatic sarcoma induced by 3,4-benzopyrene treatment (means ± SD)

Group	Numbers	Bcl-2	t value	*P* value
Lung carcinoma	15	4.159±1.019	3.529	0.001
Somatic sarcoma	30	5.374±1.121		

Table 3. Expression of *bcl-2* mRNA in lung carcinoma and somatic sarcoma induced by 3,4-benzopyrene treatment

Group	Tota	Negative	Positive	Strongly positive	*P* value
Lung carcinoma	15	0	10	5	0.003
Somatic sarcoma	30	0	11	19	

In situ hybridization revealed a positive expression of *CYP1A1* mRNA in the neoplasm tissue cells. Consistent with their protein expression, subcutaneous malignant neoplasm tissues and lung carcinoma tissues showed a similar percentage of *CYP1A1* mRNA positive cells and intensity of *CYP1A1* mRNA signals with no statistical difference between the two groups (P>0.05) (**Figure 4**) (**Table 5**). Moreover, Western blot analysis indicated a similar overexpression of the CYP1A1 protein in lung carcinoma tissues and subcutaneous malignant neoplasm tissues with no statistical difference between the two groups (**Figure 4**).

NF-κB expression in advanced malignant skin neoplasm and lung carcinoma

Immunohistochemical staining showed expression of NF-κB protein in the cytoplasm and nucleus of neoplasm cells, and all samples had positive NF-κB protein expression. Compared with lung carcinoma tissues, subcutaneous malignant neoplasm tissues had a similar percentage of NF-κB positive cells and intensity of NF-κB signals with no statistical difference between the two groups (P>0.05) (**Figure 5**) (**Table 6**).

In situ hybridization revealed positive expression of *NF-κB* mRNA in neoplasm tissue cells. Consistent with protein expression, subcutaneous malignant neoplasm tissues and lung carcinoma tissues showed a similar percentage of *NF-κB* mRNA positive cells and intensity of *NF-κB* mRNA signals with no statistical difference between the two groups (P>0.05) (**Figure 5**) (**Table 7**). Similarly, Western blot analysis indicated similar overexpression of NF-κB protein in lung carcinoma tissues and subcutaneous malignant neoplasm tissues with no statistical difference between the two groups (**Figure 5**).

Discussion

Tumorigenesis in tissues and organs of the body is an unexpected event. Even in the lung, most carcinomas are derived from epithelial

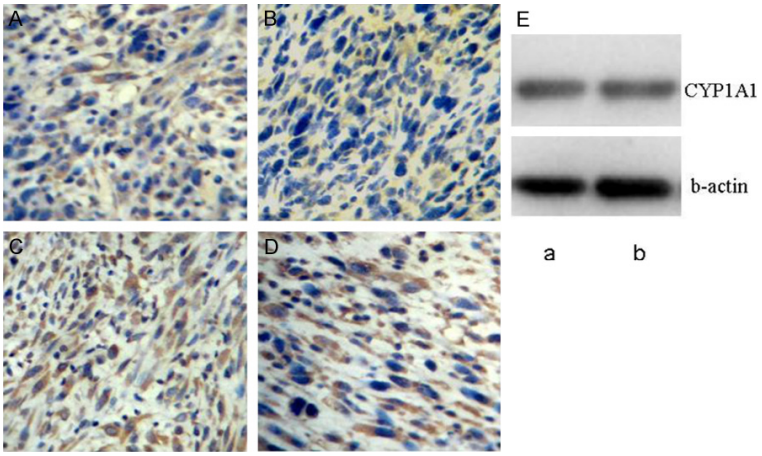

Figure 4. CYP1A1 expression in rat malignant neoplasms. A. Immunohistochemical staining of CYP1A1 protein in rat lung carcinomas (poorly differentiated squamous cell carcinoma). The percentage of positive cells was score 2 and the intensity of positive cells was evaluated as score 2. B. Immunohistochemical staining of CYP1A1 in rat malignant neoplasms under skin (poorly differentiated fibrosarcoma). The percentage of positive cells was score 2 and the intensity of positive cells was evaluated as score 2. C. In situ hybridization of *CYP1A1* mRNA expression showed strong positive staining of *CYP1A1* mRNA in rat lung carcinomas (poorly differentiated squamous cell carcinoma); D. In situ hybridization illustration of *CYP1A1* mRNA showed strong positive staining of *CYP1A1* mRNA in rat malignant neoplasms under skin (poorly differentiated fibrosarcoma); E. Western blot analysis of CYP1A1 protein expression in (a) rat lung carcinomas and (b) rat malignant neoplasms under skin. The expression level of CYP1A1 protein in rat malignant neoplasms under the skin was similar to that in rat lung carcinomas.

Table 4. Expression of CYP1A1 protein in lung carcinoma and somatic sarcoma induced by 3,4-benzopyrene treatment (means ± SD)

Group	Numbers	Bcl-2	t value	*P* value
Lung carcinoma	15	4.836±0.984	1.316	0.195
Somatic sarcoma	30	4.458±0.870		

Table 5. Expression of *CYP1A1* mRNA in lung carcinoma and somatic sarcoma induced by 3,4-benzopyrene treatment

Group	Total	Negative	Positive	Strongly positive	*P* value
Lung carcinoma	15	0	8	7	0.741
Somatic sarcoma	30	0	17	13	

cells in the larger and smaller airways, and lung carcinoma derived from supporting tissues is rare. It has been reported that cancer stem cells are involved in tumorigenesis [25] and some epithelial cells with stem cell-like properties may play a role in lung tumorigenesis [26]. It is of value to know whether epithelial cells in the lining of bronchi have more potential for carcinogenesis compared with other cells; in other words, whether the lung is more vulnerable to carcinogenic attack than other organs and tissues, leading to a high incidence of lung cancer. In this study, we observed that the incidence of tumor formation induced by subcutaneous injection of 3,4-benzopyrene was significantly higher that induced by intrapulmonary injection of 3,4-benzopyrene. This result suggests that lung may actually have a protective mechanism against carcinogenesis. As a respiratory organ, the lung has a high risk of carcinogen exposure and continuous carcinogen exposure can resu-lt in DNA damage and mutation in epithelial cells, which induces carcinogenesis. However, lung tissue may undergo continuous adjustment of adaptive responses, enhanced toxin scavenging, and regulation of the ability to repair damage. Thus, although the lung may not be more vulnerable to carcinogenic attack compared with other organs, continuous carcinogenic attack is the major cause of the high incidence of lung cancer.

Additionally, it is possible that the lung exhibits a reduced risk of carcinogenesis through regulation of the expression of some key gene such as Bcl-2. Bcl-2 is a regulator of apoptosis and a key element in cancer development and progression [27]. Bcl-2 protein is overexpressed in a variety of human cancers [28]. Overexpression of Bcl-2 protein can inhibit apoptosis of cancer cells and promote their proliferation. Thus, the *Bcl-2* gene has been classified as an oncogene. During the cell cycle, Bcl-2 reduces intracellular levels of dNTPs by inhibiting ribonucleotide reductase activity and triggers DNA replication stress [29], thereby

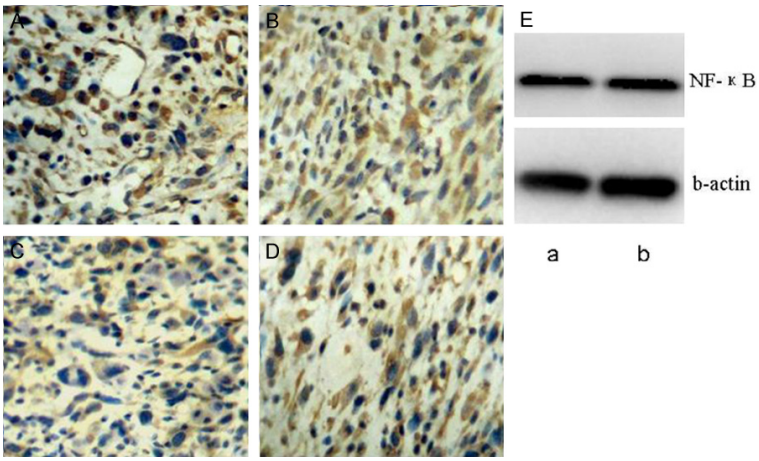

Figure 5. NF-κB expression in rat malignant neoplasms. A. Immunohistochemical staining of NF-κB protein in rat lung carcinomas (poorly differentiated squamous cell carcinoma). The percentage of positive cells was score 3 and the intensity of positive cells was evaluated as score 3. B. Immunohistochemical staining of NF-κB in rat malignant neoplasms under skin (poorly differentiated fibrosarcoma). The percentage of positive cells was score 3 and the intensity of positive cells was evaluated as score 3. C. In situ hybridization of *NF-κB* mRNA expression showed strong positive staining of NF-κB mRNA in rat lung carcinomas (poorly differentiated squamous cell carcinoma); D. In situ hybridization of *NF-κB* mRNA showed strong positive staining of NF-κB mRNA in rat malignant neoplasms under skin (poorly differentiated fibrosarcoma); E. Western blot analysis of NF-κB protein expression in (a) rat lung carcinomas and (b) rat malignant neoplasms under skin. The expression level of NF-κB protein in rat malignant neoplasms under the skin was similar to the levels in rat lung carcinomas.

Table 6. Expression of NF-κB protein in lung carcinoma and somatic sarcoma induced by 3,4-benzopyrene treatment (means ± SD)

Group	Numbers	Bcl-2	t value	*P* value
Lung carcinoma	15	4.914±1.046	1.068	0.291
Somatic sarcoma	30	4.588±0.923		

Table 7. Expression of *NF-κB* mRNA in lung carcinoma and somatic sarcoma induced by 3,4-benzopyrene treatment

Group	Total	Negative	Positive	Strongly positive	*P* value
Lung carcinoma	15	0	6	9	0.212
Somatic sarcoma	30	0	16	14	

promoting cell growth and proliferation. Bcl-2 can also mediate cell apoptosis by interacting with other pathways. It has been reported that the glucocorticoid receptor mediates apoptosis in small-cell lung cancer through interaction with Bcl-2 [30]. In our previous study [17], we observed that *Bcl-2* gene expression was significantly upregulated by 3,4-benzopyrene treatment in rat lung tissues at the early stage of carcinogenesis. Thus, Bcl-2 has been considered a critical determinant of the susceptibility of cells to apoptosis [31].

Pulmonary inflammation can contribute to the development of lung cancer in humans [32]. In the lungs, nuclear factor-kappa B (NF-κB) is a central effector of inflammatory responses, which are frequently activated in non-small cell lung cancer [33]. NF-kB is an important transcription factor and regulates a variety of pathophysiologic processes involved in cell survival and death [34]. Studies have shown that NF-kB is associated with inflammation and carcinogenesis [35], and that the knockdown of the NF-κB p65 subunit suppresses xenograft tumor growth of lung cancer cells in nude mouse by inhibiting Bcl-2 expression [33]. Benzo[a]pyrene (BaP) is one of the major carcinogens of lung cancer. Phase I metabolic enzymes such as cytochrome P450 (CYP) monooxygenases [36] transform benzo[a]pyrene (BaP) into dibenzo[a]pyrene (DBaP) in vivo, rendering it genotoxic. Cytochrome P450 CYP1A1 detoxifies the potential carcinogens in tobacco smoke [18], but it sometimes converts compounds into potent genotoxins. It is reported that Bcl-2 overexpression is linked to CYP1A1 and NF-κB in lung cancer [21, 33].

In the present study, we observed positive Bcl-2 expression in lung carcinoma tissues, but the expression of Bcl-2 mRNA and protein in subcutaneous malignant neoplasm tissues was significantly higher than that in lung carcinoma. Both CYP1A1 and NF-κB were positively expressed in both lung carcinoma tissues and subcutaneous malignant neoplasm tissues with no significant difference between the tissues. These results indicate that a differential Bcl-2 expres-

sion pattern in subcutaneous malignant neoplasm tissues and lung carcinoma tissues may be related to the different incidence of carcinogenesis induced by intrapulmonary injection and subcutaneous injection of 3,4-benzopyrene. Once carcinogen-induced DNA damage triggers DNA replication stress, carcinogenesis of epithelial cells may occur through the activation of the Bcl-2 pathway and interaction with other signaling pathways. Compared with other tissues, activation of signaling pathways in lung tissues may be different because of the adaptive response to continuous attack by multiple risk factors. Although the role of the Bcl-2 signaling pathway in lung carcinogenesis remains unclear, we predict that the lung may have acquired protective mechanisms to prevent carcinogenesis of epithelial cells in the presence of continuous attack from multiple risk factors, which may be involved in the inhibition of Bcl-2 signaling.

Acknowledgements

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Disclosure of conflict of interest

None.

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