Increased susceptibility to urethane-induced lung tumors in mice with decreased expression of connexin43

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Gap junction intercellular communication capacity and connexin expression are reportedly decreased in human lung cancer. The mechanisms by which connexins, the gap junction proteins, act as tumor suppressors are unclear. In order to understand the involvement of connexins in tumorigenesis, we analyzed the effect of the heterologous deletion of Gja1 [the connexin43 (Cx43) gene] on the development of lung adenomas in mice. Heterozygous (Cx43+/−) and wild-type mice (Cx43+/+) were treated or not with single doses of urethane at 15 and 17 days after birth. Twenty-five weeks later, both the number and size of nodules were increased in Cx43−/− mice as compared with Cx43+/+ mice. Moreover, the lesions were histologically more aggressive in the heterozygous mice. However, no increase in spontaneous lesions was observed in the lungs of untreated Cx43+/− mice. Heterozygous mice effectively presented lower expression of Cx43 genes and decreased amounts of Cx43. In conclusion, our results indicate that deletion of one allele of the Cx43 gene clearly favors the carcinogenic effect of urethane administration and results in a higher susceptibility to lung adenoma formation in mice.

Introduction

The maintenance of metabolic cooperation between cells is crucial for tissue homeostasis. In mammalian cells, intercellular channels control such processes by allowing cell-to-cell exchange of small hydrophilic molecules and ions (<1–2 kDa). These structures, known as gap junctions, are made up of protein subunits, the connexins, which belong to a multigene family of at least 20 members (1). Gap junctions are widely distributed in most tissues and animal species (2–4) and a reduced communication capacity has frequently been associated with tumor progression (5).

The epithelium of the pulmonary alveolus presents two cell types, known as alveolar pulmonary types I (APTI) and II (APTII), which are morphologically and functionally distinct (6). APTII cells synthesize, secrete and recycle all the surfactant components that regulate alveolar surface tension in mammalian lungs (7). These cells are also considered to be the progenitor cells, being responsible for the renewal of epithelium in normal or pathological conditions (8). Recent observations have shown that alveolar pulmonary epithelial cells communicate through gap junctions (9–15). Transcripts of a widely distributed type of connexin, connexin43 (Cx43), is expressed in both APTI and APTII cells (9–11). Gap junction intercellular communication (GJIC) between APTI and APTII cells is probably mediated through gap junction channels made up of Cx43 (16). In human and mouse lung carcinomas the neoplastic phenotype is frequently associated with a reduction in Cx43 and GJIC capacity (17,18). Moreover, the recovery of GJIC after transfection with Cx43 cDNA in human lung carcinoma cells is followed by a tumor suppressing effect (19).

Urethane-induced pulmonary adenomas in mice have been extensively used as a model to study lung tumors and to provide insights into the molecular events involved in carcinogenesis. Two compounds, vinyl carbamate and its epoxy derivative, may be the proximate and ultimate electrophilic metabolites responsible for genotoxicity and carcinogenicity of urethane. Epoxycarbamates interacts with DNA to form 7-(2-oxoethyl)guanine adducts (20–23). Recently it has been confirmed that both solid and papillary murine pulmonary adenomas chemically induced by urethane originate from APTII cells (24).

Mouse strains in which important tumor suppressor genes are knocked out are useful for understanding their involvement in carcinogenesis. Cx43−/− mice developed by Reama et al. (25) have been used in several research studies and represent a useful model to show the role of Cx43 in physiological and pathological processes, including cancers (26,27). Thus, studies of Cx43 heterozygous mice may be useful to understand the involvement of Cx43 in lung neoplasia. In this study we describe a higher susceptibility to pulmonary lesions in Cx43−/− mice after urethane administration.

Materials and methods

Abbreviations: Cx32KO, Cx32 knockout; Cx43, connexin43; DTT, dithiothreitol; GJIC, gap junction intercellular communication; PCNA, proliferating cell nuclear antigen.

doi:10.1093/carcin/bgh193
These mice were weaned at the age of 4 weeks. They were housed under controlled conditions (22 ± 2°C, 65 ± 15% relative humidity, air exchange rate 15 times/h, 12 h light–12 h dark cycle) in filter top cages at the Animal Facility of the Department of Pathology of the Faculty of Veterinary Medicine and Zootechny of the University of São Paulo. The animals received a pelleted standard diet (Lab Chow; Purina, Curitiba, Brazil) and tap water ad libitum during the study.

Genotyping of mice by PCR
DNA from the tail of each mouse was analyzed by PCR as previously described (28). The reaction products were loaded on an agarose gel (1.5%, diluted in Tris-buffered saline). The typical amplified band for the Gial gene was 520 bp and that for the neo gene 294 bp (data not shown). Once the mice had been genotyped they were separated into the different experimental groups of the study.

Experimental design
Mouse pulmonary adenomas were induced by treatment with urethane, an ethyl ester of carbamic acid extensively used in murine models of carcinogenesis (29–31). Groups of 15-day-old male or female Cx43−/− or Cx43+/+ mice received two s.c. injections of urethane (1.5 mg/kg) at intervals of 48 h (32). Animals that died during the study were not replaced. After 25 weeks the animals were anesthetized by i.p. injection of sodium pentobarbital (250 mg/kg body wt) and then killed by sectioning the abdominal aorta to evaluate the presence of pulmonary lesions.

Nodele counting and histological analysis
Once the animals had been killed, the lungs were removed as suggested by Dr Jerrold M.Ward (National Cancer Institute, Frederick, MA, personal communication) The trachea was sectioned and a needle was introduced between the cartilage rings. The alcoholic fixative methacarn was carefully instilled to infiltrate and to fix the lung structures. The number of external nodules in each pulmonary lobe was counted and the diameter of each lesion was measured immediately after instillation of the fixative. Lungs were kept in methacarn for 12 h. Then they were routinely processed and embedded in paraffin. Histological sections (5 μm) were stained with haematoxylin and eosin and analyzed in a Nikon E-800 microscope. The pulmonary lesions were classified as solid, papillary or solid/papillary (33). Areas of lung adenomas were then measured with the aid of an image analysis system. For this purpose, each lesion was drawn round with an electronic pen and its area was immediately calculated by Image Pro-Plus software (Media Cybernetics Inc., USA).

Spontaneous lesion incidence
For this study the animals were housed under the same conditions as described above and were killed at 25 weeks of age. The lungs were examined for the presence of adenomas. After gross examination, the whole lung was routinely processed for paraffin embedding and 5 μm sections were examined under the microscope.

RNA isolation, cDNA construction and real time PCR analysis
Total RNA was extracted from the tissues by a single step technique with TRZol Reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer’s protocol. Only lung lesions > 3 mm were sampled for analysis. The quality of the RNA samples was determined by electrophoresis through agarose gels and staining with ethidium bromide. The 18S and 28S bands were measured after cutting the gels and evaluating them with NIH Image software. The RNA was subjected to cDNA synthesis using oligo(dT) primers and the TakMan probe for Cx43 and GAPDH quantification were used to detect the housekeeping gene GAPDH. The TakMan probes carried a 5′ reporter dye, 6-carboxy fluorescein (FAM for Cx43) or (VIC for GAPDH) and a 3′ non-fluorescent quencher dye (NFQ) and a minor groove binder (MGB). The final concentrations of 0.9 and 0.25 μM were used for the primers and probes, respectively.

Statistical analysis
Statistical evaluation of tumor incidence data was performed with the ANOVA test. Values of P < 0.05 were considered significant.

Results
Incidence of macroscopic lesions in mice after urethane treatment
All mice were weighed once a week over the experimental period. No difference in weight gain was observed between either males or females with different genotypes (data not shown). The incidence, number and size of gross pulmonary nodules observed in the Cx43+/− and Cx43−/− groups are shown in Table I. First, all animals exhibited lung nodules 2 weeks after urethane injection, independent of their genotype. The only exception was a Cx43−/− male. Second, a higher incidence of macroscopic lesions was clearly observed in

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Cx43+/− male mice. Moreover, the highest nodule multiplicity was observed in Cx43+/− males (20±8 lesions/mouse), significantly different from Cx43+/− male mice (10±5). Cx43+/− male mice also showed a higher incidence of pulmonary lesions in comparison with Cx43+/− females (P < 0.05). The average number of pulmonary lesions in Cx43+/− females did not differ significantly from Cx43+/− females (P > 0.05). The total number of lesions was 1.7 times higher in Cx43+/− mice (364 and 292 lesions in males and females, respectively) as compared with Cx43+/− mice (217 and 176 lesions in males and females, respectively). Pulmonary lesions were also larger in Cx43+/− mice than in Cx43+/− mice. For instance, in the lungs of Cx43+/− male mice 11 nodules larger than 3 mm were observed.

Spontaneous macroscopic lesion incidence after 25 weeks  
Twenty-five-week old Cx43+/− and Cx43+/+ male or female mice were examined for spontaneous lung lesions. No pulmonary lung lesion was observed in any mouse (data not shown).

Histological classification and microscopic quantification of adenomas  
Microscopic quantification of lung adenomas is shown in Table II. No significant difference was observed in the lesion areas even if they were slightly larger in Cx43+/− mice. However, Cx43+/− males exhibited significantly higher numbers of microscopic lesions (P < 0.05) when compared with their Cx43+/+ counterparts.

Classification of the lung lesions into three different histological groups, as shown in Figure 1, was proposed. The results obtained using the histopathological classification are shown in Table III. These results were compared between male groups and female groups only. Cx43+/− mice showed higher numbers of papillary adenomas than Cx43+/+ mice. The number of solid adenomas, which was highest in Cx43+/− females, was similar between Cx43+/− and Cx43+/+ males. However, the percentage of solid/papillary adenoma was found to be higher in Cx43+/− males than in their Cx43+/− counterparts. The opposite was found for females, with the percentage of solid/papillary adenomas being lower in Cx43+/− females than in Cx43+/− females. It seems that a higher incidence of papillary adenomas coincided with the Cx43+/− genotype.

**Real time PCR**  
The end-point of real time PCR analysis is the threshold cycle or Ct. The Ct is determined from a log–linear plot of the PCR signal versus the cycle number. Thus, Ct is an exponential and not a linear term. For this reason, any statistical presentation using raw Ct values should be avoided. Our results from real time PCR analyses are shown in Table IV.

A difference of 0.5 in the relative amount of cDNA amplification (equivalent to 1 Ct difference) represents twice the amount of Cx43 gene mRNA. Cx43+/− mice showed lower Cx43 gene expression than Cx43+/+ mice under all the conditions analysed. Without urethane treatment Cx43+/− mice have ~50% of the Cx43 gene expression with urethane treatment. After urethane treatment there was no difference in Cx43 gene expression between male and female Cx43+/− mice. However, Cx43 gene expression was increased in male and female Cx43+/+ mice in comparison with untreated mice of the same genotype. Incidentally, only female Cx43+/− showed an increase in Cx43 gene expression after treatment.

To study Cx43 gene expression in tumors, we used only lesions > 3 mm. In these samples we were unable to observe any expression in Cx43+/− mice. However, in Cx43+/+ mice the females showed higher expression in comparison with male mice of the same genotype.
Table III. Histopathological classification of pulmonary adenomas obtained 25 weeks after urethane injection

<table>
<thead>
<tr>
<th>Group</th>
<th>Adenoma (%) of total lesions</th>
<th>Total lesion no.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Solid</td>
<td>Solid/papillary</td>
</tr>
<tr>
<td>Cx43+/+ male</td>
<td>41 ± 1</td>
<td>36 ± 2*</td>
</tr>
<tr>
<td>Cx43+/- male</td>
<td>42 ± 2</td>
<td>23 ± 9</td>
</tr>
<tr>
<td>Cx43+/+ female</td>
<td>71 ± 2*</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>Cx43+/- female</td>
<td>52 ± 1</td>
<td>31 ± 2*</td>
</tr>
</tbody>
</table>

Mice were killed 25 weeks after urethane (1.5 g/kg) treatment by i.p. injection of sodium pentobarbital (250 mg/kg). Means in the same column indicated with an asterisk are significantly different according to the Tukey–Kramer test (P < 0.05). Values represent means ± SEM of 21, 18, 19 and 24 different mice.

Table IV. Levels of Cx43 mRNA determined by quantitative RT–PCR

<table>
<thead>
<tr>
<th>Group</th>
<th>Difference in cycles between Cx43 and GAPDH</th>
<th>Normalized Cx43 amount relative to female Cx43+/+ mouse</th>
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<tbody>
<tr>
<td>Cx43+/+ male</td>
<td>6.7 ± 0.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Cx43+/- male</td>
<td>8.1 ± 0.7</td>
<td>0.5</td>
</tr>
<tr>
<td>Cx43+/+ female</td>
<td>7.7 ± 0.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Cx43+/- female</td>
<td>6.1 ± 0.2</td>
<td>1.8</td>
</tr>
<tr>
<td>Cx43+/+ male</td>
<td>7.9 ± 0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Cx43+/- female</td>
<td>6.2 ± 0.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Cx43+/+ female</td>
<td>7.2 ± 0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Cx43+/- male</td>
<td>9.7 ± 0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Cx43+/+ female</td>
<td>8.0 ± 0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Cx43+/- female</td>
<td></td>
<td>NDT</td>
</tr>
</tbody>
</table>

Mice were killed 25 weeks after urethane (1.5 g/kg) treatment by i.p. injection of sodium pentobarbital (250 mg/kg). Using reverse transcriptase, cDNA was synthesized from 500 μg total RNA. Aliquots of cDNA were used as templates for real time PCR reactions containing both primers and probe for Cx43 or primers and probe for GAPDH. Each reaction contained cDNA derived from 20 ng total RNA. Eight different lungs from each group were performed and four replicates of each reaction were performed by RT–PCR. For nodules (>3 mm), three different nodules from each group were used and four replicates of each reaction were performed by RT–PCR. Nu, untreated; wu, treated with urethane; tu, tumor sample; NDT, undetected.

Fig. 1. Histological classification of adenomas in mouse lungs visualized after 25 weeks urethane (1.5 g/kg) treatment. (A) Solid adenoma. (B) Solid/papillary adenoma. (C) Papillary adenoma. 20× objective.

Fig. 2. Western blot analysis of Gja1 expression in (A) lung and (B) heart tissue (control). Mice were killed 25 weeks after urethane (1.5 g/kg) treatment by i.p. injection of sodium pentobarbital (250 mg/kg). Each lane was loaded with 150 μg protein. Lane 1, size markers; lanes 2–5, homogenates from untreated animals; lanes 6–9, homogenates from animals treated with urethane. Lanes 2 and 6, Cx43+/+ males; lanes 3 and 7, Cx43+/- females; lanes 4 and 8, Cx43+/+ males; lanes 5 and 9, Cx43+/- females. These blots represent three different lungs and hearts, respectively, by lane. These experiments were repeated four times for each sample with similar results.

Immunoblotting of Cx43

Cx43+/- mouse hearts showed a lower level of Cx43 protein compared with Cx43+/+ mouse hearts. The same pattern of Cx43 expression was observed in lungs (Figure 2).

Immunohistochemical staining

Untreated lungs from male or female Cx43+/+ mice present less intense immunostaining for Cx43 in comparison with their wild-type counterparts, as expected (Figure 3A–D). Urethane-treated male or female Cx43+/- or Cx43+/+ mice presented the same immunostaining pattern as untreated mice; heterozygous mice also presented less intense immunostaining for Cx43 (Figure 3E–H). In lung tumor samples a stronger punctate immunostaining for Cx43 was observed in wild-type male mice in comparison with heterozygous mice. This pattern was seen in all tumor types, independent of their classification (solid, solid/papillary or papillary) (Figure 4A–F). However, if tumor tissues were compared with their respective surroundings, less intense immunostaining was verified.
PCNA-positive cell quantification

The results of PCNA-positive cell quantification are shown in Table V and Figure 5. Male and female Cx43‡ mice presented a higher PCNA labeling index in the three histopathological types of adenomas (P < 0.05).

Discussion

Decreased expression of Cx43 has previously been described in lung cancer tissue (18) and cells (17). On the basis of this, it has been suggested that Cx43 may exert some suppressive...
effect on human lung carcinoma cells (19). Because Cx43 is a widely distributed connexin, we were interested in verifying whether Cx43 \( ^{+/+} \) mice could be used as a model to characterize the biological involvement of this protein in chemically induced carcinogenesis of the lung.

In this study Cx43 \( ^{+/−} \) mice presented a higher susceptibility to urethane-induced lung adenomas. The loss of one allele of Cx43 is associated with an increase in the number of lung lesions developed during the 25 week experimental period. These results suggest that Cx43 plays an important function in

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**Fig. 4.** Expression of Cx43 in lung tumors. Mice were killed 25 weeks after urethane (1.5 g/kg) treatment by i.p. injection of sodium pentobarbital (250 mg/kg). (A–F) Samples from male mice. (G) Negative control. Arrowheads show connexin staining. Nuclei were stained with propidium iodide. 40× objective. See online supplementary material for a color version of this figure.
tissue homeostasis during lung carcinogenesis. Up to now these mice have not been reported to be more sensitive to any chemical carcinogen. For example, no higher susceptibility to skin cancer or to fibrosarcoma was observed in Cx43$^{+/−}$ mice treated with dimethylbenzanthracene or 12-O-tetradecanoylphorbol-13-acetate (27). It was suggested in that study that the expression of other connexins in skin cells could compensate for the Cx43 deficiency and prevent a higher susceptibility to skin cancer. On the other hand, our study shows for the first time that a deficiency in only one allele of a connexin gene can contribute to development of the carcinogenic process. Other studies previously showed that mice in which both alleles of Cx32 were deleted, Cx32 knockout (Cx32KO) mice, presented a higher incidence of both spontaneous and chemically induced liver cancers (35). In another study, Dagli et al. (36), working with transgenic mice expressing a dominant negative form of Cx32,

Table V. Quantification of PCNA immunostained nuclei in mouse lung tumors

<table>
<thead>
<tr>
<th>Groups (no. of slides)</th>
<th>Morphological adenoma types (%)</th>
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<th></th>
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<tbody>
<tr>
<td></td>
<td>Solid</td>
<td>Papillary</td>
<td>Solid/papillary</td>
</tr>
<tr>
<td></td>
<td>(no. of lesions)</td>
<td>(no. of lesions)</td>
<td>(no. of lesions)</td>
</tr>
<tr>
<td>Cx43$^{+/+}$ male (10)</td>
<td>7 ± 2 (11)</td>
<td>12 ± 2 (5)</td>
<td>10 ± 2 (8)</td>
</tr>
<tr>
<td>Cx43$^{+/−}$ male (10)</td>
<td>18 ± 2* (19)</td>
<td>20 ± 5* (17)</td>
<td>16 ± 2* (11)</td>
</tr>
<tr>
<td>Cx43$^{+/+}$ female (10)</td>
<td>7 ± 1 (14)</td>
<td>9 ± 1 (3)</td>
<td>7 ± 1 (4)</td>
</tr>
<tr>
<td>Cx43$^{+/−}$ female (10)</td>
<td>14 ± 3* (8)</td>
<td>18 ± 7* (6)</td>
<td>13 ± 3* (12)</td>
</tr>
</tbody>
</table>

Mice were killed 25 weeks after urethane (1.5 g/kg) treatment by i.p. injection of sodium pentobarbital (250 mg/kg). Means in the same column indicated with an asterisk are significantly different according to the Tukey–Kramer test ($P < 0.05$). Values represent means ± SEM of 10 different mice (slide) chosen at random. The numbers of lesions studied in each histological group after classification are listed in parentheses. These experiments were repeated twice for each slide with similar results.

Fig. 5. PCNA staining of lung adenomas 25 weeks after urethane treatment (1.5 g/kg). (A) Cx43$^{+/+}$ mice (solid adenoma). (B) Cx43$^{+/−}$ mice (solid adenoma). (C) Cx43$^{+/+}$ mice (solid/papillary adenoma). (D) Cx43$^{+/−}$ mice (solid/papillary adenoma). (E) Cx43$^{+/+}$ mice (papillary adenoma). (F) Cx43$^{+/−}$ mice (papillary adenoma). 40× objective. See online supplementary material for a color version of this figure.
observed higher numbers of liver preneoplastic lesions, hepatocellular adenomas and carcinomas, with significantly higher multiplicity. This study showed that maintenance of GJIC capacity is important for carcinogenesis, in order to coordinate cell growth both positively and negatively. Recently, Yoon et al. (37) verified that Cx32 is important for pneumotoxicity of the carcigen benzene. According to these authors, the results of pulmonary tumorigenesis in Cx32KO mice are not conclusive. This statement may indicate that, according to our results, Cx43 is the main connexin type involved in lung tumorigenesis.

Cytochrome P450 2E1 (CYP2E1) is the main enzyme responsible for urethane metabolism in lungs (38). Lung carcinogenesis using urethane involves the development of nodular lesions called adenomas, originating from single APTII cells that have developed mutations induced by the carcigen metabolites (24). Lung adenomas are reportedly seen earlier or later in mouse life, depending on the dose and age of injection of the carcigen (39). Mouse strain is also important in the susceptibility to urethane. It has been reported that strain A is the most susceptible, while strain C57BL is less susceptible to urethane (39); Swiss mice and, thus, strain CD1 (which is derived from the Swiss strain) presents an intermediate susceptibility to the development of adenomas associated with urethane.

Our intention in this study was to quantify lung adenomas at the age of 25 weeks. Adenomas in lung are classified into three categories, with papillary adenomas being characterized as the least differentiated stage (33). On the other hand, solid/papillary adenomas are a transition stage between less aggressive adenomas (solid) and aggressive stages (papillary adenomas). Cx43+/− females showed the greatest number of adenomas classified as solid, probably reflecting a more aggressive response to urethane. On the other hand, both male and female Cx43+/− mice presented more papillary adenomas than their Cx43+/+ counterparts. This finding may support a previous finding showing that aberrant Cx43 gene expression is involved in the progression of lung carcinogenesis (17).

Before urethane treatment both male and female heterozygous mice presented ~50% Cx43 expression in comparison with Cx43+/+ mice. However, after treatment expression increased in wild-type mice and was twice that for Cx43+/− female mice. In Cx43+/− male mice gene expression did not increase with treatment. In lung tumors mRNA expression was not detected in Cx43+/− mice, but we were able to detect it in Cx43+/+ mice. These results were in accordance with the minor lesion incidence in female mice. This increase in gene expression probably contributes to restoring dye coupling between APTII cells. This issue is currently under investigation.

Our results also revealed that after urethane treatment female mice developed fewer tumors than did male mice. In fact, this is a frequent finding in carcinogenesis experiments and it is still unexplained. The increase in Cx43 expression in females could be a sex-specific effect triggered through estrogens. Indeed, the AP-1 site in the promoter region of connexin genes can be activated in the presence of c-jun and c-fos. As reported, this further regulates connexin gene expression in the presence of estrogen (40). By western blot and immunohistochemistry we confirmed that Cx43+/− mice present lower Cx43 amounts in both lung tissue and tumor cells. It has been previously shown that transfection of Cx43 cDNA into human lung carcinoma cells reduced their tumorigenicity when injected into nude mice (19). The decrease in Cx43 mRNA levels in lung carcinoma cells also correlated with Cx43 protein expression (17). These results reinforce the idea that Cx43 is acting as a tumor suppressor.

Cx43 involvement in the induction of lung adenomas could be related to K-ras activation since it has been found to be mutated in mouse lung tumors induced by urethane (41). Activated ras indirectly has the capacity to disrupt GJIC (42). It is possible that Cx43+/− mice, due to lower GJIC, were more susceptible to the effect of ras than Cx43+/+ mice. Consequently, an even lower GJIC capacity could be developed in these mice, so that essential molecules involved in growth control would not be able to diffuse appropriately. cAMP permeates easily through Cx43 channels (43), control the cell cycle and could inhibit the growth of lung epithelial cells (44). If a cell lacks functional gap junctions, its growth would not be suppressed by neighboring negative signals or would be stimulated by the accumulation of positive signals. This could lead to dysregulated growth (45).

According to this, it is interesting to note that adenomas from Cx43+/− mice presented a higher percentage of S phase-positive cells (~18%) than Cx43+/+ mice (~10%). Thus, the different urethane-induced adenomas in Cx43+/− mice can proliferate faster than their counterparts.

We believe that the function of some proteins that regulate the cell cycle is compromised when Cx43 is not normally expressed. Certain possibilities arise from recent studies undertaken by Olbina and Eckhart (46), who proposed that Cx43 inhibits cell growth by a mechanism that does not require functional gap junction channels. Peebles et al. (47) have described a relationship between protein disulfide isomerase and Cx43 expression, whose reduction follows neoplastic transformation of alveolar type II cells. This protein is able to regulate transcription factor activity (48). It would be very interesting to know whether protein disulfide isomerase is involved in carcinogenicity induced by urethane in Cx43+/− mice.

In summary, these results indicate that Cx43 expression is important for the maintenance of lung homeostasis. Cx43+/− mice showed a higher susceptibility to urethane lung carcinogenesis. Since this susceptibility was higher in males, we may argue that other connexins mostly present in alveolar cells from males were unable to replace Cx43. It is possible that in the females hormonal control can provide a compensatory activity further regulating Cx43. Taken together, these results confirm a tumor suppressor role of Cx43 in lung carcinogenesis.

Supplementary material

Supplementary material can be found at http://www.carcin.oupjournals.org/

Acknowledgements

This work is part of the PhD thesis of J.L. Avanzo in the Experimental and Comparative Pathology Program of the Faculty of Veterinary Medicine and Zootechny of the University of São Paulo. This research was supported by grant 01/06820-2 from the Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP). J.L.A. was supported by a fellowship from FAPESP 01/06821-9. This work is part of the International Cooperation Program CAPES/COFECUB (Proc. 386/02).
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Received December 2, 2003; revised May 6, 2004; accepted May 16, 2004