Effect of IFN-γ encapsulated liposomes on major signal transduction pathways in the lymphocytes of patients with lung cancer

MAYSA ALHAWAMDEH[1], BELAL ALMAJALI[2], WAFA HOURANI[3], HAMID ALI NAGI AL-JAMAL[4], ABDULLAH SALEH AL-WAJEEH[5], NESRIN RIAD MWAFI[6], YOUSEF AL-HAJAYA[1], HANAN KAMEL M. SAAD[4], DIANA ANDERSON[7], MAHMOUD ODEH[8] and IBRAHEAM A. TARAWNEH[9]

[1]Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Mutah University, Al-Karak 61710; [2]Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, Al-Ahliyya Amman University, Amman 19111; [3]Faculty of Pharmacy, Philadelphia University, Amman 19392, Jordan; [4]School of Biomedicine, Faculty of Health Sciences, Universiti Sultan Zainal Abidin, Kuala Nerus, Terengganu 21300, Malaysia; [5]Metabolomics Department, Anti-Doping Lab Qatar, Doha 27775, Qatar; [6]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Mutah University, Al-Karak 61710, Jordan; [7]Division of Medical Sciences, Faculty of Life Sciences, University of Bradford, Bradford, BD7 1DP, UK; [8]Business Faculty, Zarqa University, Zarqa 13110, Jordan; [9]School of Graduate Studies, Management and Science University, Shah Alam, Selangor 40100, Malaysia

Received June 29, 2023; Accepted September 28, 2023

DOI: 10.3892/ol.2023.14141

Abstract. Globally, lung cancer affected 2.2 million individuals and caused 1.8 million deaths in 2021. Lung cancer is caused by smoking, genetics and other factors. IFN-γ has anticancer activity. However, the mechanism by which IFN-γ has an effect on lung cancer is not fully understood. The present study aimed to assess the effect of IFN-γ on the peripheral lymphocytes of patients with lung cancer compared with healthy controls. The efficacy of IFN-γ against oxidative stress was assessed using a comet repair assay and the effects of IFN-γ on p53, PARP1 and OGG1 genes and protein levels in lymphocytes was evaluated by RT-qPCR and western blotting. DNA damage was significantly reduced in the lymphocytes of patients treated with IFN-γ. However, there was no effect in the cells of healthy individuals after treatment with naked IFN-γ [IFN-γ (N)] and liposomal IFN-γ [IFN-γ (L)]. Following treatment with IFN-γ (N) and IFN-γ (L), the p53, PARP1 and OGG1 protein and gene expression levels were significantly increased (P<0.001). It has been suggested that IFN-γ may induce p53-mediated cell cycle arrest and DNA repair in patients. These findings supported the idea that IFN-γ (N) and IFN-γ (L) may serve a significant role in the treatment of lung cancer, via cell cycle arrest of cancer cells and repair mechanisms.

Correspondence to: Dr Hamid Ali Nagi Al-Jamal, School of Biomedicine, Faculty of Health Sciences, Universiti Sultan Zainal Abidin, 2nd Floor, Maimunah Block, Gong Badak Campus, 174 Jalan Sultan Mahmud Road, Kuala Nerus, Terengganu 21300, Malaysia
E-mail: aljamalhamid@unisza.edu.my

Key words: Lung cancer, IFN-γ, lymphocytes, liposomes, p53, PARP1, OGG1

Introduction

Lung cancer accounts for ~13% of annual cancer cases worldwide and is the second most common type of cancer in both male and female patients (1). Investigation of proteins including proteins like Carcinoembryonic Antigen (CEA), autoantibodies such as P53 autoantibodies, and gene expression profiles in the blood or airway epithelium has yielded promising biomarker candidates for the early detection of lung cancer, such as epidermal growth factor receptor, c-ros oncogene1(ROS1), KRAS expression (2). Interferon-γ (IFN-γ) is the only type II IFN member that isa dimerized soluble molecule and consisting of 143 amino acids (3,4). IFN-γ performs roles with antiviral, immunoregulatory and anti-tumor properties, via interactions with specific cell-surface receptors such as IFN-γ receptor (IFNGR) (5).

Researchers have long sought a rapid, safe, and effective therapy for malignant tumors. However, traditional cancer treatments, such as surgery, radiotherapy (RT), and chemotherapy still fall short. For example, surgical excision often fails due to cancer recurrence and metastasis. Radiotherapy which uses high-energy X-rays, damages normal tissues, and traditional chemotherapy is limited by severe multidrug resistance and side effects such as nausea and Vomiting, anemia, and hair loss (6). To overcome these challenges and improve prognosis, intelligent nanoplatforms have been developed for improved diagnostic and therapeutic outcomes (7). These nanoplatforms use naturally existing nanoparticles, such as

bacterial viruses (bacteriophages or phages), plant viruses, nucleic acid nanoparticles (such as DNA origami), protein nanoparticles and liposomes (8). These bionanoparticles possess unique properties in terms of composition, structure, shape and function, which make them valuable tools for cancer imaging, diagnosis and therapy (9).

Liposomal IFN-γ [IFN-γ (L)] is a cytokine that serves a key role in the maturation and function of certain immune cells (10). The encapsulation of IFN-γ in liposomes improves its pharmacokinetic profile, prolonging its half-life and enhancing its stability in the bloodstream (11). This enhanced formulation offers several advantages, such as targeted delivery to specific tissues and reduced systemic toxicity, making it a promising candidate for various therapeutic applications (12). Studies have reported the potential of IFN-γ (L) to stimulate antitumor immunity, control viral infections and modulate the immune response in autoimmune diseases (13,14). IFN-γ (L) inhibits melanoma growth and metastasis by inducing antitumor immunity (15). The second generation murine IFN-γ (L) has been reported to exhibit antitumor and antiangiogenic effects (16). If liposomes can overcome the current limitations like targeted delivery, and reduced systemic toxicity, they could be considered next generation protein therapeutics due to their ability to increase protein and peptide (PPs) solubility and provide controlled sustained release of PPs to decrease side effects of traditional therapy including autoimmunity and non-specific inflammation (17).

Inactivation of the tumor suppressor gene, p53, by somatic mutations has been reported to be associated with certain malignant neoplasms, and its reactivation represents an attractive therapeutic strategy for cancers (18,19). p53 has also demonstrated the ability to induce DNA repair pathways to minimize DNA damage (20). The regulation of specific DNA repair is controlled via p53-mediated transcriptional genes depending on the type of DNA damage. The p53 gene can induce crucial DNA repair genes including base excision repair (BER), non-homologous end-joining and nucleotide excision repair (21). Human 8-oxo guanine-DNA glycosylase (OGG1) serves a crucial role in the repair pathway of reactive oxygen species-induced damage, through stepwise base excision repair (BER) (22). In addition, OGG1 interacts with poly ADP-ribose polymerase 1 (PARP1), a DNA-damage sensor protein involved in DNA repair and numerous other cellular processes (23). However, the effect mechanism of IFN-γ on lung cancer lymphocytes is still not clear. The present study examined the DNA protective effects of IFN-γ (L) on lymphocytes from patients with lung cancer compared with healthy individuals through the study of p53, OGG1 and PARP1 at the gene and protein levels after treatment with IFN-γ (L).

Materials and methods

Reagents. All chemicals utilized in the present study were purchased from Sigma-Aldrich (Merck KGaA) including IFN-γ (98% purity; cat. no. 17001), fetal bovine serum (FBS; cat. no. F7524), RPMI 1640 medium (RPMI-1640; cat. no. R8758) and penicillin-streptomycin solution (cat. no. P4333). Before using IFN-γ, the lyophilized powder was reconstituted in double-distilled water to create the stock solution. It was then diluted in RPMI-1640 medium containing 10% FBS and kept at 20˚C.

Dose-response tests were performed to identify the best naked IFN-γ [IFN-γ (N)] and IFN-γ (L) dosages. Various doses (50, 100, 200,300 U/ml were administered at different time intervals (24, 48, 72 h) at 37 ˚C to assess their effectiveness. Based on this, 100 U/ml IFN-γ (N) and 100 U/ml IFN-γ (L) were administered at a constant dosage. The 75 μM H_2O_2 at 37˚C for 24 h was used to induce the oxidative stress and increase the DNA damage in lymphocytes from healthy individuals and lung cancer patients to be used as the positive control (PC).

Cell viability. Based on our previous study (17), cell viability was assessed using the Cell Counting Kit-8 (CCK-8), Sigma-Aldrich (Shanghai, China) at 37˚C for 4 h. In all tests, doses expected to produce a cell viability of ~75% were used and incubated with the treatment for 24 h.

Sample preparation and enzyme-modified comet assay. Healthy non-smoking volunteers and patients with lung cancer (including non-small cell lung cancer and small cell lung cancer) provided informed consent to participate. Ethical approval for the present study was received from The Leeds East Research Ethics Committee (approval no. 12/YH/0464; Leeds, UK), The University of Bradford Research Ethics Sub-Committee on Research in Human Subjects (approval no. 0405/8; Bradford, UK) and The Research Support and Governance Office, Bradford Teaching Hospitals, NHS Foundation (approval no. RE DA 1202; Bradford, UK). Whole blood samples were collected and labeled for identification. Samples were diluted in RPMI and mixed with 10% dimethyl sulfoxide. The diluted blood solution was divided and transferred to -80˚C storage. The DNA repair capability of human lymphocytes from five healthy volunteers and five patients with lung cancer was determined using an Endonuclease III (Nth) and hOGG1 FLARETM Test kit (Trevigen; cat. no. CA:4055-100-FK), according to the manufacturer's instructions. Data analyesd by using Komet 6 software and Kinetic Imaging (Andor Technology Ltd, Belfast) to determine the % DNA tail and Olive tail moment (OTM).

Lymphocyte isolation. A total of 3 ml whole blood was diluted 1:1 with 0.9% saline and layered on top of 3 ml Lymphoprep™ (Axis-Shield Diagnostics, Ltd.) in 15 ml falcon tubes. The tubes were centrifuged for 20 min at 800 x g at 4˚C. Lymphocytes were harvested and washed with saline. Cells were re-suspended in RPMI and used for *in vitro* experiments.

Preparation and characterization of liposomes. Liposomes were prepared using the thin film rehydration method (24) and all measurements were performed in triplicate.

Determination of IFN-γ encapsulation efficiency. The IFN-γ encapsulation efficiency of liposomes was determined by an indirect procedure based on the determination of uncoated free IFN-γ in the supernatant utilizing reversed-phase high-performance liquid chromatography, as previously described (25,26). Each sample was assessed in triplicate and the loading of IFN-γ was expressed as percentage encapsulation efficiency.

Table I. Primers for RT-qPCR analysis.

Gene	Primer sequence (5'-3')	(Refs.)
p53	F: GGATCCTAATACGACTCACTA	(27)
	R: GGCAGTGACCCGGAAGGCA	
PARP1	F: CCTGATCCCCCACGACTTT	(28)
	R: GCAGGTTGTCAAGCATTTC	
OGG1	F: GGTGGCCCTAAAGGACTCTC	(29)
	R: AAGGTGCTTGGGGAATTTCT	
GAPDH	F: GGAGCGAGATCCCTCCAAAAT	(28)
	R: GGCTGTTGTCATACTTCTCATGG	

All primer sequences were selected based on previous studies and verified using The Primer-BLAST, NCBI database. F, forward; R, reverse; PARP1, poly ADP-ribose polymerase 1; OGG1, 8-oxo guanine-DNA glycosylase.

Reverse transcription-quantitative (RT-q)PCR. Isolated lymphocytes were seeded in 6-well plates (1x10^6 cells/well) and treated with 100 U/ml IFN-γ (N) and IFN-γ (L) for 24 h at 37˚C. A total of 2 mg total isolated RNA was subjected to RT using an iScript™ cDNA synthesis kit (Bio-Rad Laboratories, Inc.) according to the manufacturer's protocol. Each RT-qPCR experiment was performed three times in a 10 ml reaction mixture. The primers (MilliporeSigma; Merck KGaA) were verified using Primer-BLAST, NCBI database (ncbi.nlm.nih.gov/tools/primer-blast/) and presented in Table I. Data were analyzed using the $2^{-\Delta\Delta Cq}$ method (16) and normalized against the internal reference gene GAPDH in each sample.

Western blotting analysis. Lymphocytes were seeded in 6-well plates at a density of 1x10^6 cells/well. The treated cells with IFN-γ (N) and IFN-γ (L) were incubated overnight at 37˚C in the presence of 5% CO_2, washed with cold PBS and lysed by adding 150 µl lysis buffer with 15 µl fresh protease inhibitor cocktail (Thermo Fisher Scientific). Total protein levels were determined using the Bio-Rad Bradford assay kit (Bio-Rad Laboratories, Inc.) with each experiment repeated three times. Tris buffers (pH 6.8 and 8.8) were prepared for resolving and stacking gels. The catalysts APS and TEMED and a final concentration of 10.4% SDS were added for polyacrylamide gel polymerization with 30 µg protein/well. The blotting membranes were incubated overnight at 4˚C with the primary antibody. GAPDH rabbit monoclonal primary antibody (cat. no. ab8245) was used as a loading control. The primary antibodies [GAPDH (1:10,000), p53 (1:1,000; cat. no. ab26); P21 (1:1,000); cat.no. ab109520); BCL-2 (1:1000); cat. no. ab182858] (Abcam) were diluted with TBS-T containing 1% (w/v) BSA (Sigma fraction V; Sigma Chemicals). Proteins were transferred to a blotting nitrocellulose membrane using the iBlot® Gel Transfer Device (Invitrogen) for 7 min at a constant voltage of 25V. After transfer, the nitrocellulose membranes were incubated with the blocking solution contained 1% (w/v) BSA in Tris-buffered saline containing 0.1% Tween 20 and incubated for 1 hour at room temperature with HRP-Donkey Anti-Rabbit IgG (CAT: ab7083, Abcam, UK). The blots were rinsed and visulaized by enhanced chemiluminescence substrate detection reagent [ECL substrate kit; cat. no. ab133406 (Abcam, UK). Relative expression of the protein was determined using image j software (version 1.54f; National Institutes of Health).

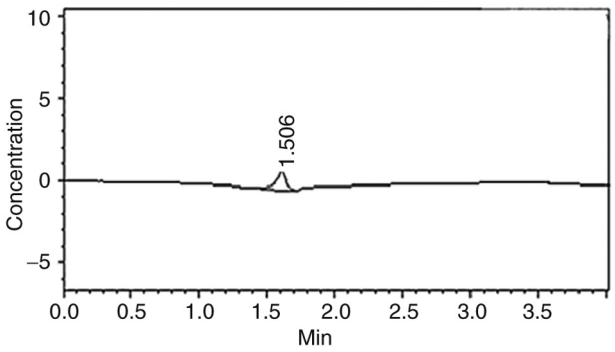

Figure 1. HPLC calibration curve chromatogram of free drug, IFN-γ, with peak identified at 1.5 min. HPLC, high performance liquid chromatography.

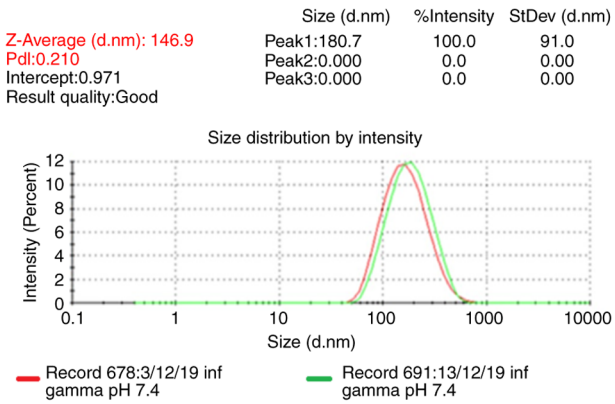

Figure 2. Measurement of particle size-distribution by DLS using Zetasizer Nano ZS-90 Model ZEN3600 (Malvern Panalytical Ltd; Spectris). DLS, dynamic light scattering.

Statistical analysis. GraphPad Prism 8 (Dotmatics) was used for statistical analysis and One-way ANOVA followed by Dunnett's post hoc test were conducted. Data are presented as the mean and SEM). $P<0.05$ was considered to indicate a statistically significant difference.

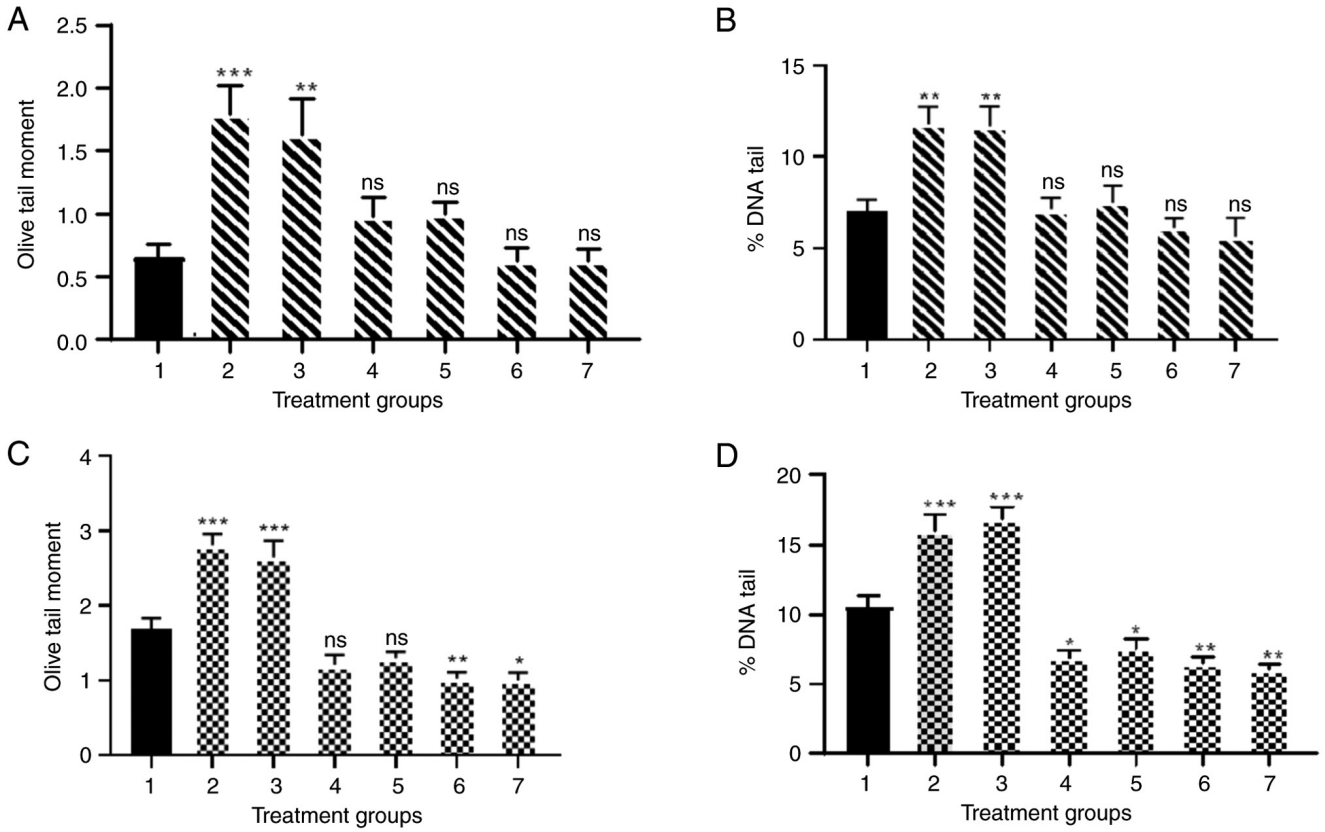

Figure 3. DNA damage in samples from healthy individuals and patients with lung cancer. (A) Olive tail moment and (B) percentage DNA tail of samples from healthy individuals. 1, NC; 2, PC + FPG; 3, PC + hOGG1; 4, 100 U/ml IFN-y (N) + endonuclease III; 5, 100 U/ml IFN-γ (N) + hOGG1; 6, 100 U/ml IFN-γ (L) + endonuclease III; and 7, 100 U/ml IFN-γ (L) + hOGG1. (C) Olive tail moment and (D) percentage DNA tail of samples from patients with lung cancer. 1, NC; 2, PC + endonuclease III; 3, PC + hOGG1; 4, 100 U/ml IFN-γ (N) + endonuclease III; 5, 100 U/ml IFN-γ (N) + hOGG1; 6, 100 U/ml IFN-γ (L) + endonuclease III; and 7, 100 U/ml IFN-γ (L) + hOGG1. Experiments were repeated at least three times. Data are presented as the mean ± SEM. *P<0.05, **P<0.01 and ***P<0.001 compared with NC. NC, untreated cells; IFN-γ (N), naked IFN-γ; IFN-γ (L), liposomal IFN-γ; hOGG1, human 8-oxo guanine-DNA glycosylase; PC, positive control of 75 μm H_2O_2; ns, not significant; FPG, formamidopyrimidine (fapy)-DNA glycosylase) repair enzyme.

Results

Encapsulation efficiency of IFN-γ liposome. The encapsulation efficiency of IFN-γ was calculated as follows: Encapsulation efficiency (%)=[(Total IFN-γ)-(Free IFN-γ)]/total IFN-γ) x 100. As the initial concentration of IFN-γ was 1.38 μg/ml and the concentration of free IFN-γ was 0.38 μg/ml, Therefore, the concentration of encapsulated IFN-γ was 1 μg/ml and the encapsulation efficiency was calculated to be 72%. (Fig. 1).

Particle size of IFN-γ liposome. The Z-Average particle size represents the intensity-weighted mean hydrodynamic size of the entire ensemble of particles, as measured using dynamic light scattering (DLS) was 146.9 nm with polydispersity index=0.210. To ensure liposome stability, samples were stored at 4˚C and the particle size was measured three times, the results showed no notable increases in the particle size over 10 days (Fig. 2).

IFN-γ has a low cytotoxicity effect against lymphocytes. CCK-8 assay (Fig. 3) indicated that the viability of lymphocytes from three healthy individuals and three patients with lung cancer in different treatment groups was >75% after 24 h treatment. Dose-response experiments were performed to determine the optimal doses of IFN-γ (N) and IFN-γ (L) used throughout the study with a fixed dose of 100 U/ml IFN-γ (N) and 100 U/ml of IFN-γ (L) determined to be the optimal dose and used during the present study.

IFN-γ (L) reduced DNA damage in the lymphocytes of patients with lung cancer. The results demonstrated the concentration-response for 100 U/ml IFN-γ (N) and IFN-γ (L) in the presence of endonuclease III and hOGG1 enzymes using % DNA tail and OTM, which indicated the extent of DNA damage in lymphocytes. The DNA of lymphocytes from healthy volunteers treated with 100 U/ml IFN-γ (N) and IFN-γ (L) demonstrated no significant change in DNA damage compared with untreated cells (Fig. 3A and B). However, lymphocyte DNA from patients with lung cancer (Fig. 3C and D) showed a significant decrease in % DNA tail for IFN-γ (N) (P<0.05) and (P<0.01) for IFN-γ (L) in lymphocytes from patients with lung cancer. Moreover, the IFN-γ (L) with endonuclease III showed a significant reduction in OTM compared with untreated cells (P<0.01). hOGG1 enzymes with IFN-γ (L) also demonstrated a significant decrease in DNA damage in lymphocytes from patients with lung cancer (P<0.05).

IFN-γ upregulates the gene expression of p53, PARP1 and OGG1 genes in lymphocytes from patients with lung cancer. The gene expression levels of p53, PARP1 and OGG1 were evaluated using RT-qPCR. The results indicated that 100 U/ml

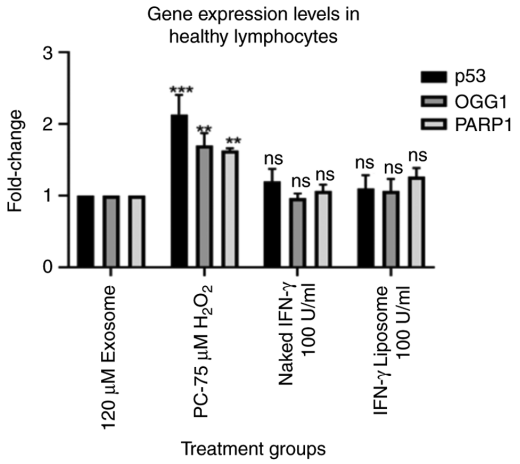

Figure 4. Effect of 100 U/ml IFN-γ (N) and IFN-γ (L) on gene expression levels of p53, OGG1 and PARP1 in lymphocytes from healthy individuals. No significant effects were recorded compared with the NC. Data are presented as the mean ± SEM of three experiments. **P<0.01 and ***P<0.001 compared with the NC. ns, not significant; OGG1, 8-oxo guanine-DNA glycosylase; IFN-γ (N), naked IFN-γ; IFN-γ (L), liposomal IFN-γ; PARP1, poly ADP-ribose polymerase 1; NC, negative control.

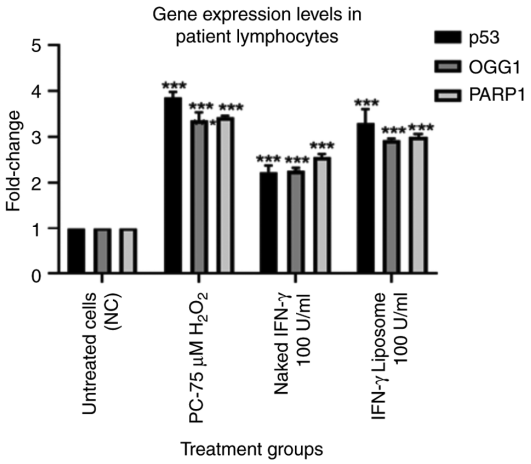

Figure 5. Significant upregulation of p53, OGG1 and PARP1 in lymphocytes from patients with lung cancer after treatment with 100 U/ml IFN-γ (N) and 100 U/ml IFN-γ (L) compared with the NC. Data are presented as the mean ± SEM of three experiments. ***P<0.001 compared with the NC. ns, not significant; OGG1, 8-oxo guanine-DNA glycosylase; IFN-γ (N), naked IFN-γ; IFN-γ (L), liposomal IFN-γ; PARP1, poly ADP-ribose polymerase 1; NC, untreated cells.

IFN-γ (N) and 100 U/ml IFN-γ (L) treatments had no detectable effects on the mRNA expression levels of p53, PARP1 and OGG1 in lymphocytes from healthy individuals (Fig. 4). IFN-γ treatment significantly increased the mRNA expression levels of p53, PARP1 and OGG1 in lymphocytes from patients with lung cancer (P<0.001). However, IFN-γ (L) upregulated the targeted genes markedly more than the naked form (Fig. 5).

IFN-γ increases protein expression levels of p53, OGG1 and PARP1 in lymphocytes from patients with lung cancer. The results of the present study demonstrated that p53, OGG1 and PARP1 protein expression levels in the lymphocytes of healthy individuals were not significantly affected by 100 U/ml IFN-γ (N) or 100 U/ml IFN-γ (L) (Fig. 6). However, p53, OGG1 and PARP1 protein expression levels in lymphocytes from patients with lung cancer showed a statistically significant increase when compared with the untreated cells (Fig. 7). Treatment with 100 U/ml IFN-γ (N) and 100 U/ml IFN-γ (L) significantly increased p53 levels in lymphocytes from patients with lung cancer by 1.8 and 1.9-fold, respectively. Moreover, compared with the control group, the OGG1 levels for both the naked and liposomal forms of 100 U/ml IFN-γ increased by 1.9-fold. Furthermore, 100 U/ml IFN-γ (N) increased the protein expression levels of PARP1 by 1.2-fold, whereas 100 U/ml IFN-γ (L) increased it by ~1.8-fold. IFN-γ (L) had a greater impact on the protein levels of targeted proteins compared with the naked form.

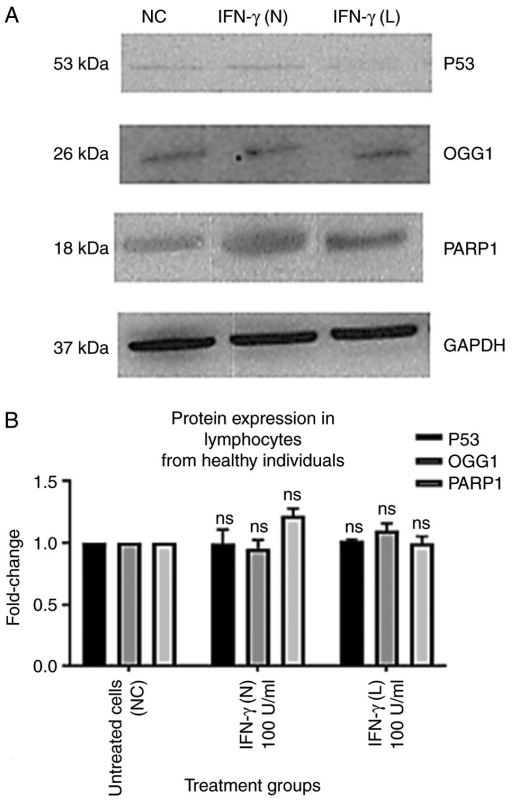

Figure 6. Effect of 100 U/ml IFN-γ (N) and 100 U/ml IFN-γ (L) on the protein expression levels of p53, OGG1 and PARP1 in lymphocytes from healthy individuals. All data from the treatment groups were compared with the NC and normalized against the internal reference protein, GAPDH. The experiment was repeated three times in three different individuals. The treatment groups included NC, 100 U/ml IFN-γ (N) and 100 U/ml IFN-γ (L). IFN-γ in both forms did not demonstrate any significant effect on p53, OGG1 and PARP1 protein levels compared with the NC. (A) Immunoblot analysis of p53, OGG1 and PARP1 proteins in lymphocytes from healthy individuals treated with 100 U/ml IFN-γ (N) and 100 U/ml IFN-γ (L). (B) Bar graphs presented fold changes in protein expression levels. Data are presented as the mean ± SEM of three experiments. NC, untreated cells; ns, not significant; OGG1, 8-oxo guanine-DNA glycosylase; IFN-γ (N), naked IFN-γ; IFN-γ (L), liposomal IFN-γ; PARP1, poly ADP-ribose polymerase 1.

Discussion

The purpose of the present study was to analyze the effect of IFN-γ on the peripheral lymphocytes of patients with lung cancer and the ability of IFN-γ to protect against oxidative stress.

Lymphocytes were selected as the model cells for the present investigation. High levels of DNA damage in lymphocytes may be caused by genetically impaired DNA repair

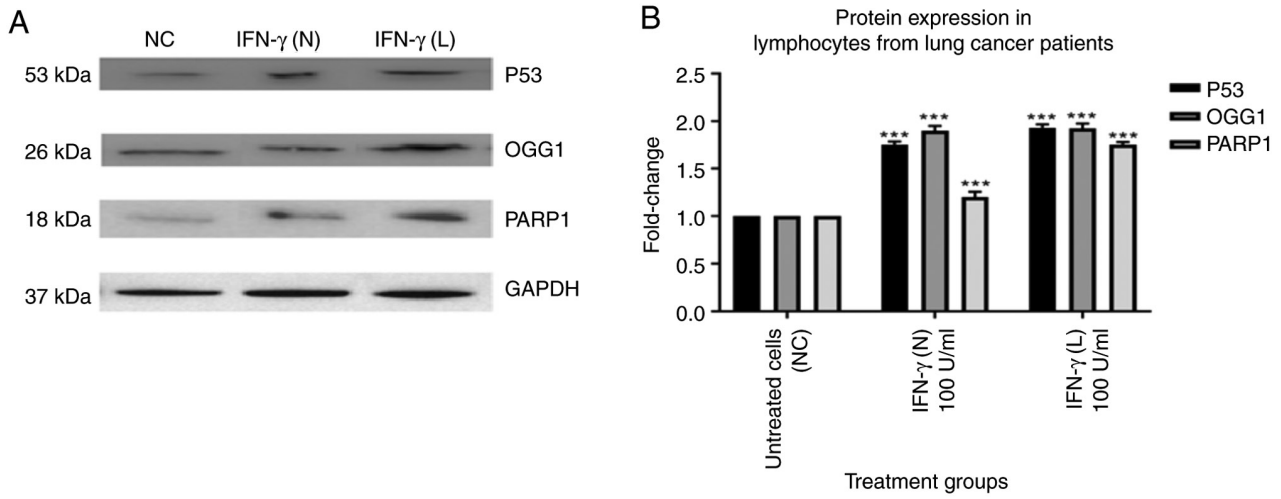

Figure 7. Effect of 100 U/ml IFN-γ (N) and 100 U/ml IFN-γ (L) on the protein expression levels of p53, OGG1 and PARP1 in lymphocytes from patients with lung cancer. All data from the treatment groups were compared with the NC and normalized against the internal reference protein, GAPDH. The experiment was repeated three times in three different individuals. The treatment groups included NC, 100 U/ml IFN-γ (N) and 100 U/ml IFN-γ (L). IFN-γ in both forms significantly increased the protein expression levels of p53, OGG1 and PARP1. The protein expression levels of p53, OGG1 and PARP1 in lymphocytes from patients with lung cancer showed a significant increase after treatment with IFN-γ compared with the NC. (A) Immunoblot analysis of the p53, OGG1 and PARP1 proteins in lymphocytes from patients with lung cancer treated with 100 U/ml IFN-γ (N) and 100 U/ml IFN-γ (L). (B) Bar graphs presenting fold changes in protein expression levels. Data are presented as the mean ± SEM of three experiments. ***P<0.001 compared with the NC. ns, not significant; NC, untreated cells; OGG1, 8-oxo guanine-DNA glycosylase; IFN-γ (N), naked IFN-γ; IFN-γ (L), liposomal IFN-γ; PARP1, poly ADP-ribose polymerase 1.

mechanisms (27). Peripheral lymphocytes are an excellent model for evaluating the sensitivity of the genome to mutagens, which is determined by measuring genotoxic events triggered by chemical or physical agents (28).

The results of the hOGG-1 and endonuclease III comet modified test showed that IFN-γ (N) and IFN-γ (L) were able to repair DNA damage in human lymphocytes derived from patients with lung cancer and healthy persons compared with untreated cells. IFN-γ (L) decreased DNA damage more effectively than IFN-γ (N) compared with untreated cells. These findings were consistent with previous reports in which lymphocytes from patients with lung cancer were treated with IFN-γ in both forms and which revealed that DNA damage was reduced compared with untreated cells (29,30). Nonetheless, the reduction in DNA damage caused by IFN-γ (L) was greater than the reduction caused by IFN-γ (N). This may be a consequence of the increased biocompatibility and cellular reactivity of liposomes as compared with compounds larger particles (24).

The tumor-suppressor, p53, provides a protective effect against the development of cancer by serving a crucial part in genomic stability-maintaining homeostasis and repairing processes (31). In addition, certain DNA repair mechanisms, including the BER pathway which depends on the activity of the OGG1 and PARP1 proteins, are important to protect genetic integrity and prevent mutations that can cause disease or cell death (32). Therefore, it is important to unravel the effect of IFN-γ on the gene expression and protein expression levels of p53, OGG1 and PARP1.

In the present study, there was a significant upregulation of the p53 gene and increase in the protein expression level of p53 in lymphocytes from patients with lung cancer after 24 h of treatment with both the liposome and naked forms of IFN-γ. These findings suggested that IFN-γ may encourage p53-mediated cell cycle arrest and DNA repair in patients with lung cancer and that the protective effects caused by IFN-γ might be dependent on the tumor suppressor activity of the p53 gene. This is similar to a previous study which reported that IFN-γ activated p53 expression in melanoma cancer, resulted in the triggering of certain cellular stressors, such as those brought on by DNA damage and replication stress caused by misregulated oncogenes (33).

Previous research has shown that p53 may also influence the transcriptional expression of BER genes, including OGG1 and PARP1 (34). Similarly, the findings of the present study demonstrated that protein and mRNA expression levels of OGG1 were increased in lymphocytes from patients with lung cancer after treatment with IFN-γ (L) and IFN-γ (N). Moreover, PARP1 levels were significantly affected by both forms of IFN-γ in lymphocytes from patients with lung cancer and healthy individuals.

However, the p53, OGG1 and PARP1 protein and mRNA expression levels in lymphocytes from healthy individuals after treatment with IFN-γ liposome and naked forms were barely detectable. The findings were supported by previously reported research in which the MDM2 proto-oncogene maintains p53 at a low level in normal cells (35,36).

Furthermore, the present study demonstrated that expression of p53, OGG1 and PARP1 genes in lymphocytes from healthy individuals and patients with lung cancer was up-regulated by stimulation with H_2O_2 which used as a positive control , these results were consistent with a previous study, which reported that H_2O_2 induced apoptosis in H_9C_2 cells by an increase in p53 expression (30,37). Taken together, the findings of the present study suggested that IFN-γ may prevent lung cancer by stopping tumor cell cycles via induction of the expression of p53, OGG1 and PARP1 genes and increasing protein levels, and that liposomes may be a more effective alternative drug delivery strategy in certain conditions such as severe side effects and chemotherapy resistance.

The potential mechanism of the effect of IFN-γ on lymphocytes is complicated, and further work is needed to evaluate the mechanism *in vitro* and *in vivo*. The mechanism of IFN-γ effect on lymphocytes is intricate and not fully understood. The study may not have comprehensively explained all facets of this complex mechanism, indicating the need for further research in this area. The study primarily relied on *in vitro* (cell culture) models, which may not completely represent the intricacies of immune responses that occur in living organisms (*in vivo*). Findings from *in vitro* experiments might not always directly apply *in vivo* situations.

Acknowledgements

The authors would like to thank University of Bradford in the UK, Mutah University, and Philadelphia University in Jordan for use of laboratories and machines.

Funding

The present study was funded by the University of Bradford (grant no. 43091/205100/DB071).

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DA, MA and HANAJ conceptualized the study; MA performed the study methodology; WH, YAH, MO, HKMS and IAT performed the formal analysis; DA investigated the present study; ASAW supplied the resources for the present study; MA and BA wrote the original draft preparation; HANAJ, BA, WH, NRM and ASAW analyzed and interpreted data, reviewed and edited the manuscript; NRM, YAH, MO and IAT performed study visualization; and DA supervised, performed project administration and acquired funding for the present study. All authors have read and approved the final version of the manuscript. MA, BA and HANAJ confirmed the authenticity of all the raw data.

Ethics approval and consent to participate

Ethical approval was obtained to perform the Comet repair assay, RT-qPCR and western blotting for the study of IFN-γ (N) and IFN-γ (L). The present study received ethical approval from The Leeds East Research Ethics Committee (approval no. 12/YH/0464; Leeds, UK), The University of Bradford Research Ethics Sub-Committee on Research in Human Subjects (approval no. 0405/8; Bradford, UK) and The Research Support and Governance Office, Bradford Teaching Hospitals, NHS Foundation (approval no. RE DA 1202; Bradford, UK). Informed consent was obtained from all participants prior to participation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Oo AM, Mohd Adnan LH, Nor NM, Simbak N, Ahmad NZ and Lwin OM: Immunomodulatory effects of flavonoids: An experimental study on natural-killer-cell-mediated cytotoxicity against lung cancer and cytotoxic granule secretion profile. Proceedings Singapore Healthcare 30: 279-285, 2020.
2. Daud NNNNM, Septama AW, Simbak N, Bakar NHA and Rahmi EP: Synergistic effect of flavonoids from artocarpus heterophyllus heartwoods on anticancer activity of cisplatin against H460 and MCF-7 cell lines. Nat Product Sci 25: 311-316, 2019.
3. Miller CH, Maher SG and Young HA: Clinical use of interferon-γ. Ann N Y Acad Sci 1182: 69-79, 2009.
4. Ferreira VL, Borba HHL, Bonetti AdF, Leonart LP and Pontarolo R: Cytokines and interferons: Types and functions. Autoantibodies Cytokines 13, 2018.
5. Castro F, Cardoso AP, Gonçalves RM, Serre K and Oliveira MJ: Interferon-gamma at the crossroads of tumor immune surveillance or evasion. Front Immunol 9: 847, 2018.
6. Hishinuma S, Ogata Y, Tomikawa M, Ozawa I, Hirabayashi K and Igarashi S: Patterns of recurrence after curative resection of pancreatic cancer, based on autopsy findings. J Gastrointest Surg 10: 511-518, 2006.
7. Li Y, Bao Q, Yang S, Yang M and Mao C: Bionanoparticles in cancer imaging, diagnosis, and treatment. View 3: 20200027, 2022.
8. de Ruiter MV, Klem R, Luque D, Cornelissen JJ and Castón JR: Structural nanotechnology: Three-dimensional cryo-EM and its use in the development of nanoplatforms for in vitro catalysis. Nanoscale 11: 4130-4146, 2019.
9. Yang Z and Chen H: The recent progress of inorganic-based intelligent responsive nanoplatform for tumor theranostics. View 3: 20220009, 2022.
10. Koizumi S-i, Wakita D, Sato T, Mitamura R, Izumo T, Shibata H, Kiso Y, Chamoto K, Togashi Y, Kitamura H and Nishimura T: Essential role of Toll-like receptors for dendritic cell and NK1. 1+ cell-dependent activation of type 1 immunity by Lactobacillus pentosus strain S-PT84. Immunol Lett 120: 14-19, 2008.
11. Ramos TI, Villacis-Aguirre CA, Santiago Vispo N, Santiago Padilla L, Pedroso Santana S, Parra NC and Alonso JRT: Forms and methods for interferon's encapsulation. Pharmaceutics 13: 1533, 2021.
12. Jailani MTM, Totten J, Nevin J and Halbert G: ID 180. Fabrication, characterisation and in vitro study of dual-loaded irinotecan/cisplatin liposomes. J Pharmacy Bioallied Sci 12, 2020.
13. Hirai M, Kimura R, Takeuchi K, Hagiwara Y, Kawai-Hirai R, Ohta N, Igarashi N and Shimuzu N: Structure of liposome encapsulating proteins characterized by X-ray scattering and shell-modeling. J Synchrotron Radiat 20: 869-874, 2013.
14. Zhao H, Wu L, Yan G, Chen Y, Zhou M, Wu Y and Li Y: Inflammation and tumor progression: Signaling pathways and targeted intervention. Signal Transduct Target Ther 6: 263, 2021.
15. Kalm M, Andreasson U, Björk-Eriksson T, Zetterberg H, Pekny M, Blennow K, Pekna M and Blomgren K: C3 deficiency ameliorates the negative effects of irradiation of the young brain on hippocampal development and learning. Oncotarget 7: 19382, 2016.
16. Wirth M, Plattner V and Gabor F: Strategies to improve drug delivery in bladder cancer therapy. Expert Opin Drug Deliv 6: 727-744, 2009.
17. Pisal DS, Kosloski MP and Balu-Iyer SV: Delivery of therapeutic proteins. J Pharm Sci 99: 2557-2575, 2010.
18. Yu H, Gao HY, Guo H, Wang GZ, Yang YQ, Hu Q, Liang LJ, Zhao Q, Xie DW, Rao Y and Zhou GB: Upregulation of wild-type p53 by small molecule-induced elevation of NQO1 in non-small cell lung cancer cells. Acta Pharmacol Sinica 43: 692-702, 2022.
19. Almajali B, Johan MF, Al-Wajeeh AS, Wan Taib WR, Ismail I, Alhawamdeh M, Al-Tawarah NM, Ibrahim WN, Al-Rawashde FA and Al-Jamal HAN: Gene expression profiling and protein analysis reveal suppression of the C-Myc oncogene and inhibition JAK/STAT and PI3K/AKT/mTOR signaling by thymoquinone in acute myeloid leukemia cells. Pharmaceuticals 15: 307, 2022.

20. Williams AB and Schumacher B: p53 in the DNA-damage-repair process. Cold Spring Harb Perspect Med 6: a026070, 2016.
21. Xu X, Jin S, Ma Y, Fan Z, Yan Z, Li W, Song Q, You W, Lyu Z, Song Y, *et al*: miR-30a-5p enhances paclitaxel sensitivity in non-small cell lung cancer through targeting BCL-2 expression. J Mol Med (Berl) 95: 861-871, 2017.
22. Slupphaug G, Kavli B and Krokan HE: The interacting pathways for prevention and repair of oxidative DNA damage. Mutat Res 531: 231-251, 2003.
23. Noren Hooten N, Kompaniez K, Barnes J, Lohani A and Evans MK: Poly(ADP-ribose) polymerase 1 (PARP-1) binds to 8-oxoguanine-DNA glycosylase (OGG1). J Biol Chem 286: 44679-44690, 2011.
24. Alhawamdeh M, Isreb M, Aziz A, Jacob BK, Anderson D and Najafzadeh M: Interferon-γ liposome: A new system to improve drug delivery in the treatment of lung cancer. ERJ Open Res 7: 00555-2020, 2021.
25. Van Slooten M, Boerman O, Romøren K, Kedar E, Crommelin D and Storm G: Liposomes as sustained release system for human interferon-γ: Biopharmaceutical aspects. Biochim Biophys Acta 1530: 134-145, 2001.
26. Haggag YA, Matchett KB, Falconer RA, Isreb M, Jones J, Faheem A, McCarron P and El-Tanani M: Novel ran-RCC1 inhibitory peptide-loaded nanoparticles have anti-cancer efficacy in vitro and in vivo. Cancers 11: 222, 2019.
27. Fouad YA and Aanei C: Revisiting the hallmarks of cancer. Am J Cancer Res 7: 1016, 2017.
28. Collins AR: The comet assay for DNA damage and repair: principles, applications, and limitations. Mol Biotechnol 26: 249-261, 2004.
29. Alhawamdeh MF: Applying a new technique, the interferon gamma liposomal delivery system to improve drug delivery in the treatment of lung cancer. University of Bradford, 2021.
30. Rivlin N, Brosh R, Oren M and Rotter V: Mutations in the p53 tumor suppressor gene: important milestones at the various steps of tumorigenesis. Genes Cancer 2: 466-474, 2011.
31. Yin T, Wang P, Li J, Wang Y, Zheng B, Zheng R, Cheng D and Shuai X: Tumor-penetrating codelivery of siRNA and paclitaxel with ultrasound-responsive nanobubbles hetero-assembled from polymeric micelles and liposomes. Biomaterials 35: 5932-5943, 2014.
32. Lieschke E, Wang Z, Kelly GL and Strasser A: Discussion of some 'knowns' and some 'unknowns' about the tumour suppressor p53. J Mol Cell Biol 11: 212-223, 2019.
33. Chatterjee N and Walker GC: Mechanisms of DNA damage, repair, and mutagenesis. Environ Mol Mutagen 58: 235-263, 2017.
34. Fukui T, Matsui K, Kato H, Takao H, Sugiyama Y, Kunieda K and Saji S: Significance of apoptosis induced by tumor necrosis factor-α and/or interferon-γ against human gastric cancer cell lines and the role of the p53 gene. Surg Today 33: 847-853, 2003.
35. Thiem A, Hesbacher S, Kneitz H, di Primio T, Heppt MV, Hermanns HM, Goebeler M, Meierjohann S, Houben R and Schrama D: IFN-gamma-induced PD-L1 expression in melanoma depends on p53 expression. J Exp Clin Cancer Res 38: 397, 2019.
36. Midgley CA and Lane DP: p53 protein stability in tumour cells is not determined by mutation but is dependent on Mdm2 binding. Oncogene 15: 1179-1189, 1997.
37. Zhang M, Zhang Y, Xu E, Mohibi S, de Anda DM, Jiang Y, Zhang J and Chen X: Rbm24, a target of p53, is necessary for proper expression of p53 and heart development. Cell Death Differ 25: 1118-1130, 2018.