Constitutive over expression of IL-1β, IL-6, NF-κB, and Stat3 is a potential cause of lung tumorgenesis in urethane (ethyl carbamate) induced Balb/c mice

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Abstract

**Background:** Lung cancer is a leading cause of cancer death. There has been found a substantial gap in the understanding of lung cancer genesis at the molecular level. We developed urethane (ethyl carbamate) induced lung tumor mice model to understand the mechanism and molecules involved in the cancer genesis. There might be many molecules involved, but we subsequently emphasized here the study of alternation in the expression of NF-κB, Stat3, and inflammatory cytokines interleukin-1β and interleukin-6 to hypothesize that the microenvironment created by these molecules is promoting tumor formation. **Materials and Methods:** 7–8 week old Balb/c mice of either sex were given intraperitoneal (i.p.) injection of urethane (1g/kgbw) for eight consecutive weeks. Histopathological analysis was done to detect abnormality or invasions occurred in the lung tissues. Automated cell counter was used to count the number of inflammatory cells. The expression of NF-κB, Stat3, and IL-1β was observed at translational level by western blot, while the expression of IL-1β and IL-6 was observed at transcriptional level by semiquantitative reverse transcriptase polymerase chain reaction (RT-PCR) method. Secretion of IL-1β and IL-6 in the blood was measured by enzyme-linked immunosorbent assay (ELISA) method at different time intervals. **Results:** Histopathological analysis showed various lung cancer stages hyperplasia, atypical adenomatous hyperplasia, and adenocarcinoma. Increased population of inflammatory cells, persistant expression of NF-κB, Stat3, pStat3, and IL-1β at translational level, while at transcriptional level constitutive enhanced expression of IL-1β and IL-6 followed by increased secretion of IL-1β and IL-6 in the blood were observed in urethane-injected mice in comparison to phosphate buffer saline (PBS) injected mice at 12, 24, and 36 weeks. **Conclusions:** Overexpression of key molecules such as NF-κB, Stat3, pStat3, IL-1β, and IL-6 might have caused chronic inflammation, leading to the progression of lung cancer. **Keywords:** Adenocarcinoma, NF-κB, proinflammatory cytokines, Stat3, urethane

BACKGROUND

Lung cancer has become one of the most common causes of cancer death in human. Worldwide death toll due to lung cancer is approximately 1.2 million per year. The number of lung cancer affected individuals throughout the world has been increased several times in recent years. Lung cancer is a highly complex neoplasm consisting of two common histological types. The two types are small cell lung carcinoma (SCLC)
and nonsmall cell lung carcinoma (NSCLC). NSCLC are further classified as adenocarcinoma (AD), squamous cell carcinoma (SCC), and large cell neuroendocrine carcinoma. [4] Out of these types and subtypes, AD is a most common form of lung cancer.[5] Chronic inflammation is implicated in multistages of carcinogenesis to contribute tumor initiation and progression by several mechanisms including acceleration of cell cycle, cell proliferation and stimulation of tumor neo-vascularization.[6,7]

Urethane or ethyl carbamate (EC) is a chemical carcinogen present in tobacco leaves, tobacco smoke, [8] and in fermented food products, such as bread, yogurt, and cheese, [9] formed by the reaction of urea with alcohol [10, 11]. The concentration of EC (urethane) in some food and beverages[12-14] has been documented in the Table 1. Urethane specifically promotes the development of lung tumors from nonciliated airway epithelial (Clara) or type II alveolar epithelial cells in mice.[15,16] Balb/c mice are considered as an intermediate-susceptible for the development of lung tumor by urethane administration.[17] Urethane-induced lung AD has been well characterized and accepted as model for human lung AD.[18,19] The pathways connecting transcription factors (TFs) and cytokine-dependent inflammation and tumor formation in the lung induced by urethane have not been well deciphered so far.

**MATERIALS AND METHODS**

**Chemicals**

Urethane (Sigma chemical company, St Louis, MO), ethylenediamine tetracetic acid (EDTA) (Merck, Mumbai, India), protease inhibitor cocktail (Thermo scientific, Rockford, USA), page ruler™ plus prestained protein ladder (Fermentas, USA); primary antibodies β-Actin (C4), (SC-47778); NF-κB p65 (C-20), (SC-372); Stat3 (C-20), (SC-482); pStat3 (Tyr705), (SC-7993); interleukin-1β (IL1β), (I3767, Sigma); secondary antibodies goat anti-rabbit IgG HRP-conjugated, (SC-2030); donkey anti-goat IgG HRP-conjugated, (SC-2033); rabbit anti-mouse IgG HRP-conjugated, (HPO5, Genei, Bangalore); super signal west pico chemiluminescent substrate, 134078 (Pierce, Thermo Scientific, Rockford, USA). SC stands for Santa Cruz Biotechnology (CA, USA).

**Animals and experimental design**

7–8 weeks old Balb/c mice of either sex, approximately having equal body weight were taken. Mice were distributed into two groups. Each group contains 8 mice numbered separately and one group was intraperitoneal (i.p.) injected with urethane dissolved in phosphate buffer saline (PBS), while another group was i.p. injected with PBS (control). All the mice were kept in hygienic condition at room temperature (RT) with a 12-h light/dark cycle, and provided with standard animal feed and water *ad libitum*. Tumors were induced by i.p. injection of urethane 1g/kg bw of mice for eight consecutive weeks and 6 to 8 month latency period. Mice were sacrificed for observation at 0, 12, 24, and 36 weeks from group of urethane-injected mice and PBS-injected mice. Body weight of each mouse was recorded weekly up to their sacrifice. Animal ethical rules were followed as per the guidelines given by the Animal Ethical Committee Institute of Medical Sciences (IMS), Banaras Hindu University (BHU), Varanasi, India.

**Histopathology**

Lung’s tissues were fixed over night in Bouin’s solution followed by washing and dehydration in a graded series of ethanol. Finally, lung tissues samples were embedded in the paraffin. Paraffin-containing lung tissues were cut into the 5-µm-thick sections at the median transverse level. Sections were de-paraffinized using xylene and graded series of ethanol. Sections were mounted on glass slides, stained, and counterstained with hematoxylin and eosin (H&E), respectively, for microscopic examination.

**Peripheral blood cell counting**

40 µl blood samples were collected from the retro-orbital sinus, by using a capillary pipette containing EDTA anticoagulant. The samples were used for complete blood cell count by automated Hematology analyzer KX-21 (Sysmex Corporation, Japan).

**SDS-PAGE and western blot**

Lung tissues from urethane-injected mice and PBS-injected mice were used for protein extraction using lysis buffer. Lysis

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**Table 1: Concentration of urethane or ethyl carbamate in various foods and beverages**

| Reported by  | Food and beverage       | Concentration of ethyl carbamate |
|-------------|-------------------------|---------------------------------|
| Hasnip et al. (2007) | Spirit drinks            | 0.01–6.2 mg/L                   |
|             | Whisky                  | <0.01 mg/L                      |
|             | Beer                    | <0.01 mg/L                      |
|             | Wine                    | 0.01–0.025 mg/L                 |
|             | Sake                    | 0.08–0.17 mg/L                  |
|             | Vinegar                 | 0.033 mg/L                      |
|             | Cake                    | <0.01 mg/kg                     |
|             | Bread                   | <0.01 mg/kg                     |
|             | Sauerkraut              | 0.029 mg/kg                     |
|             | Yeast extract           | 0.041 mg/kg                     |
|             | Cheese                  | <0.01 mg/kg                     |
|             | Soybean paste           | <0.01 mg/kg                     |
|             | Soy sauce               | 0.01–0.084 mg/kg               |
|             | Yogurt                  | <0.01 mg/kg                     |
|             | Christmas pudding       | 0.02 mg/kg                      |
| EFSA 2007   | Stone fruit brandy      | 0.01–0.22 mg/L                  |
| Park et al 2007 | Fermented soy sauce   | 0.01–0.1 mg/kg                  |
buffer contains 50 mM Tris-Cl (pH-7.4), 150 mM NaCl, 0.5% Triton X-100, 5 mM EDTA (pH-8), and protease inhibitors cocktail. The concentration of each protein sample was estimated by Bradford assay, equalized and 50 µg/well was loaded on each lane of 10% SDS-PAGE for transcription factor TF and 12% sodium dodecyl sulphate-polyacrylamide gel electrophoresis SDS-PAGE for cytokines. Separated proteins were transferred to the polyvinylidene difluoride membranes (PVDF) at 20V/4°C for overnight. The membranes were blocked with 5% nonfat dry milk in tris-buffer saline tween-20 TBST (1xTBS, 0.2% Tween 20) buffer at RT for 1 h. The membrane was washed thrice with TBST and incubated separately in the TBST buffer containing either primary antibody NF-κB p65, Stat3, pStat3 (Tyr705), IL-1β, or β-Actin separately, each having dilution of 1:1000. Separate PVDF membrane with protein samples were incubated with each primary antibody, thrice washed with TBST and then incubated separately into horse-radish peroxidase conjugated species-specific secondary antibody with a dilution of 1:5000 at RT for 1 h. After washing thrice with TBST to each PVDF membrane, the chemiluminescence substrate was poured all over membrane. The primary antibody specific proteins made visible by detecting chemiluminescence emission by exposing to the kodak X-ray film. To confirm equal protein loading, membranes were stripped and reprobed with mouse monoclonal anti-β-actin primary antibody.

Isolation of total RNA and semiquantitative PCR (RT-PCR)

Expression of IL-1β and IL-6 in lungs was studied by semiquantitative RT-PCR. For this, total RNA was isolated using Trizol (Invitrogen, Carlsbad, CA, USA) as per manufacturer’s instructions. The tissue was homogenized using Trizol reagent and chloroform was added for phase separation. Colorless upper aqueous phase was gently taken out in separate tube and precipitated with isopropanol. Precipitated RNA was centrifuged at 10,000 rpm at 4°C. The pellet was retained to wash with 70% ethanol. Washed pellet was air dried and dissolved in diethyl pyrocarbonate (DEPC) -treated double distilled water. The concentration and purity of RNA was determined using nanodrop spectrophotometer (ND-1000, Wilmington, USA). First, strand of cDNA was synthesized by using AMV RT-PCR kit (105566, Banglore Genei, India) as follows: 1–5 µl (0.1–1 µg) total RNA, 1 µl Oligo (dT)18 , and RNase free water to make total volume 10 µl. The vial containing sample solution was placed at 65°C for 10 min and at RT for 2 min. The sample was spun briefly and other components, e.g., RT enzyme, dNTP, RNase inhibitors, and RT buffer, were added. Sample solution was properly mixed, briefly centrifuged, and incubated at 42°C for 1 h, followed by incubation at 95°C for 2 min to stop the reaction. Finally, samples containing single cDNA strand were centrifuged for few seconds and kept on ice till PCR.

The amplification of IL-1β and IL-6 was done using MJ mini thermal cycler (Bio Rad, California, USA). The primers and amplicon sizes were as follows: IL-β forward primer 5’-GACCTTCCAGGATGAGGACA-3’, reverse primer 5’-AGGCCACAGTATTTTGTGC-3’ with a product size 273bp; IL-6 forward primer 5’-TCTCCAGCAACGAGGAGAAT-3’ reverse primer 5’-TGTGATCTGAAAACCTGCTGC-3’ with a product size 348bp; β-actin forward primer 5’-AGCCATGTACGTAGCCATCC-3’ reverse primer 5’-CTCTCAGCTGTTGCGTGAA-3’ with a product size 227bp.

The parameters for the amplification of IL-1β and IL-6 were as follows: 94°C for 2 min, 94°C for 45 s, 52°C for 30 s, 72°C for 60 s for 35 cycles at 72°C for 2 min. The PCR products were separated on 2% agarose gel by electrophoresis along with DNA ladder of 100 bp. Gel was visualized under UV light in gel documentation system alpha imager EC (Alpha innotech, Japan).

Enzyme-linked immunosorbent assay

IL-1β and IL-6 level in mice serum were measured by using highly specific quantitative sandwich Enzyme-linked immunosorbent assay (ELISA) kit (EMIL1B & EM2IL6 Pierce Biotechnology, Thermo Scientific, Rockford, USA). ELISA was performed according to the manufacturer’s instructions. 50 µl of biotinylated antibody reagent was added to each well of ELISA plate and then 50 µl test samples was added in duplicate to each well. Reagent in well were mixed and incubated for 2 h at RT, followed by three gentle washing with wash buffer. 100 µl of streptavidin-HRP solution was added to each well and incubated for 30 min at RT. 100 µl of TMB substrate solution was added, mixed by shaking and incubated 30 min for color development. Reaction was terminated by adding 100 µl of stop solution. Finally optical density was measured at 450 nm in chemiluminescent ELISA plate reader machine.

Electrophoretic mobility shift assay

For the isolation of nuclear proteins, the pellet from minced lung tissues were resuspended in a modified RIPA lysis buffer containing 50 mM Tris, pH-7.4, 1% NP-40, 150 mM NaCl, 1 mM EDTA, 0.5% sodium deoxycholate, and protease inhibitor cocktail (Thermo Scientific, Rockford, USA) with repeated vortexing on ice lysates were spun at 14,000 rpm for 25 min at 4°C to obtain supernatant designated as nuclear proteins. The concentration of proteins was determined by spectrophotometric method and the proteins were stored in aliquots at -80°C till further use. For binding reaction
and detection, light shift chemiluminescent Electrophoretic mobility shift assay (EMSA) and chemiluminescent nucleic acid detection module were used (Thermo scientific, Rockford, USA). Briefly, 100 fmol of labeled probe was incubated with nuclear extract (10 µg proteins) in a total volume of 20 µl for 30 min at RT with binding buffer. To prevent nonspecific binding of nuclear proteins, 1 µl of poly dI:dC was added. Protein-DNA complexes were separated from unbound DNA using 6% native PAGE. NF-κB and Stat3 binding oligonucleotide consensus sequence were as follows: NF-κB binding oligonucleotide consensus sequence biotin probe-5’- biotin -AGTTGAGGGGACTTTCCCAGGC-3’, Stat3 binding oligonucleotide consensus sequence biotin probe- 5’-biotin-GATCCTTCTGGGAATTCCTAGATC-3’.

**Statistical analysis**

All the experiments were conducted three times and were reproducible. The bands were analyzed and quantitated using computer-assisted densitometry (Alpha innotech corporation, Japan). For RT-PCR and western blots, the band intensity was measured. Data were expressed as mean ± SEM; pair-wise comparisons were made by student’s t-test. P < 0.05 were considered as significant.

**RESULTS**

**Effect of urethane on body weight**

The initially (at zero week) average body weight recorded for urethane-injected group and PBS-injected group were 20.75 g and 17.88 g, respectively. Body weight of urethane-injected mice decreased up to fourth week (from 20.75 to 16.42 g), followed by continuous increase in the body weight up to 28 weeks (from 16.42 to 33.30 g). After 28 weeks, body weight of urethane-injected mice again decreases (from 33.30 to 30.05 g) but the body weight of PBS-injected mice increased continuously up to 36 weeks (from 17.88 to 34.05 g) [Figure 1].

**Effect of urethane on the lung organ index**

Lung organ index of urethane-injected and PBS-injected mice were calculated at different time interval, i.e., at 0, 12, 24, and 36 week after sacrifice of mice. The lung organ index surprisingly increases 1.4, 1.6, and 1.72 folds at 12, 24, and 36 week, respectively, in the urethane-injected mice as compared to PBS-injected mice [Figure 2].

Organ index = weight of organ (g)/body of weight of mice (g) x 100

**Histopathological analysis of lung tissues section**

The histopathological slide of urethane-injected lung tissues showed different adenoma stages [Figure 3]. Variable degree of atypia or hyperplasia shows abnormality with increase in the number of normal cells and its arrangement [Figure 3b]. Atypical adenomatous hyperplasia (AAH) shows severe cellular atypia with minimal septal inflammation and minimal septal fibrosis, cuboidal to low columnar in shape, sparse cytoplasm, inconspicuous nucleoli, distinct boundary

![Figure 1: Line graphs showing the average body weight (in grams) of urethane-injected and PBS- (phosphate buffer saline) injected control Balb/c mice. Body wt. was taken up to 36 weeks. (Data reported as the mean ± SEM for n = 8 per group and compared against PBS-injected mice.)](image-url)
with small lesions [Figure 3c]. AD shows the epithelial cells making up the tumor mass, severe septal inflammation and septal fibrosis, cuboidal to columnar in shape, moderate to abundant amount of eosinophilic cytoplasm, pleomorphic nuclei are located centrally at base occasional prominent nucleolus or refractile pseudoinclusions, irregular boundary with severe lesions [Figure 3d].

**Quantitation of inflammatory cells in peripheral blood**

Counting of total leukocytes, lymphocytes, monocytes, and neutrophils in peripheral blood samples of urethane-injected mice was done. The peripheral blood samples were collected at 0, 12, 24, and 36 week after urethane and PBS injection in mice [Figure 4]. The number of monocytes 2.92, 4.19, and 3.68 folds [Figure 4a], neutrophils 1.75, 2.21, and 2.09 folds [Figure 4b], lymphocytes 1.39, 1.71, and 2.26 folds [Figure 4c], and total leukocytes 1.41, 1.54, and 2.45 folds [Figure 4d] were higher in urethane-injected mice compared to PBS-injected mice at 12, 24, and 36 week, respectively.

**Translational expression of transcription factors and pro-inflammatory cytokines**

Western blotting was performed to check the expression of TF pStat3, Stat3, NF-κB, and proinflammatory cytokines IL-1β at translational level in lung tissue of urethane-injected and PBS-injected mice at 0, 12, 24, and 36 week [Figure 5]. Immunoblots revealed almost equal expression of pStat3, Stat3, NF-κB, and IL-1β protein in the PBS-injected mice lung tissue samples at 0, 12, 24, and 36 week [Figure 5], while in urethane-injected mice lung tissue samples the expression level of pStat3, Stat3, NF-κB, and IL-1β proteins comparatively increased by 2.39, 2.36, 1.69, and 3.68 folds, respectively, at 12 week [Figure 5], 6.03, 5.48, 2.23, and 10 folds, respectively, at 24 week [Figure 5], and 3.01, 5.34, 2.44, and 9.77 folds, respectively, at 36 week [Figure 5] in comparison to PBS-injected mice lung tissue samples. The expression level of these proteins in urethane-injected and PBS-injected mice lung tissue samples at 0 week was almost equal.

**Gene expression of pro-inflammatory cytokines at transcription level**

The effect of urethane on the expression of proinflammatory cytokines IL-1β and IL-6 at mRNA level was assessed in lung tissues of urethane-injected and PBS-injected mice at 0, 12, 24, and 36 week [Figure 6] by semiquantitative RT-PCR. The expression level of IL-1β and IL-6 was almost equal in PBS-injected mice lung tissue samples at 0, 12, 24, and 36 week [Figure 6], whereas in urethane-injected mice lung tissue samples showed upregulated expression of IL-1β and IL-6 by 5.6 and 4.5 folds, respectively, at 12 week, 9.4 and 2.8 folds, respectively, at 24 week [Figure 6], while 10 and 6.6 folds, respectively, at 36 week [Figure 5] compared to PBS-injected mice lung tissue samples. At zero week in urethane-injected mice lung tissues and PBS-injected mice tissues shows equal expression level of both IL-1β and IL-6.

**Production and secretion of pro-inflammatory cytokines**

To determine the synthesis and secretion of pro-inflammatory cytokines (IL-1β and IL-6) level in blood, ELISA was performed with the serum samples isolated at 0, 12, 24, and 36 week [Figure 7] after urethane injection and PBS injection. IL-1β protein level was found elevated by 1.28, 1.72, and 2.26 folds at 12, 24 and 36 week, respectively, in
the serum of urethane-injected mice as compared to PBS-injected mice [Figure 7a]. The level of IL-6 protein was also found elevated by 1.3, 1.97, and 2.43 folds at 12, 24, and 36 week, respectively, in the serum of urethane-injected mice as compared to PBS-injected mice [Figure 7a]. The level of IL-1β and IL-6 increased with time in urethane-injected mice serum samples, while in the PBS-injected mice serum samples the levels of both cytokines were approximately constant along with time [Figure 7].

**EMSA of NF-κB and Stat3**

To show the urethane-mediated activation and binding of TF, NF-κB, and Stat3 present in the urethane-injected lung tissues of mice, NF-κB and Stat3 specific biotin-labeled oligonucleotide consensus sequence was used to perform EMSA. Results suggest shift in the consensus oligonucleotide sequence of NF-κB [Figure 8; Lane 1] and Stat3 [Figure 9; Lane 1] after comparatively more binding of TF, NF-κB, and Stat3 of nuclear extract of urethane primed lung tissues in comparison to PBS primed nuclear extract [Figure 8; Lane 2 and Figure 9; Lane 2], respectively, in NF-κB and Stat3. Lane 3 of Figures 8 and 9 show NF-κB and Stat3 specific biotin-labeled oligonucleotide consensus sequence without nuclear extract, respectively.

**DISCUSSION**

In the present study, our motivation was to understand the possible mechanism and cause of lung tumor development after i.p. injection of urethane in Balb/c mice model. We report here that the urethane-induced lung tumor genesis in Balb/c mice is due to chronic inflammation occurred in the lung. The chronic inflammation is due to increase in the expression of pro-inflammatory cytokines (IL-1β and IL-6), activation and expression of NF-κB [Figure 8] and Stat3 [Figure 9] in the urethane-injected mice lung tissues. Upregulated expression might have subsequently created microenvironment to play a pivotal role in the urethane-induced lung tumor formation.

After tumor development, the average body weight of urethane-injected mice decreased in comparison to PBS-injected mice [Figure 1], whereas the lung organ index of urethane-injected mice increased as compared to lung organ index of PBS-injected mice [Figure 2]. This increase in the lung organ index of urethane-injected mice is due to
increase in the size and weight of lungs and overall decrease in the body weight after tumor genesis and development. The step-wise development of tumors were also observed in urethane-injected lung tissues of mice [Figures 3b–3d]. AAH is a precursor lesion of AD as we observed in our result [Figure 3], which is in accordance with earlier reported.[20]

The multiple doses of i.p. injection of urethane in mice increased the population of circulating inflammatory cells (monocytes, neutrophils, and lymphocytes) in peripheral blood [Figure 4]. All inflammatory cells latter suppose to get recruited at the site of lung tissues damage[21] and secrete large amount of inflammatory cytokines and chemokines to promote the outgrowth of neoplastic cells to trigger
probably persistent inflammation. At transcriptional level, we observed upregulated expression of pro-inflammatory cytokines IL-1β and IL-6 in urethane-injected mice lung tissues as compared to PBS-injected mice lung tissues, as shown in Figure 6, which is similar to the observation of other researcher in the gastric cancer tissues. The significant upregulated expression level of these cytokines up to 24 week and thereafter the expression level become almost constant in urethane-injected lung tissues but not in the PBS-injected mice lung tissues suggests that urethane persistently upregulates the expression level of pro-inflammatory cytokines which have created microenvironment for the activation of possible signaling pathways for lung carcinogenesis. This fact is well supported by the earlier reports that IL-1β and IL-6 activates the NF-κB and Stat3. The upregulated expression of TF such as NF-κB, Stat3, and pStat3 in the urethane-injected lung tissues [Figure 5], which is up to 24 week and there after saturation in the expression level was observed up to 36 week in urethane-injected lung tissues. Shows the constitutive expression of these TF such constitutive expression might be essential requirement for tumor genesis and progression. In contrast, we observed significantly lower but equal expression level of above TF in PBS-injected mice lung tissue [Figure 5], where tumor genesis was absent.

However, these results suggest that urethane is responsible for the upregulation, activation, and expression of NF-κB and Stat3 via pro-inflammatory cytokines IL-1β and IL-6 which is well supported by earlier reports that cytokines modulate the expression of genes involved in cell-cycle progression and inhibition of apoptosis via the NF-κB and Stat3 pathways. The NF-κB and Stat3 both TF have been identified as an important promoter of tumorgenesis (AD) in oral keratinocytes and gastrointestinal tract as we also observed in the case of mice lung adenocarcinoma. TF, NF-κB, and Stat3 are key molecules to regulate the genes responsible for the production of pro-inflammatory cytokines and chemokines. Probably activated NF-κB [Figure 8] and Stat3 [Figure 9] bind separately or in cooperate to each other within promoter region of many target genes to promote constitutive expression of cytokines, inflammatory cells proliferation, metastasis and angiogenesis, as well as protect tumor cells from apoptosis by regulating associated cancerous genes. Moreover, several other target genes responsible for chronic inflammation might got upregulated by the both TF. Our ELISA results suggested the enhanced production and secretion of pro-inflammatory cytokines in urethane-injected mice serum than those in PBS-injected mice serum, indicate the role of tumor cells and infected pro-inflammatory cells in creating chronic inflammatory microenvironment by secreting IL-1β & IL-6 to enhance cell proliferation and uninterrupted growth of alveolar epithelial cells leading to tumor genesis and development in the urethane-induced Balb/c mice lung.

Moreover, TF NF-κB and Stat3 seem to create a potential molecular bridge between IL-1β and IL-6 mediated inflammation and lung tumor development in urethane-injected mice. Our results support the above hypothesis that pro-inflammatory IL-1β and IL-6 cytokines initially secreted by circulatory inflammatory cells might have
upregulated the expression and activation of NF-κB and Stat3. Consequently, the constitutive expression of NF-κB and Stat3 promoted the transcription of pro-inflammatory cytokine genes in tumor cells [Figure 6] and tumor-associated stromal cells creating a sustained chronic inflammatory state within the tumor-microenvironment. [40]

In conclusion, urethane-induced lung tumor generation in Balb/c mice model enable us to understand the mechanisms involved in the genesis and development of tumor in the lungs along with the molecules or factors involved in the cancer progression. The upregulation of potential inflammatory cytokines IL-1β and IL-6 followed by the activation and upregulation of TF, NF-κB, and Stat3 lead to chronic inflammation, subsequently the upregulation of cancerous genes to make the lung cells cancerous leading to lung’s tumor formation.

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