Indian Journal of Biochemistry & Biophysics
Vol. 62, June 2025, pp. 607-614
DOI: 10.56042/ijbb.v62i6.10703

Benzo (a)pyrene exposure reduces CD54 expression on alveolar macrophages and alters apoptosis and inflammatory responses in the lungs

Pooja Chauhan[1], Nitin Bhardwaj[1]*, Sumit Rajaura[1], Rambabu[2], Ashutosh Singh[3] & Mohd Z Ahmed[4]

[1]Department of Zoology and Environmental Science, Gurukula Kangri (Deemed to be University), Haridwar-249 404, Uttarakhand, India

[2]Department of Botany, Kirori Mal College, New Delhi-110 007, Delhi, India

[3]Department of Biochemistry, University of Lucknow, Lucknow-226 007, Uttar Pradesh, India

[4]Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh-11451, Saudi Arabia

Received 26 April 2024; *revised 03 March 2025*

The study investigates the role of intracellular cell adhesion molecule-1 (ICAM-1, CD54) expression in alveolar macrophages and associated molecular alterations in benzo (a)pyrene (BaP)-induced lung tumor-bearing mice that provide more profound insight into immune mechanisms in lung cancer. Lung tumors were induced by administering BaP (50 mg/kg body weight, twice a week for four weeks) orally. A single-alveolar cell suspension was stained with fluorescently conjugated antibodies for the demarcation of alveolar macrophages (F4/80 and CD11b cells). CD54 expression on different alveolar macrophages was analyzed based on CD11b/F4/80 gating. The mortality and cell cycle were studied by 7-AAD and PI staining, respectively, and flow cytometric analysis. Our results suggest that CD54 expression is significantly decreased on $CD11b^+$ alveolar macrophages and $CD11b^+/F4/80^+$ interstitial macrophages. BaP treatment increased cell mortality, and cell cycle progression was inhibited. The expression of proapoptotic (BAX and Caspase 3) and antiapoptotic (Bcl-2 and Cytochrome C) genes was reduced, but the expression of proinflammatory (IL-6, IL-10, and TNF-α) and anti-inflammatory (IFN-γ) genes was significantly increased, but that of TGF-β was decreased. Overall, BaP-induced tumors suppress CD54 expression on alveolar macrophages, induce cellular mortality, inhibit the cell cycle, and alter the expression of pro-apoptotic, anti-apoptotic, and anti-inflammatory genes in murine lungs.

Keywords: Cell cycle, Flow Cytometry, Gene expression, Inflammation, Lung cancer

The development of lung cancer is significantly influenced by polycyclic aromatic hydrocarbons (PAHs), one of the more than 60 carcinogens found in tobacco smoke[1]. Benzo (a)pyrene (BaP) is one of the most potent carcinogens that causes lung cancer among the various PAHs[2]. BaP is metabolically transformed into an epoxide derivative, which binds to DNA and combines to form a DNA adduct that promotes cancer[3]. It leads to oxidative DNA damage by inducing reactive oxygen species (ROS)[4].

The initiation of a tumor stimulates immune responses within the lungs. The defensive cellular milieu in the lungs includes neutrophils, dendritic cells, alveolar epithelial cells (AECs) and alveolar macrophages (AMs). AMs survive longer and replenish 40% in a year[5]. AMs represent the first line of defense and are phagocytic and antigen-presenting cells that remove cellular debris and apoptotic cells and elicit immune responses. They exhibit structural and functional plasticity in various diseases and function as immune regulators by secreting cytokines[6]. Macrophages can be classified into two main categories: M1 macrophages, which are typically activated by IFN-γ, and M2 cells, which are alternatively triggered by IL-4. In contrast to M2 macrophages, which are both pro- and anti-inflammatory, M1 macrophages promote inflammation[7]. AMs are closely associated with AECs and dendritic cells and communicate with AECs to initiate immunosuppression under inflammatory conditions[8].

CD54, or ICAM-1, belongs to the immunoglobulin supergene family and has five extracellular immunoglobulin-like domains that are involved in adhesive contact between cells. Various inflammatory mediators and proinflammatory cytokines increase surface CD54 expression[9]. Drugs may down-regulate CD54 expression on airway mast cells (AMs) in airway tissues as a therapeutic mechanism in the lung

*Correspondence:
Phone: +91-9927031937 (Mob)
E-mail: nitin.bhardwaj@gkv.ac.in; nitindna2001@gmail.com
Suppl. Data available on respective page of NOPR

inflammatory process[10]. CD54 may contribute to carcinogenesis and increase the propensity for malignant tumors to spread, as it is differentially expressed in a variety of benign and malignant disorders[11, 12].

The evaluation of CD54 expression and its soluble form in lung cancer has attracted a significant amount of attention in recent years[13]. CD54 and LFA-1 interactions are crucial for accumulating lymphocytes inside the lungs. Activated pulmonary vasculature endothelial cells and type II pneumocytes are the primary sites of CD54 expression[14]. Thus, CD54 is induced in altered AECs, pulmonary lymphocytes, and fibroblasts in lung cancer[15]. Tumor-associated macrophages have been shown to exhibit similar induction of CD54 expression, which has been linked to macrophage polarization[16]. A crucial role for CD54 in carcinogenesis is indicated by the elevation of the expression of this receptor by tumor resident and infiltrating cells at both the main and secondary sites[17]; however, the modulatory effect of CD54 expression on various AMs under tumorigenic conditions has not been studied.

In the present study, we investigated how CD54 expression varies among different kinds of alveolar macrophages. We also investigated cell death, cell cycle inhibition, and the expression of proapoptotic, antiapoptotic, proinflammatory, and anti-inflammatory genes in response to BaP-induced lung cancer.

Materials and Methods

Animals

Swiss male mice (10–12 weeks old, 30–35 g body weight) were utilized in all the experiments. All the experiments followed the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The Gurukula Kangri Institutional Animal Ethics Committee approved the study. (IAEC Code: GKV/AHF/14/2020).

Chemicals and antibodies

FITC-conjugated anti-mouse CD11b, APC-conjugated anti-mouse F4/80, APC-conjugated rat IgG2bκ, FITC-conjugated rat IgG2bκ, and 7-aminoactinomycin D (7-AAD) were obtained from BioLegend (San Diego, CA, USA). Purified rat anti-mouse CD16/CD32 and hamster anti-mouse CD54 antibodies were purchased from BD Biosciences (San Diego, CA, USA). Primescript™ First Strand cDNA Synthesis Kit, TB Green Premix Ex Taq, and PCR Master Mix were obtained from Takara (Kyoto, Japan). BaP, propidium iodide (PI), RPMI, HEPES, and TRI reagents were obtained from Sigma–Aldrich (India). Fetal bovine serum (FBS) was obtained from Himedia (South Logan, UT). Primer synthesis was carried out by Eurofins. The sequences of the primers used are provided in (Suppl. Table 1).

Development of a mouse model of lung cancer by BaP administration

A mouse model of lung cancer was generated as previously described[18-20]. Briefly, mice were given BaP suspended in corn oil (50 mg/kg body weight, twice a week for four weeks) *via* oral gavage; the control group received vehicle alone[19, 20]. After 4 months, the mice were dissected, and the lungs were excised and cleared. The tissues were crushed using a plunger, and single-cell suspensions of the lungs were suspended in RPMI medium supplemented with 10% FBS[21].

Flow cytometry

CD54 expression analysis on alveolar macrophages

To evaluate the different types of alveolar macrophages (AMs), single-cell suspensions were blocked with an anti-CD16/32 antibody for 10 min. The cells were stained with an anti-mouse CD11bFITC antibody, F4/80 panmacrophage marker, and anti-mouse CD54PE antibody for 20 min at 4°C and analyzed on a flow cytometer[22, 23]. CD54 expression was analyzed in CD11b/F4/80-gated cells[24].

Apoptosis and cell cycle analysis

Apoptosis was analyzed by staining the cells with 7-aminoactinomycin-D (0.5 µg/mL), and cell mortality was measured by flow cytometric analysis[25]. For cell cycle analysis, alveolar single-cell suspensions were stained with PI and analyzed *via* flow cytometry. The PI staining patterns were further analyzed to determine the percentages of cells in the G1/G0, S, and G2M phases[26-28]. Flow cytometric experiments were carried out on a BD FACS Verse flow cytometer, and the results were analyzed using Facsuite software. A minimum of 10000 events were enumerated on a flow cytometer.

Reverse transcription and quantitative real-time RT–PCR

RNA isolation and cDNA synthesis

Total RNA was isolated from 1×10^6 pulmonary cells by using TRIzol reagent (Sigma). The RNA pellet was cleaned twice with 75% ethanol, air-dried and suspended in nuclease-free water. The purity of

the isolated RNA was assessed by measuring the absorbance at 260/280 and 260/230 nm wavelengths with a NanoDrop spectrophotometer. The RNA integrity was examined by running 5µg of RNA on a 1.2% formaldehyde agarose gel, and 1 µg of RNA was utilized to synthesize cDNA[28, 29].

Gene expression analysis using qPCR

cDNA amplification was performed *via* RT–qPCR. The expression of antiapoptotic (Bax, BCL2, Cyt C, and Caspase 3) and anti-inflammatory (IFN-γ, TNF-α, TGF-β IL-6 and IL10) genes was analyzed in the present study. mRNA levels were quantified by quantitative PCR (RT–qPCR) using Applied Biosystems QuantStudio3 and SYBR Green[30]. Samples were analyzed in triplicate and normalized to 18S rRNA expression using the $2^{-\Delta\Delta Ct}$ method[30].

Statistical analysis

SigmaPlot software was used for statistical analysis. The mean ± standard error of the mean (SEM) was calculated for all the data. The significance of differences between the control and BaP-treated groups was measured by Student's t test. A p value less than 0.05 was considered to indicate statistical significance.

Results

BaP-induced tumors altered CD54 expression on alveolar macrophages

Various macrophages in the lungs were classified based on the intensity profile of CD11b/F4/80 panmacrophage staining[23]. Three types of macrophages, *i.e.*, alveolar macrophages 1 ($CD11b^+$, AM1), interstitial macrophages (IM) ($CD11b^+F4/80^+$ IM), and alveolar macrophages 2 ($F4/80^+$ AM2), were identified based on the expression of these receptors (Suppl. Fig. 1). The expression levels of CD54 were examined in CD11b/F4/80-gated cells. The mean fluorescence intensities (MFIs) of CD54 expression in $CD11b^+$, $CD11b^+/F4/80^+$, and $F4/80^+$ macrophages were 36826, 55078, and 68864, respectively (Fig. 1 Panels a, c, and e), respectively, and decreased to 24838, 48461, and 66378 after BaP treatment

(Fig. 1 Panels b, d, and f). The cumulative data suggest that the MFI of CD54 was reduced by 19% in $CD11b^+$ AM, 34% in $CD11b^+/F4/80^+$, and 27% in $F4/80^+$ AM after induction of tumors in the lungs. These results confirmed the downregulation of CD54 expression in BaP-treated lungs.

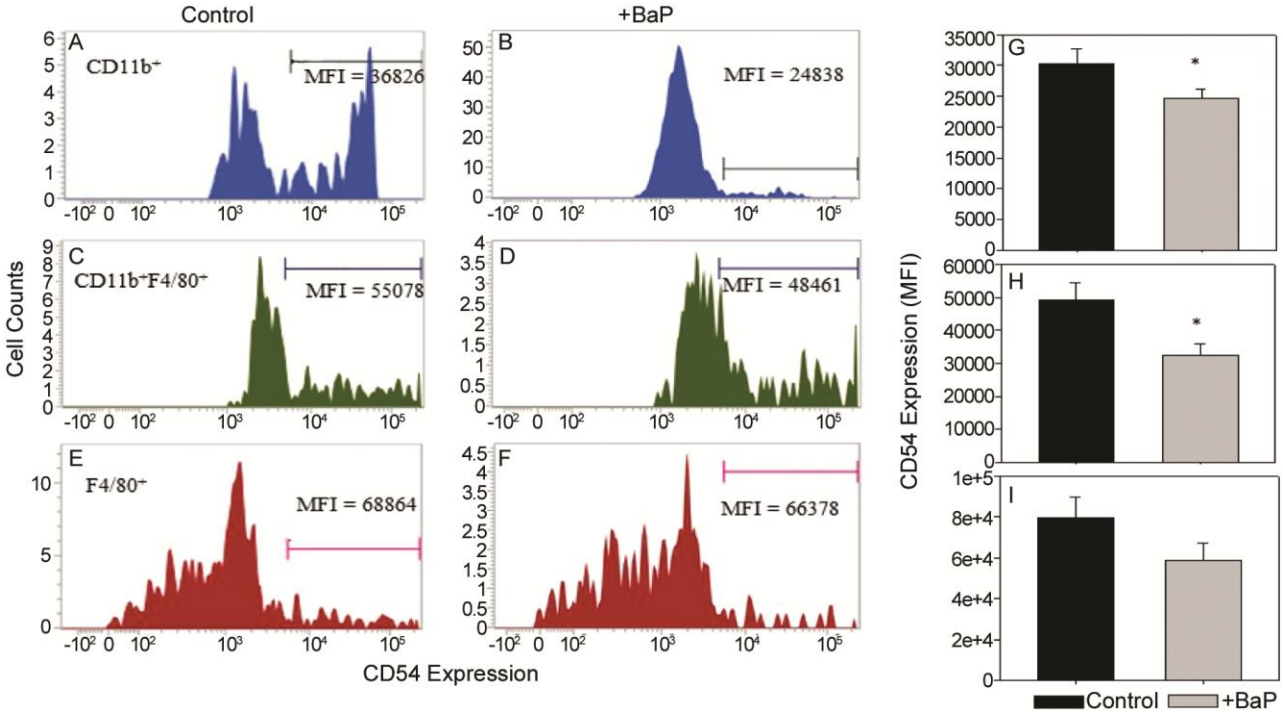

Fig. 1 — BaP treatment reduced CD54 expression in alveolar macrophages. Single-cell suspensions from control and BaP-treated mice were stained with anti-mouse CD11b, F4/80 and CD54 monoclonal antibodies, followed by flow cytometry analysis. CD54 expression on various macrophages was analyzed *via* Cd11b/F4/80 staining. Histograms in panels a, c, e and b, d, f show the CD54 expression on various macrophages in control and BaP-treated mice, respectively. Panels g, h and i show the cumulative changes in the MFI of CD54 expression on various alveolar macrophages. The data are presented as the means ± SEMs; n=6 in both groups.*P<0.05 Student's *t*test.

BaP treatment induces mortality inside the lungs

The alterations observed in AMs may result in altered mortality inside the lungs. Cellular mortality was analyzed by staining the cells with 7-AAD, which is an intercalator of double-stranded DNA[31]. A representative histogram suggested that the proportion of dead cells increased from 14.15% to 22.29% in BaP-treated lungs (Fig. 2A & B). The cumulative data suggested a significant increase in the number of dead cells (17% greater) in the BaP-treated mice (Fig. 2C).

BaP treatment inhibited cell cycle progression inside the lungs

Cell cycle analysis was performed by staining with PI dye. The representative images in Figure 3 show the proliferative activities of cells in the lungs of

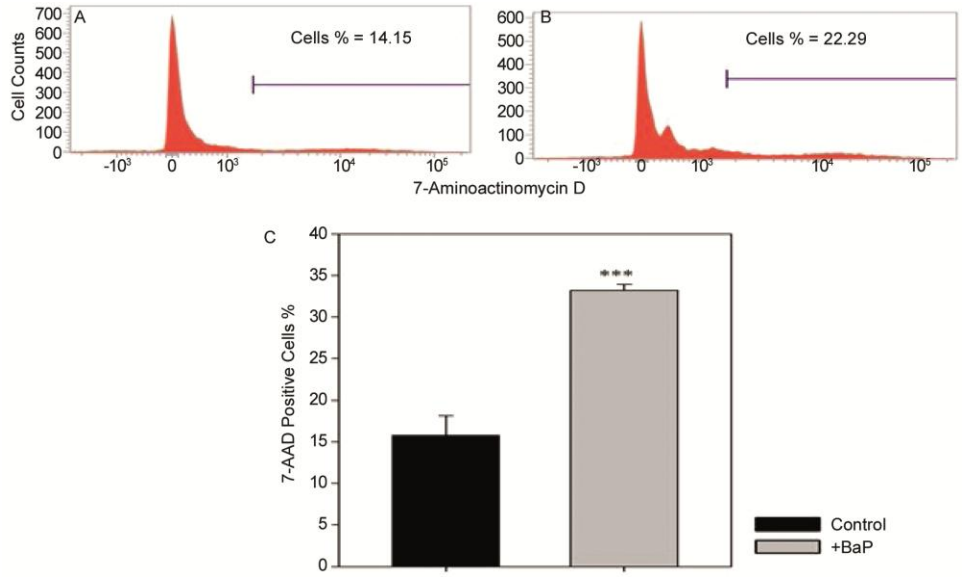

Fig. 2 — BaP administration enhanced mortality inside the lungs. For analysis of dead cells, single-cell suspensions from Bap-induced tumorigenic lungs were stained with 7-AAD dye and subjected to flow cytometric analysis. Histograms in panelsa and b represent the proportions of 7-AAD$^+$ cells among the control and treated cells, respectively. The cumulative changes are depicted in bar graph c. The data are presented as the mean ± SEM; n=6 in each group. ***P<0.0005, Student's *t*test.

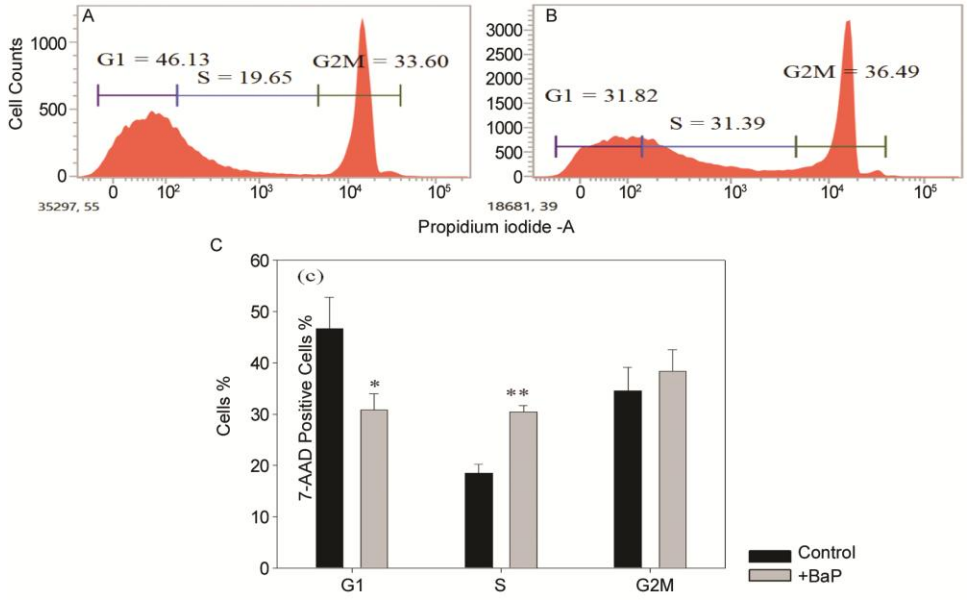

Fig. 3 — BaP administration inhibited the cell cycle progression of pulmonary cells. For cell cycle analysis, cells were stained with PI and analyzed *via* flow cytometry. The representative histograms in panels a and b show the percentages of cells in different phases of the cell cycle. Panel c shows the cumulative changes in the cell cycle patterns. The data are presented as the means ± SEMs; n=6 in each group. *P<0.05, **P<0.005, Student's *t* test

control and BaP-treated mice. The percentages of G1- and S-phase cells were 46.69 ± 6.04 and 18.50 ± 1.69, respectively, in the lungs of control mice (Fig. 3). BaP-induced tumors cause the G1 to S phase transition, as the number of cells in the G1 phase decreased to 30.79 ± 3.20 but increased to 30.46 ± 1.21 in S phase (Fig. 3C). The cells in the G2M phase, on the other hand, were nearly similar to the control cells. These results clearly suggest that BaP-induced tumors alter the proliferative activities of alveolar cells.

Changes in the relative expression of antiapoptotic and inflammatory genes

In the lungs of tumor-bearing mice, the expression of proapoptotic (BAX and Caspase 3) and antiapoptotic (Bcl-2 and Cytochrome C, Caspase 3) genes as well as anti-inflammatory (IL6, IL10, IFN-γ, TNF-α, and TGF-ß) genes was examined. The expression of proapoptotic and antiapoptotic genes was significantly reduced under BaP-induced tumor conditions. The relative expression levels of BAX (0.24 ± 0.22), BCL2 (0.02 ± 0.01), and Caspase 3 (0.24 ± 0.22) in the lungs were significantly decreased (Fig. 4A-D). Cytochrome C levels decreased to a much greater extent. The expression of the anti-inflammatory gene IL6 and IL10 were 3.5 and 5 folds greater in the lungs of tumor-bearing mice than in those of control mice (Fig. 4E & F). The expression levels of IFN-γ (15.22 ± 4.99) and TNF-α (11.61 ± 2.54) were significantly increased in the tumorigenic mice after BaP administration, while the expression level of TGF-β (0.21 ± 0.19) decreased significantly.

Discussion

Lung cancer accounts for approximately 20% of all cancers. It is the most lethal type of cancer, with 18.4% mortality in both men and women, and its incidence is increasing by 0.5% per year. Polycyclic aromatic hydrocarbons are the major constituents of tobacco smoke and cause 90% of lung carcinogenesis[32, 33]. In this study, we investigated the effects of BaP-induced tumors on CD54 receptor expression in alveolar macrophages and inflammatory responses inside the lungs.

AMs provoke an immune response and inhibit tumor progression. We studied the surface expression of CD54 on different macrophages. CD54 is a transmembrane intercellular adhesion molecule expressed on epithelial, macrophage, dendritic, and neutrophil cells[34]. CD54 contributes to tumor

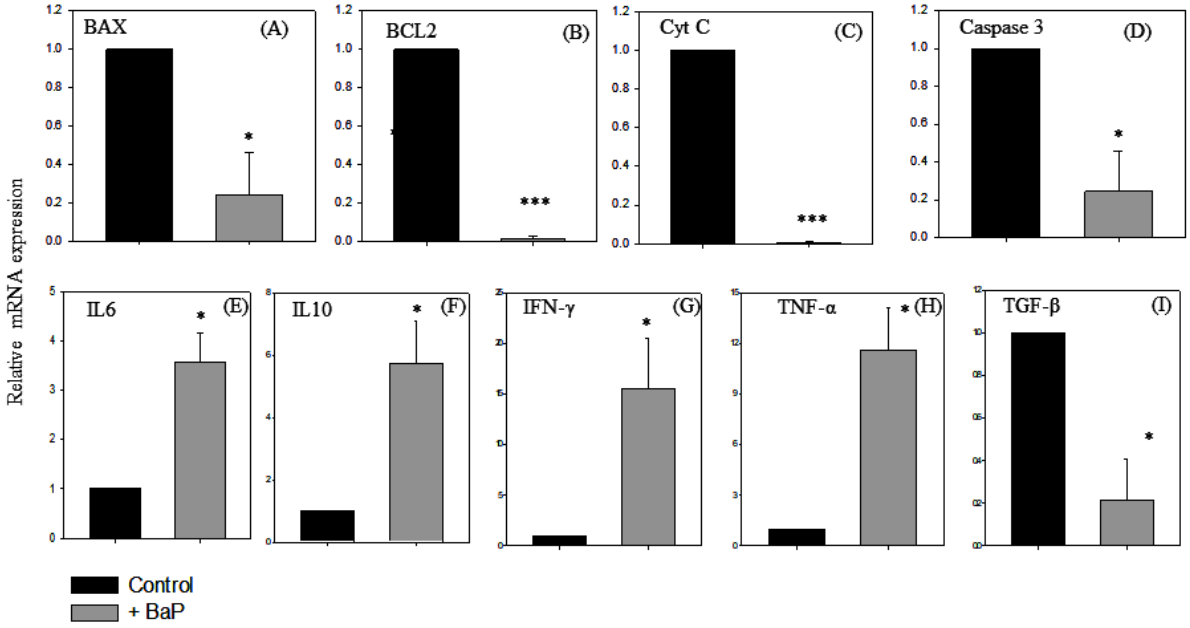

Fig. 4 — Changes in apoptotic and anti-inflammatory gene expression in alveolar cells following tumor induction. RNA was isolated from pulmonary cells by using TRIzol (TRI) reagent. First-strand cDNA was prepared by utilizing the First Strand cDNA Synthesis Kit and amplified by qRT–RTa. The RNA transcripts of different genes were measured by the SYBR Green method. The relative expression levels of the apoptotic genes BAX, BCL2, Cyt C, and Caspase 3 are shown in panels a to d. The expression levels of the anti-inflammatory genes IL6, IL10, IFN-γ, TNF-α and TGF-β are shown in panels e to i. The mRNA levels were normalized to 18S rRNA levels using the $2^{-\Delta\Delta C}$T method. The data are presented as the means ± SEMs of three independent experiments. **P*<0.05, ****P*<0.0005, Student's *t* test.

progression and inflammatory responses by interacting with the β2 integrins leucocyte function antigen-1 (CD11a) and Mac-1 (CD11b). CD54 expression contributes to pulmonary inflammation in various treatments, such as radiation, autoimmune disease, and toxic administration. It regulates leucocyte trafficking and influences metastatic behaviour by influencing macrophage and granulocyte recruitment[35].

Based on the surface expression data, three different macrophages were recognized, *i.e.*, AM1, $CD11b^+$, interstitial macrophages (IM), $CD11b^+F4/80^+$ and alveolar macrophages 2 (AM2) $F4/80^+$. We observed decreased surface expression of the CD54 receptor on all the macrophage populations. The decrease in CD54 expression was most pronounced in the IM (34%), followed by the AM2 (27%) and AM1 (19%) populations. We speculate that the decreased CD54 expression in the tumor microenvironment could help with immune suppression and leucocyte trafficking inside the lungs. Earlier, BaP exposure was shown to cause acute inflammation, which reduces proinflammatory cytokine production, and as a result, ICAM-1 expression decreases in inflammatory regions[36]. Pouniotis *et al*. (2005) reported a mean 34% reduction in CD54 expression on alveolar macrophages in cancer patients, which also correlates well with our observations[37].

The changes observed in the pulmonary immune response could lead to cell death inside the lungs. We estimated cell mortality by staining with 7-AAD dye, which intercalates in DNA[38]. According to our observations, tumor-bearing mice had a considerable increase in the fraction of $7\text{-}AAD^+$ cells. We also studied cellular proliferation by PI staining. Our results suggest that the number of cells in the G1 phase decreased, while the number of cells in the S phase increased. It seems that the G1-S phase transition occurs in response to tumors. Conversely, cells in the G2 phase were nearly identical to control cells, indicating that the S-G2M transition was inhibited in tumor-bearing mice. These observations indicate that BaP-induced tumors promote cell death and inhibit cell multiplication in pulmonary cells.

Furthermore, we investigated the mechanisms of the inflammatory response and cellular mortality inside the lungs. We analyzed the expression of proapoptotic (BAX, Caspase 3), antiapoptotic (BCL2, Cyt C), proinflammatory (IL6 and TNF-α) and anti-inflammatory (IFN-γ and TGF-ß) cytokines. A significant decrease in the expression of apoptotic genes and an increase in the expression of anti-inflammatory genes were observed. BAX and Bcl2 belong to the Bcl2 protein family, which maintains mitochondrial integrity and inhibits apoptosis[39]. A decrease in the expression of the BAX and Bcl2 genes initiates the cell death process *via* an evolutionarily conserved programmed cell death pathway. A decrease in cytochrome C results in caspase-independent apoptosis, which is an indicator of cell death caused by an alternate mechanism, such as necrosis[40]. This finding indicates that BaP-induced cell mortality is caused by extrinsic and intrinsic mechanisms.

Alveolar cells release various cytokines, such as IL6, IFN-γ, TNF-α, and TGF-β, under tumorigenic conditions. IL-6 and IL-10 upregulation is associated with a prominent immune response and is also related to progression and apoptosis inside the lungs of tumor-bearing mice[41]. Moreover, TNF-α exhibits a strong antitumor effect in multiple animal cancer models. This cytokine binds nonselectively to tumor cells, endothelial cells, normal cells, and blood vessels, causing nonspecific harm to different cell types. IFN-γ overexpression directly inhibits the development of cancer cells by causing tumor cell growth arrest and apoptosis[42]. Additionally, downregulation of TGF-β induces cell apoptosis in a JNK-dependent manner in lung cancer cells[43]. It is possible that TGF-β-induced arrest of cells in the G1 phase further leads to apoptosis[44, 45].

Conclusion

BaP exposure causes the development of lung tumors. The tumor microenvironment causes alterations in the diversity of AMs, which leads to immunosuppression inside the lungs. Cellular mortality, cell cycle inhibition and decreased CD54 expression were observed in the lungs of tumor-bearing mice. Moreover, anti-inflammatory gene expression was increased, but the expression of proapoptotic and antiapoptotic genes was reduced.

Acknowledgement

Nitin Bhardwaj thanks support from Indian Council for Medical Research (ICMR) No. 56/2/Hae/BMS and University Grant Commission (UGC) start-up grant No. 30- 496/2019. Dr Mohammad Z. Ahmed extend their appreciation to Researchers Supporting Project number (RSPD2025R728), King Saud University, Riyadh, Saudi Arabia.

The flow cytometry testing was carried out in part at the FACS Facility of the Advanced Technology Platform Centre (ATPC), managed by the Regional Centre for Biotechnology (RCB), and funded by the Department of Biotechnology " (Grant No. BT.MED-II/ATPC/BSC/01/2010)".

Conflict of interest

All authors declare no conflict of interests.

References

1 Stading R, Gastelum G, Chu C, Jiang W & Moorthy B, Molecular mechanisms of pulmonary carcinogenesis by polycyclic aromatic hydrocarbons (PAHs): Implications for human lung cancer. *Semin Cancer Biol*, 76 (2021) 16.

2 Słowikowski BK, Jankowski M & Jagodziński PP, The smoking estrogens–a potential synergy between estradiol and benzo (a) pyrene. *Biomed Pharmacother*, 139 (2021) 111658.

3 Krais AM, Speksnijder EN, Melis JP, Indra R, Moserova M, Godschalk RW, van Schooten FJ, Seidel A, Kopka K, Schmeiser HH & Stiborova M, The impact of p53 on DNA damage and metabolic activation of the environmental carcinogen benzo a pyrene: effects in Trp53 (+/+), Trp53 (+/–) and Trp53 (–/–) mice. *Archtox*, 90 (2016) 839.

4 Bao Y, Chen Q, Xie Y, Tao Z, Jin K, Chen S, Bai Y, Yang J & ShanS, Ferulic acid attenuates oxidative DNA damage and inflammatory responses in microglia induced by benzo (a) pyrene. *Int Immunopharmacol*, 77 (2019) 105980.

5 Nayak DK, Zhou F, Xu M, Huang J, Tsuji M, Hachem R & Mohanakumar T, Long-term persistence of donor alveolar macrophages in human lung transplant recipients that influences donor-specific immune responses. *Am J Transplant*, 16 (2016)2300.

6 Jain N, Moeller J & Vogel V, Mechanobiology of macrophages: how physical factors coregulate macrophage plasticity and phagocytosis. *Annu Rev Biomed Eng*, 21 (2019) 267.

7 Jiang M, Qi Y, Huang W, Lin Y & Li B, Curcumin reprograms TAMs from a protumor phenotype towards an antitumor phenotype *via* inhibiting MAO-A/STAT6 pathway. *Cells*, 21 (2022) 3473.

8 Mu M, Gao P, Yang Q, He J, Wu F, Han X, Guo S, Qian Z & Song C, Alveolar epithelial cells promote IGF-1 production by alveolar macrophages through TGF-β to suppress endogenous inflammatory signals. *FrontImmunol*, 11 (2020) 1585.

9 Espagnolle N, Balguerie A, Arnaud E, Sensebé L, & Varin A, CD54-mediated interaction with pro-inflammatory macrophages increases the immunosuppressive function of human mesenchymal stromal cells. *Stem cell Rep*, 8 (2017), 961.

10 Fattal-German M, Ladurie FL, Cerrina J, Lecerf F & Berrih-Aknin S, Modulation of ICAM-1 expression in human alveolar macrophages *in vitro*. *Eur Respir J*, 9 (1996) 463.

11 Huang WC, Chan ST, Yang TL, Tzeng CC & Chen CC, Inhibition of ICAM-1 gene expression, monocyte adhesion and cancer cell invasion by targeting IKK complex: molecular and functional study of novel α-methylene-γ-butyrolactone derivatives. *Carcinogenesis*, 10 (2004) 1925.

12 Rosette C, Roth RB, Oeth P, Braun A, Kammerer S, Ekblom J & Denissenko MF, Role of ICAM1 in invasion of human breast cancer cells. *Carcinogenesis*, 26 (2005) 943.

13 Kotteas EA, Boulas P, Gkiozos I, Tsagkouli S, Tsoukalas G & Syrigos KN, The intercellular cell adhesion molecule-1 (icam-1) in lung cancer: implications for disease progression and prognosis. *Anticancer res*, 34 (2014) 4665.

14 Guzman J, Izumi T, Nagai S & Costabel U, ICAM-1 and integrin expression on isolated human alveolar type II pneumocytes. *Eur Respir J*, 7 (1994) 736.

15 Jiang Z, Woda BA, Savas L & Fraire AE, Expression of ICAM-1, VCAM-1, and LFA-1 in adenocarcinoma of the lung with observations on the expression of these adhesion molecules in non-neoplastic lung tissue. *Mod Pathol*, 12 (1998) 1189.

16 Yang M, Liu J, Piao C, Shao J & Du J, ICAM-1 suppresses tumor metastasis by inhibiting macrophage M2 polarization through blockade of efferocytosis. *Cell Death Dis*, 6 (2015) 1780.

17 Bui TM, Wiesolek HL & Sumagin R, ICAM-1: A master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. *J LeucoBiol*, 108 (2020) 787.

18 Chauhan P, Bhardwaj N, Rajaura S, Chandra H, Singh A, Babu R, & Gupta NJ. Benzo (A) pyrene exposure alters alveolar epithelial and macrophage cells diversity and induces antioxidant responses in lungs. *Toxicol Rep*, 13 (2024) 101777.

19 Salem ML, El-Ashmawy NE, Abd El-Fattah EE & Khedr EG, Immunosuppressive role of Benzo a pyrene in induction of lung cancer in mice. *Chem Biol Interact*, 333 (2021) 109330.

20 Chauhan P, Bhardwaj N, RajauraS & Gupta N, Selective elimination of younger erythrocytes in blood circulation and associated molecular changes in benzo (a) pyrene induced mouse model of lung cancer. *Toxicol Res*, 13 (2024) 115.

21 Rajaura S, Babu R, Bhardwaj N, Chauhan P, Singh A & Afzal M, Aflatoxin B1 administration causes inflammation and apoptosis in the lungs and spleen. *Toxicon*, 238 (2024) 107581.

22 Duan M, Steinfort DP, Smallwood D, Hew M, Chen W, Ernst M, Irving LB, Anderson GP & Hibbs ML, CD11b immunophenotyping identifies inflammatory profiles in the mouse and human lungs. *Mucosal Immunol*, 9 (2016) 550.

23 Dai B, Xu J, Li X, Huang L, Hopkins C, Wang H, Yao H, Mi J, Zheng L, Wang J & Tong W, Macrophages in epididymal adipose tissue secrete osteopontin to regulate bone homeostasis. *Nat Commun*, 13 (2022) 427.

24 Grothey A, Heistermann P, Philippou S & Voigtmann R, Serum levels of soluble intercellular adhesion molecule-1 (ICAM-1, CD54) in patients with non-small-cell lung cancer: correlation with histological expression of ICAM-1 and tumour stage. *Br j cancer*, 77 (1998) 801.

25 Rajaura S, Chauhan P, Chandra H & Bhardwaj N, Aflatoxin B1 administration induces reactive oxygen species production and apoptosis of erythrocytes in mice. *Toxicon*, 221 (2023) 106963.

26 Bhardwaj N & Saxena RK, Elimination of young erythrocytes from blood circulation and altered erythropoietic patterns during paraquat induced anemic phase in mice. *PLoS One*, 9 (2014) 99364.

27 Bhardwaj N & Saxena RK, Selective loss of younger erythrocytes from blood circulation and changes in erythropoietic patterns in bone marrow and spleen in mouse anemia induced by poly-dispersed single-walled carbon nanotubes. *Nanotoxicology*, 17 (2015) 1032

28 Bhardwaj N, Singh A, Chandra H & Gupta KK, Paraquat treatment modulated the stress erythropoiesis response in bone marrow and liver of the splenectomized mice. *Chem Biol Lett*, 26 (2022) 306.

29 Bhardwaj N & Singh A, Paraquat treatment modulates integrin associated protein (CD47) and basigin (CD147) expression and mitochondrial potential on erythroid cells in mice. *Environ Tox Pharmacol*, 58 (2018) 37.

30 Bhardwaj N, Kumar A & Gupta NJ, Altered dynamics of mitochondria and reactive oxygen species in the erythrocytes of migrating red-headed buntings. *Front Physiol*, 14 (2023) 1111490.

31 Ishaq M, DeGray G, Mou K, Aguilera A, Yang J, Lempicki RA, Hazen A & Natarajan V, Zap70 signaling pathway mediates glucocorticoid receptor-dependent transcriptional activation: role in the regulation of annexin 1 expression in T cells. *J Immunol*, 179 (2007) 3851.

32 Hecht SS, Kassie F & Hatsukami DK, Chemoprevention of lung carcinogenesis in addicted smokers and ex-smokers. *Nat Rev Cancer*, 9 (2009) 476.

33 Hoffmann I & Hoffmann D, The changing cigarette: chemical studies and bioassays. *Tob Sci Policy Public Health*, 53 (2004) 92.

34 Schulze-Koops H, Lipsky PE, Kavanaugh AF & Davis LS, Persistent reduction in IL-6 mRNA in peripheral blood mononuclear cells of patients with rheumatoid arthritis after treatment with a monoclonal antibody to CD54 (ICAM-1). *Clinic Exp Immunol*, 106 (1996) 190.

35 Shaul ME & Fridlender ZG, Tumour-associated neutrophils in patients with cancer. *Nat Rev Clin Oncol*, 10 (2019) 601.

36 Yasuda M, Tanaka Y, Tamura M, Fujii K, Sugaya M, So T, TakenoyamaM & Yasumoto K, Stimulation of β1 integrin down-regulates ICAM-1 expression and ICAM-1-dependent adhesion of lung cancer cells through focal adhesion kinase. *Cancer Res*, 61 (2001) 2022.

37 Pouniotis DS, Plebanski M, Apostolopoulos V & McDonald CF, Alveolar macrophage function is altered in patients with lung cancer. *Clin Exp Immunol*, 143 (2006) 363.

38 Zembruski NC, Stache V, Haefeli WE & Weiss J, 7-Aminoactinomycin D for apoptosis staining in flow cytometry. *Anal Biochem*, 429 (2012) 79.

39 McCurrach ME, Connor TM, Knudson CM, Korsmeyer SJ & Lowe SW, Bax-deficiency promotes drug resistance and oncogenic transformation by attenuating p53-dependent apoptosis. *Proc Natl Acad Sci U S A*, 94 (1997) 2345.

40 Mohan CD, Srinivasa V, Rangappa S, Mervin L, Mohan S, Paricharak S, Baday S, Li F, Shanmugam MK, Chinnathambi A & Zayed ME, Trisubstituted-imidazoles induce apoptosis in human breast cancer cells by targeting the oncogenic PI3K/Akt/mTOR signaling pathway. *PLoS One*, 11 (2016) 0153155.

41 Kumari N, Dwarakanath BS, Das A & Bhatt AN, Role of interleukin-6 in cancer progression and therapeutic resistance. *Tumor Biol*, 37 (2016) 11553.

42 Shen J, Xiao Z, Zhao Q, Li M, Wu X, Zhang L, Hu W & Cho CH, Anti-cancer therapy with TNF α and IFN γ: A comprehensive review. *Cell Prolif*, 51 (2018) 12441.

43 He Y, Liu H, Jiang L, Rui B, Mei J & Xiao H, miR-26 induces apoptosis and inhibits autophagy in non-small cell lung cancer cells by suppressing TGF-β1-JNK signaling pathway. *Front Pharmacol*, 9 (2019) 1509.

44 Thinon E, Morales-Sanfrutos J, Mann DJ & Tate EW, N-myristoyltransferase inhibition induces ER-stress, cell cycle arrest, and apoptosis in cancer cells. *ACS Chembiol*, 11 (2016) 2165.

45 Song J, & Shi W, The concomitant apoptosis and EMT underlie the fundamental functions of TGF-β. *ABBS*, 50 (2018) 91.