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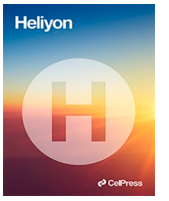

Research article

Liposomal encapsulated curcumin attenuates lung cancer proliferation, migration, and induces apoptosis

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ABSTRACT

Lung cancer is one of the most diagnosed types of cancer worldwide, accounting to one fifth of cancer-related deaths. The high prevalence of lung cancer (LC) is due to various factors such as environmental pollution or lifestyle factors such as cigarette smoking. Non-small cell lung cancer (NSCLC) is the most diagnosed type of lung cancer. Despite the availability of several lines of treatment for NSCLC, including surgery, chemotherapy, radiotherapy, immunotherapy, and combinations of these, this disease still has very low survival rate, highlighting the urgent need to develop novel therapeutics. Phytoceuticals, or plant-derived bioactives are a promising source of biologically active compounds. Among these, curcumin is particularly relevant due to its wide range of anticancer, antioxidant, and anti-inflammatory activity. However, its poor solubility causes low bioavailability, severely limiting its clinical application. Encapsulation of curcumin in nanoparticle-based delivery systems such as liposomes holds promise to overcome this limitation. In the present study, we demonstrate promising *in vitro* anticancer affect or curcumin-loaded liposomes (PlexoZome®) on A549 human lung adenocarcinoma cells. The study reveals how liposomal curcumin functionally supresses the proliferation, migration, and colony formation of these cells whilst also drastically reducing the expression of multiple cancer marker proteins. This work provides foundational data for the development of a curcumin-based nano formulation to be used as therapy for NSCLC.

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1. Introduction

The worldwide distribution of lung cancer (LC) highlights its considerable influence on public health. It is the most diagnosed cancer type globally, with 2.2 million new cases recorded and ~1.8 million deaths in 2020 and an overall poor prognosis [1]. In Australia, LC is a major health problem, with comparatively higher frequency and mortality rates compared to other forms of cancer; according to Cancer Australia, LC accounts for nearly one in every five cancer-related fatalities in recent years [2]. LC results from the abnormal growth of cells within different tissues of the lung. Depending on the cell type affected, LC is classified into two main types of LC: non-small cell lung cancer (NSCLC), and small cell lung cancer (SCLC). NSCLC is a type of LC typically caused by chain tobacco cigarette smoking [3]. On average, 80–85 % of diagnosed LC are NSCLC, making it one of the most commonly reported type [2,4].

Reportedly, LC represents a significant proportion of cancer-related mortality worldwide due to the lack of effective early diagnostic markers and therapies [5]. Although the higher prevalence of LC on the global scale has attracted a large number of therapeutic interventions, including chemotherapy, targeted therapy, immunotherapy, and radiotherapy, the effectiveness of these approaches in the controlling of cancer cell growth is limited, and treatment often causes adverse side effects compromising the patient's immune system and general health [6,7]. This, together with the fact that often LC develops treatment resistance, calls for the urgent necessity to develop alternative treatment strategies [6,8].

The use of promising natural product-based anticancer compounds with limited side effects would thus be beneficial in this context [9–11]. Curcumin is derived from turmeric or *Curcuma longa* extract and has been used in traditional medicine for centuries across many countries. Its potential anti-inflammatory and antioxidant properties make it a great dietary supplement and it has been investigated in an extensive amount of studies, discussing the benefits of using curcumin for the reduction of oxidative stress and inflammation markers in a variety of chronic diseases and illnesses [12]. Similarly, numerous studies have demonstrated the promising anticancer potential of curcumin across many cancer types, including LC [13–15]. However, the practical pharmaceutical application of curcumin is limited due to the problems such as low water solubility and poor oral bioavailability [16].

In attempts to improve the bioavailability of curcumin and fully explore its potential as a tool to combat the progression of LC, curcumin has been used in combination with Pharmako Biotechnologies trademarked liposomal technology (PlexoZome®). Through advanced delivery systems, the passive loading of curcumin in phosphatidylcholine (PC)-based liposomes was performed, using the sonication method, resulting in liposomes carrying the bioactive. Recent studies have shown how liposomes have helped deliver anticancer agents to reduce the toxic effects of other drugs when delivered alone [17]. Furthermore, the advancement in delivery techniques allows for the liposomes to be customised for the appropriate pathway of administration hence further reducing toxicity [17]. These properties of the delivery systems make them ideal to be tested *in vitro* for cancer drugs.

The aim of the present study is to explore the therapeutic potential of curcumin when used as a treatment *in vitro* in LC cells. We show that the curcumin-loaded liposomes significantly inhibit three fundamental cancer hallmarks: cell proliferation, migration, and the ability to form colonies. Furthermore, liposomal curcumin significantly downregulated the expression of several proteins involved in the promotion of cancer cell proliferation, migration/invasion/metastasis, and protection from apoptosis, providing a solid mechanistic investigation of the cancer-promoting pathways impacted by curcumin. The data collected from this study will be greatly beneficial for society in reflecting the potential of using PlexoZome® curcumin for NSCLC treatment and management.

2. Methodology

2.1. Preparation of curcumin liposomes

The liposomal formulation used throughout this study was manufactured and characterised by Pharmako Biotechnologies Pty Ltd. Zeta analysis, particle size and polydispersity index (PDI) and stability studies have been conducted. The liposomal formulation, PlexoZome® product, is a technology currently used on the market and utilises passive loading of curcumin in conjunction with phosphatidylcholine to protect and enhance the bioavailability of the raw ingredient, curcumin. Veh or Vehicle is a mixture of chemical reagents optimized by Pharmako Biotechnologies to carry the curcumin within the liposome bilayer.

2.2. Cell culture and treatment

The lung cancer cell line A549 cultured in Dulbecco's Modified Eagle Medium (DMEM), that had been supplemented with 5 % fetal bovine serum and 1 % Penicillin and Streptomycin. The culture flasks were kept in a controlled environment, with 5 % CO_2 and humidified atmosphere, and incubated at 37 °C. The 2.5 μM and 5 μM curcumin treatments were prepared by diluting the PlexoZome® curcumin stock in DMEM. To obtain optimal results, treatment was typically performed when the cells had reached 80 % confluency. In all the mentioned studies, treatment was performed for 24 h before analysis.

2.3. Cell viability - MTT assay

Cell viability was determined by running an MTT assay, a type of colorimetric test which reflects the cells metabolic activity by using the chemical 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide. The test was performed as reported in previous studies [11,18].

In a 96 well plate, 10,000 cells/well were seeded and, after overnight adhesion, treated with vehicle or 2.5, 5, 10 and 20 μM of liposomal curcumin. After 24 h of treatment, MTT solution (0.5 mg/mL final concentration) was added to each well and incubated at

37 °C for 4 h. The media was removed, and the remaining purple crystals were dissolved with 100 μL dimethyl sulfoxide (DMSO). The reading was conducted using a TECAN Infinite M1000 plate reader with a wavelength of 570 nm.

2.4. Cell colony formation

The colony formation assay was performed as reported previously [19,20]. A549 cells were seeded into a 6 well plate at low density (approximately 500 cells/well) and then placed in an incubator at 37 °C overnight for attachment. After attachment, the cells were treated with either 2.5 or 5 μM liposomal curcumin. The plate was then placed back into incubation to allow for the colonies to begin forming. 24 h after the cells were seeded the treatment was then removed and each well was washed using 1–2 mL of phosphate buffer solution (PBS) and replaced with another 2 mL of DMEM. The plates were then incubated until sufficient colonies were visible, replacing the culture media every 72 h. After colony formation, the wells were washed with 2 mL of PBS (in triplicate) and fixed with 4.7 % paraformaldehyde for 15 min. This step was followed by three washes with PBS, after which cells were stained with 2 mL of crystal violet. The plates were protected from light and left for 1 h to stain. Afterwards the stain was removed and again washed with 2 mL of PBS. The 6 well plate was inverted and photographed.

2.5. Scratch-wound migration assay

The scratch-wound migration assay was performed as reported previously [21,22]. 100,000 A549 cells/well were seeded in 6-well plates and grown until formation of a confluent monolayer. Successively, small scratch from top to bottom of each well was made with the tip of a sterile 200 μL pipette tip, and the current media (DMEM) was removed. To ensure all loose cells were removed, three washes of 2 mL of PBS were done followed by exposure of cells to either 2.5 or 5 μM liposomal curcumin. The plates are then taken for imaging using a microscope and the distances between edge of wound were recorded. Various pictures are taken of each well immediately after the scratch before (time 0 h) and after 24 h' time points.

2.6. Human oncology protein array

To perform the protein array experiment, 100,000 A549 cells/well were seeded in a 6-well plate and treated, after overnight attachment, with 5 μM liposomal curcumin for 24 h. Upon treatment, the cells were washed three times with PBS and lysed with RIPA Buffer (ThermoFisher Scientific, Australia) supplemented with protease inhibitor cocktail (Merck, Australia). The lysate was incubated on ice for 15 min followed by removal of the cell debris through centrifugation at 14,000 RPM for 15 min at 4 °C. The cleared protein samples were then quantified using the BCA Assay (ThermoFisher Scientific, Australia).

The Proteome Profiler Human XL oncology protein array kits were purchased from InVitro Technology, Australia. 300 μg of protein lysate from each sample was hybridized to the respective array membranes, and the remaining procedure was followed as per the provided instruction manual as well as using the provided reagents. Imaging of the arrays was performed using a Chemidoc system (BioRad) [23,24].

2.7. Statistical analysis

The experiments conducted for this paper was all repeated in triplicate (at the minimum). Statistical analysis was performed using

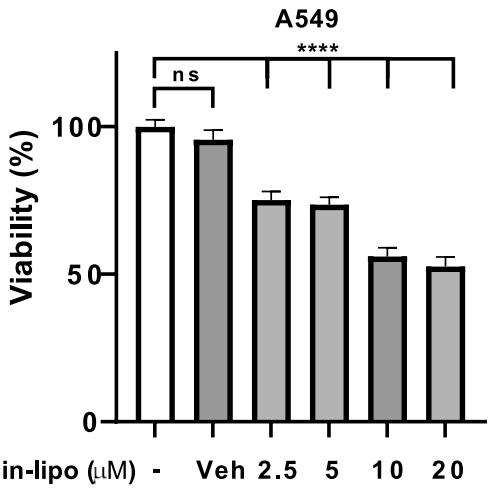

Fig. 1. Effect of PlexoZome® Curcumin on A549 cell proliferation. MTT Assay of A549 treated with varying concentrations of PlexoZome® curcumin for 24 h. Analysis was performed with one-way ANOVA, n = 3 repeats, ns = non-significant, ****$p < 0.0001$.

PRISM v.9.4 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. Cell proliferation assay - MTT

The results of the MTT assay, showing the anticancer potential of liposomal curcumin, are shown in Fig. 1. It was evident that liposomal curcumin showed a significant inhibition of A549 cell proliferation at a minimum concentration of 2.5 μM. In particular, treatment with 2.5, 5, 10, and 20 μM curcumin resulted in a reduction of cell proliferation of 24.8 %, 26.45 %, 44.03 %, and 47.37 %, respectively, compared to the untreated control (Fig. 1). Considering that the highest liposomal curcumin concentration which did not significantly impact the viability of normal human bronchial epithelial cells (BCi-NS1.1) was 5 μM (data not shown), 2.5 and 5 μM concentrations were used throughout various other experiments alongside the empty vehicle.

3.2. Cell colony formation assay

The results of the colony formation assay are shown in Fig. 2. Treatment with vehicle did not result in a change in the number of colonies formed, while treatment with 2.5 μM and 5 μM liposomal curcumin resulted in a reduction of the number of colonies of 68.58 % and 92.07 %, respectively, compared to the untreated control (Fig. 2A). Fig. 2B shows representative pictures of this assay.

3.3. Scratch-wound assay – Cell migration

To determine the impact of liposomal curcumin on A549 cell migration, a scratch-wound assay was conducted. The images were taken immediately after the scratch was made and then 24 h subsequently as seen in Fig. 3.

It is evident that over the 24-h period, 2.5 μM and 5.0 μM PlexoZome® curcumin prevented the wound from closing with a reduction of closure percentage of 26.68 % and 41.36 % respectively compared to the untreated control sample. However, the percent reduction of wound closure achieved by 2.5 μM liposomes was not statistically significant, while the 5.0 μM concentration achieved a significant inhibition of the wound closure.

3.4. Human oncology Proteome Profiler – protein array

From the protein array experiment, 21 proteins were identified as being modulated by curcumin liposomes and grouped into the core cancer pathways of migration, proliferation and anti-apoptotic with curcumin, showing a significant down regulation for all these proteins.

Of the 21 proteins, nine were identified to have anti-metastasis/anti-invasion/anti-migratory effects in the LC *in vitro*. The liposomal curcumin formulation decreased the expression of all of the proteins. Angiopoietin-like 4 was identified to of had a reduction of 11.08 % (Fig. 4A) while ENPP-2 was slightly higher with a 13.29 % decrease (Fig. 4B) and Vimentin was higher again with 19.98 % (Fig. 4C). Cathepsin B, D and S were all ascertained to have decreased when treatment with PlexoZome® Curcumin by 17.93 % (Fig. 4D), 31.99 % (Fig. 4E) and 29.17 % (Fig. 4F) respectively. Furthermore, FGF basic had a reduction in expression by 16.91 % (Fig. 4G) and eNOS with 7.58 % (Fig. 4H). The final protein identified to contribute to metastasis was MMP-3 which was 15.96 % expression reduction (Fig. 4I).

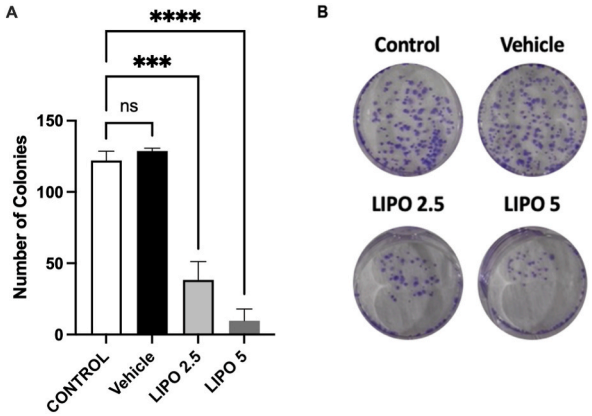

Fig. 2. Effect of PlexoZome® Curcumin on A549 cell colony formation. (A) A549 cells treated with 2.5 μM and 5 μM and PlexoZome® (liposomal) curcumin. N = 3 repeats. ***p < 0.001; ****p < 0.0001 vs. Control. Values are expressed as mean ± SEM. Analysis was performed with one-way ANOVA. (B) representative images of crystal violet stained wells. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

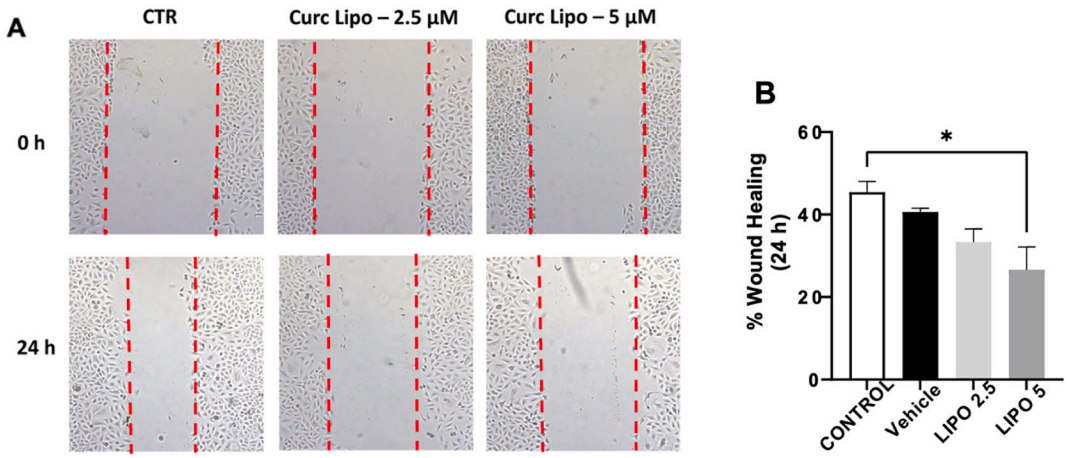

Fig. 3. Effect of PlexoZome® Curcumin on A549 cell migration (scratch wound assay) over a 24-h time period. (A) Representative pictures of treatment of the PlexoZome® Curcumin 2.5 μM and 5 μM and the wound closure over the 24 h. (B) Measurements taken of A expressed as a percentage. One-way ANOVA, n = 3, *p < 0.05. Data are shown as mean ± SEM.

The second pathway that was identified was cell proliferation. This category had the most proteins with 10 key proteins recognised to be downregulated by treatment with liposomal curcumin, as shown in Fig. 5. The expression of progranulin was significantly reduced upon treatment with liposomal curcumin by 32.69 % (Fig. 5A), while the expression of CA125/MUC16 was reduced by 6.31 % (Fig. 5B) and similarly, EGFR/ErbB1 had a reduction of 17.17 % (Fig. 5C). Although not as drastic of a reduction was evident in ErbB4, a decrease of 5.11 % (Fig. 5D) was evident as was a 12.65 % decrease for HIF-1α (Fig. 5E). The treatment of PlexoZome® curcumin had significantly decreased the expression of Axl by 27.9 % (Fig. 5F) and CCL8/MCP-1 by 7.79 % (Fig. 5G). while M-CSF showed a similar between Axl and CCL8 with a decrease of 10.23 % (Fig. 5H). The final two proteins detected were serpin B5/Maspin and serpin E1/PAI-1 which had a reduction of 13.05 % (Fig. 5I) and 20.03 % (Fig. 5J) respectively.

The final pathway identifies was anti-apoptotic (Fig. 6), and two proteins were classed into this group: B-cell lymphoma-extra-large (BCL-x, Fig. 6A) and survivin (Fig. 6B). Treatment with liposomal curcumin reduced the expression of these protein by 13.76 % and 23.6 %, respectively, compared to untreated control.

4. Discussion

The research depicted in this manuscript investigated the efficacy of liposomal curcumin in inhibiting multiple hallmarks of LC using *in vitro* experiments. Our findings provide promising evidence of curcumin PlexoZome®s' anti-cancer potential, as shown through various tests on the A549 cell line. A previous research study conducted by Adel et al. (2021) and team showing that healthy human bronchial epithelial cells respond differently to various curcumin formulations [25]. The team presented an optimized curcumin-loaded liposome that showed improved capacity to navigate through deep lung tissues with a significant proportion of bioactive. The MTT assay showed that the PlexoZome® curcumin preparation had a stronger growth inhibitory impact on A549 cells and has significantly reduced proinflammatory cytokines including tumour necrosis factor-α, interleukin-6, and interleukin-10 compared to the extract alone [25]. Furthermore, results of this study also suggested the PlexoZome® curcumin formulation performed better in the frequency and spread of absorption in lung tissue, along with its mean residence duration inside the lung tissues [25]. These data points support our study with curcumin PlexoZome® revealing a notable reduction in cell proliferation upon exposure to both PlexoZome® curcumin and control. Notably, concentrations of 2.5 μM and 5 μM exhibited similar efficacy, supporting the utilization of both concentrations in subsequent experiments.

Additionally, the cell colony formation experiment presents insight into the long-term effects of PlexoZome® curcumin therapy on A549 cells' replicative ability and their ability to form colonies when seeded at low density, a process which recapitulates metastatization of primary tumors in distant sites. Applying crystal violet staining, we found a significant decrease in colony development following liposomal curcumin treatment in comparison to control groups. The anti-colony formation efficacy using curcumin on A549 cells is consistent with prior findings, as revealed by Wang et al., in 2017. After 48 h of being treated using 10 μM curcumin, A549 cells showed reduced colony-forming ability. Also, flow cytometry analysis showed a 2.35-fold increase in cell death after 10 μM curcumin treatment compared to controls [26]. The liposomal carrier system for curcumin in our study could have improved its absorbability and bioavailability to A549 cells and thus almost similar effects were reported with both the 2.5 and 5 μM concentrations significantly reducing A594 colony formation.

The migration and invasion experiment offered important insights into liposomal curcumin's ability to inhibit cancer cell motility. Wang et al. (2020) found that the anticancer impact of curcumin was predominantly focused on its anti-invasion and anti-metastasis pathways. However, following on Wang et al.'s findings, we aimed to broaden our understanding by delving into the effects of curcumin PlexoZome® on A549 cell migration and invasion. Corresponding with the aforementioned findings, we detected in our simplified 2-D model a significant decrease in the quantity of invasive cells when compared to cells that were untreated. At curcumin

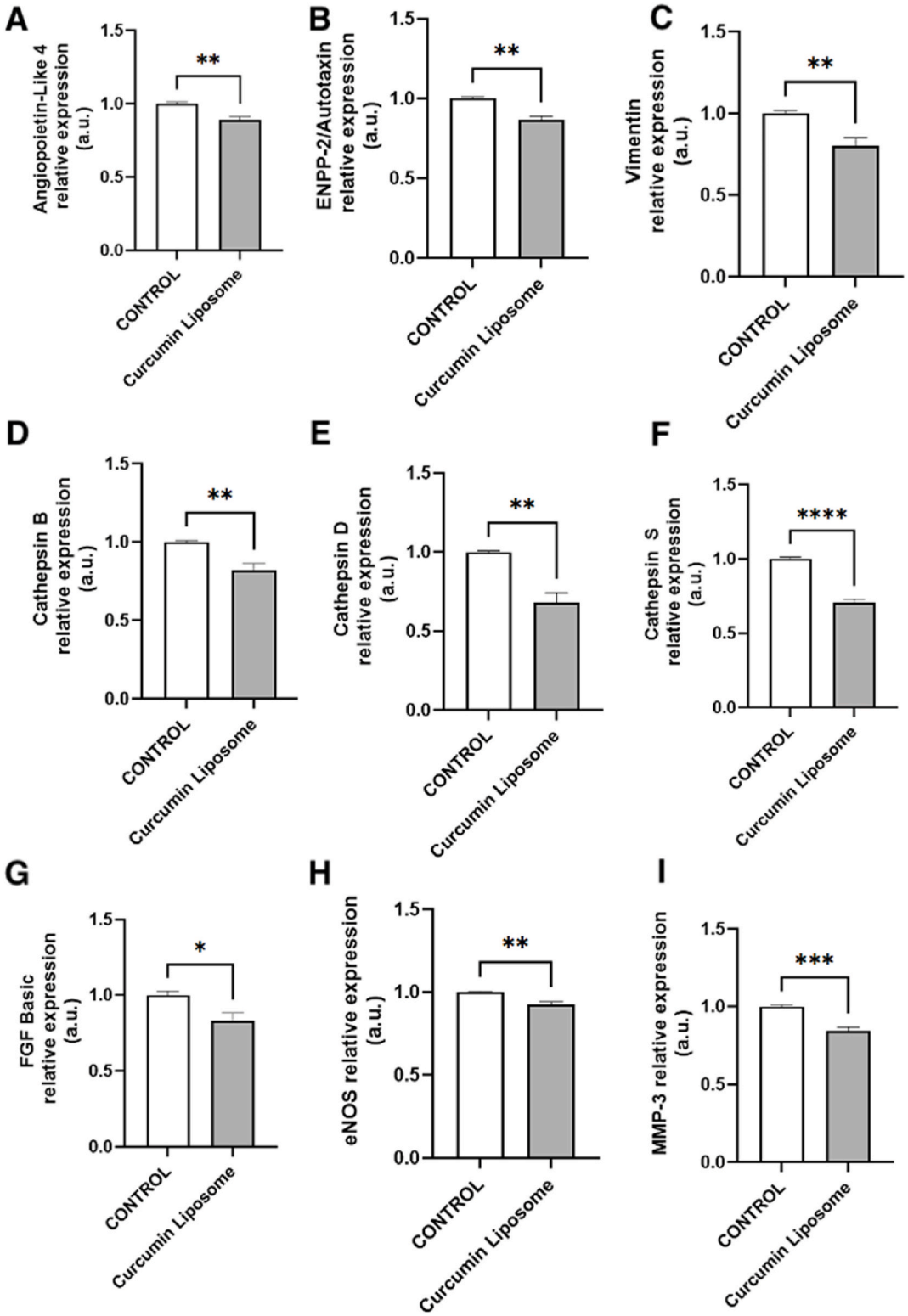

Fig. 4. Effect of PlexoZome® Curcumin on migration, metastasis and invasion protein expression in A549 cells as assessed via protein array. (A) Angiopoietin-like 4; (B) ENPP-2/Autotaxin; (C) Vimentin; (D) Cathepsin B; I Cathepsin D; (F) Cathepsin S; (G) FGF Basic; (H) eNOS; (I) MMP-3. Histograms representative of n = 4 replicates. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; ****$p < 0.0001$ vs. control. Values are expressed as mean ± SEM. Analysis was performed with one-way ANOVA.

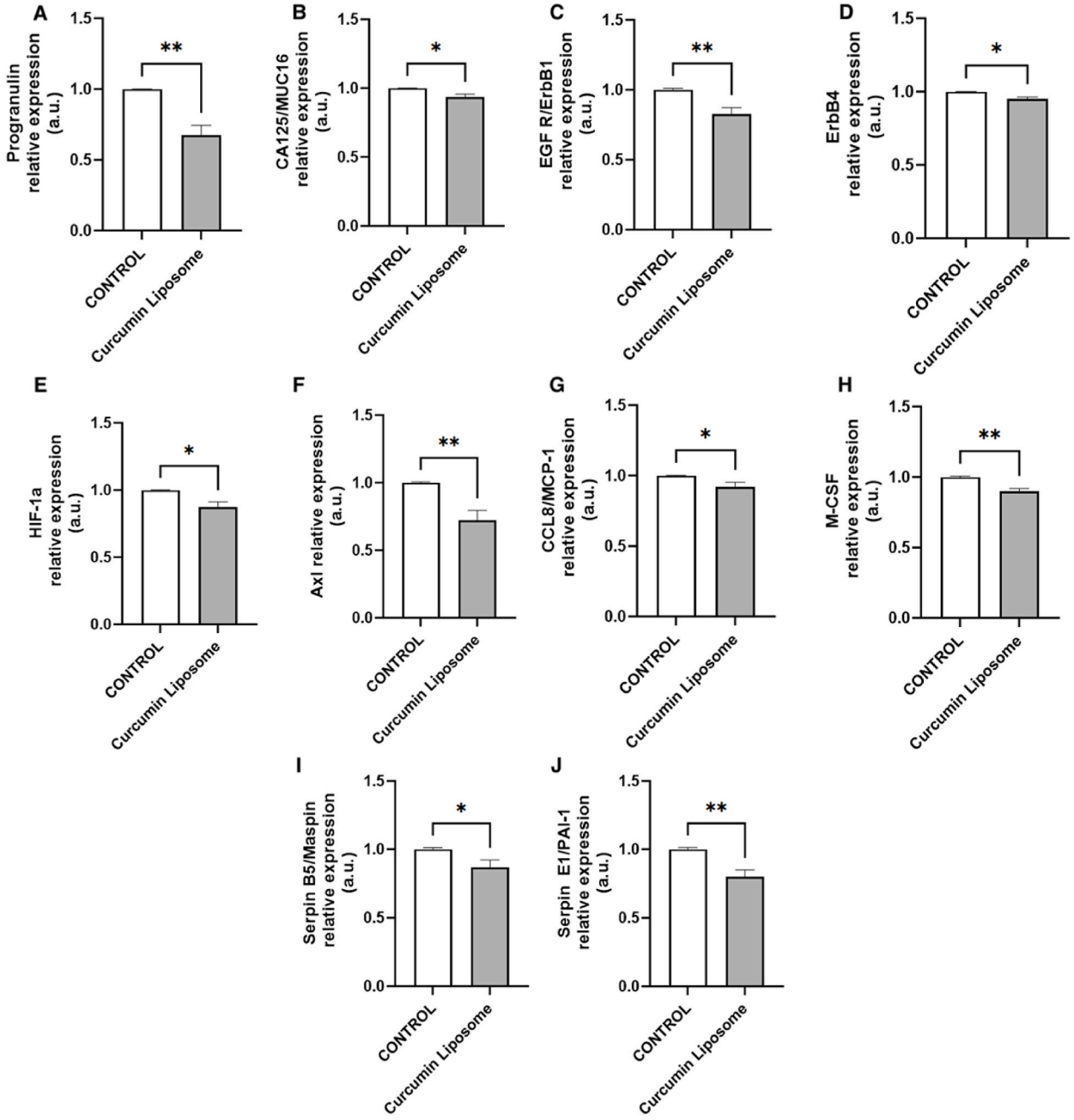

Fig. 5. Effect of PlexoZome® Curcumin on proliferation protein expression in A549 Cells as assessed via protein array. (A) Progranulin; (B) CA125/MUC16; (C) EGFR/ErbB1; (D) ErbB4; (E) HIF-1a; (F) Axl; (G) CCL8/MCP-1; (H) M-CSF; (I) Serpin B5/Maspin; (J) Serpin E1/PAI-1. Histograms representative of n = 4 replicates p*<0.05, **P < 0.01 vs control. Values are expressed as mean ± SEM, analysis was performed with one-way ANOVA.

concentrations of 5 μM,A549 cells showed a decrease to 41.36 % in migration capacity compared to the control group. This reflects the efficacy of utilising liposomal curcumin. A study conducted using free powder curcumin on the human pancreas adenocarcinoma cell lines Patu8988 and panc-1 similarly found that curcumin inhibited cell migration and invasion. By using concentrations of 10, 15 and 20 μM curcumin, Su et al. (2016) was able to prevent the cell migration in scratch wound assay from undergoing further closure with a similar trend to what was obtained in Fig. 3 of the present manuscript [27].

The protein array experiment provided important insight into the molecular mechanisms impacted by treatment with liposomal curcumin that led to the observed anticancer activity. It was found that nine of the proteins contributed to migration, metastasis, and invasion as shown in Fig. 4, and all these proteins were downregulated by liposomal curcumin. Angiopoietin-Like 4 (Angptl4) has been

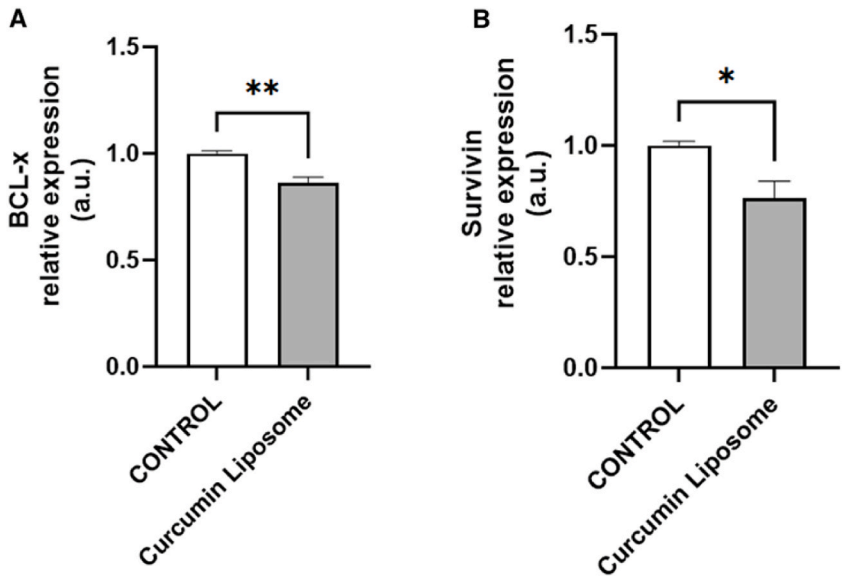

Fig. 6. Effect of PlexoZome® Curcumin on anti-apoptotic protein expressions in A549 cells as assessed via protein array. (A) BCL-x; (B) Survivin. Histograms representative of n = 4 replicates *p < 0.05, **P < 0.01 vs control. Values are expressed as mean ± SEM, analysis was performed with one-way ANOVA.

seen to be up regulated in LC and is strongly expressed in a variety of tissues and tumors [28]. Furthermore, Angptl4 promotes migration and has been shown to be a contributing factor to cancer cell metastasis [29]. As a result of this relationship and its upregulation, Angptl4 can be used as a marker for the diagnosis for LC. Autotaxin (ENPP-2) is another protein with pro-metastasis properties and has been identified as a marker for a few forms of cancer such as prostate, liver and lung [30]. The increase of ENPP-2 evidently promotes lung carcinogenesis and disease attenuation as found by C.Magkrioti (2018) when genetically deleting ENPP-2 [31]. Vimentin plays a role on the migration and invasion of cells and is also used as a critical marker for the progression of LC [32]. Cathepsin B, D and S were all detected by the protein array with a similar trend amongst the three with the liposomal curcumin downregulating the expression of all three proteins. Cathepsin increases the mortality and invasion ability of the cancerous cells [33]. The three proteins are all found to contribute to cancer cell migration making them a suitable marker for LC. Cathepsin B contributes to the infiltrative nature of tumour cells resulting in an overexpression in various types of cancer but in particular LC [34]. Similarly Cathepsin D have been seen to increase with LC enabling the cancerous cells to invade other parts of the body including blood, surrounding tissue and lymph nodes [33]. While Cathepsin S contributes to the same pathways, in addition evidence has been found on its involvement in drug resistance [35]. Fibroblast growth factor basic (FGF basic) was detected in the cancerous cells with a down regulation in the treated sample, Fig. 4G. FGF basic contributes to the cell inhibition and invasion in NSCLC [36]. Additionally, FGF basic has been shown to be able to be classed in all three pathways; metastasis, proliferation and apoptotic, however its contribution is the most significant to metastasis [37]. Endothelial nitric oxide synthase (eNOS) is upregulated in cancer due to its ability to foster cancerous cell growth [38]. The final protein which was seen to contribute to cell invasion, metastasis and migration was metalloproteinases-3 (MMP-3). MMP-3 is overexpressed in cancer and is a key inflammatory marker as it plays a role promoting cell migration and invasion. A study found that MMP-3 was significantly increased in tissue from tumour cells and surrounding areas of NSCLC [39].

Besides downregulating the aforementioned proteins involved in cell migration, invasion, and metastasis, treatment with liposomal curcumin resulted in the downregulation of ten proteins involved in the promotion of cancer cells proliferation, as shown in Fig. 5. The first includes progranulin which showed one of the highest down regulations for this group. Progranulin has many features in cancerous cells and can inhibit apoptosis, cell migration and invasion of cells. Although progranulin falls into all three categories it mostly promotes proliferation [40]. MUC16 is a protein overexpressed in cancer and commonly associated with poor prognosis. A study exploring MUC16 in NSCLC patients found a similar trend and concluded that it played a crucial role in the pathogenesis, progression, and chemoresistance of NSCLC [41]. Epidermal growth factor receptor (EGFR) is a protein found in some NSCLC cases which controls cell proliferation. Patients found to carry this protein have a higher chance of receiving targeted therapy against LC. Similarly, Erb-B2 Receptor Tyrosine Kinase 4 (ErbB4) is a part of the EGFR family and contributes to the development of healthy tissue and has tumour suppressing features [42,43]. Hypoxia inducible factor 1-alpha (HIF-1a) contributes to inflammatory pathways influencing immune cell functions [44]. The marker HIF-1a has an overexpression in LC biopsies, similarly to the data obtained in Fig. 5. The Axl receptor is apart of the Tumour-associate macrophages (TAM) family [45] and is known to trigger tumour growth while also contributing to cell proliferation, metastasis, mortality and more [46]. Chemokine ligand 8 (CCL8) similarly falls under the TAMs family and promotes aggressive tumour growth [47]. Although not necessarily apart of the TAMs family, evidence shows that Macrophage stimulating colony factor (MSC-F) has an overexpression in cervical cancer and can lead to the development of TAMs

whilst simultaneously stimulating cell proliferation [48].

Serpin B5 and Serpin E1 were also downregulated by liposomal curcumin. Both proteins are known to protect tumour cells against apoptosis reflecting how it is expected to see an upregulation in LC [49].

The final pathway identified as being impacted by treatment with liposomal curcumin was anti-apoptotic (Fig. 6), and two proteins were classed into this group including B-cell lymphoma-extra-large (BCL-x) and survivin. In cancer cells, the process of apoptosis can be blocked preventing cell death, hence reflecting its overexpression in cancer making it a hallmark for cancer [50,51]. The overexpression of these proteins can reduce the efficacy of chemotherapy [52]. Both proteins BCL-x and survivin were significantly downregulated with treatment. These data together indicate that the liposomal curcumin formulation tested has the ability to inhibit cell proliferation, migration and invasion, and colony formation, in NSCLC. Mechanistically, our liposomal curcumin formulation exerts this effect by downregulating several proteins that are fundamentally involved in these processes. The results of the present study point at the PlexoZome® formulation as a promising tool for the treatment of NSCLC, to be further investigated both as monotherapy and as an adjuvant therapy in combination with conventional treatment strategies.

Despite the promising results, our study is not exempt from limitations. One important limitation is that the results are only reported *in vitro* on 2D cell cultures. To improve the strength of these results, the used of 3D organoid models and of *in vivo* tumour xenograft systems would be invaluable. The further validation of various protein targeted by our curcumin liposomal formulation can be done for gene expression using quantitative real time-polymerase chain reaction. It would be interesting to test our curcumin liposomal in pre-clinical animal model lung cancer induced by cigarette smoke and cigarette or chemical carcinogen [53]. These studies would be necessary in order to allow the characterization of the efficacy and safety of these formulations on humans in clinical trials. The data collected from this study has been surmised in Fig. 7.

5. Conclusion

In conclusion, the data presented in this study provide proof of the promising *in vitro* anticancer potential of the PlexoZome® liposomal curcumin formulation. This formulation significantly inhibited three cancer hallmarks in A549 cells: cell proliferation, cell migration/invasion/metastasis, and colony formation. Mechanistically, we showed that the PlexoZome® liposomal curcumin formulation exerts this anticancer function by downregulating the expression of several proteins promoting lung cancer cell proliferation (Progranulin, CA125/MUC16, EGFR/ErbB1, ErbB4, HIF-1a, Axl, CCL8/MCP-1, M-CSF, Serpin B5/Maspin and Serpin E1/PAI-1), migration/invasion (Angiopoietin-like 4, ENpp-2/Autotaxin, Vimentin, Cathepsin B, Cathepsin D, Cathepsin S, FGF basic, eNOS, MMP-3), and resistance to apoptosis (BCL-x and survivin). Collectively, these data support the potential of the PlexoZome® formulation as a therapeutic for the treatment of LC. However, a thorough pre-clinical and clinical investigation of the product should be conducted to fully characterise its therapeutic potential.

Data availability statement

The data used to support the findings of this study may be released upon application to the corresponding authors, who can be contacted at Kamal.Dua@uts.edu.au and Gabriele.Derubis@uts.edu.au.

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CRediT authorship contribution statement

Sofia Kokkinis: Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Keshav Raj Paudel:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Gabriele De Rubis:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Stewart Yeung:** Writing – review & editing, Conceptualization. **Manisha Singh:** Writing – review & editing, Conceptualization. **Sachin Kumar Singh:** Writing – review & editing, Conceptualization. **Gaurav Gupta:** Writing – review & editing, Conceptualization. **Nisha Panth:** Investigation, Conceptualization. **Brian Oliver:** Writing – review & editing, Supervision, Conceptualization. **Kamal Dua:** Writing – review & editing, Visualization, Supervision, Conceptualization.

Declaration of competing interest

The authors have no conflict of interest to declare.

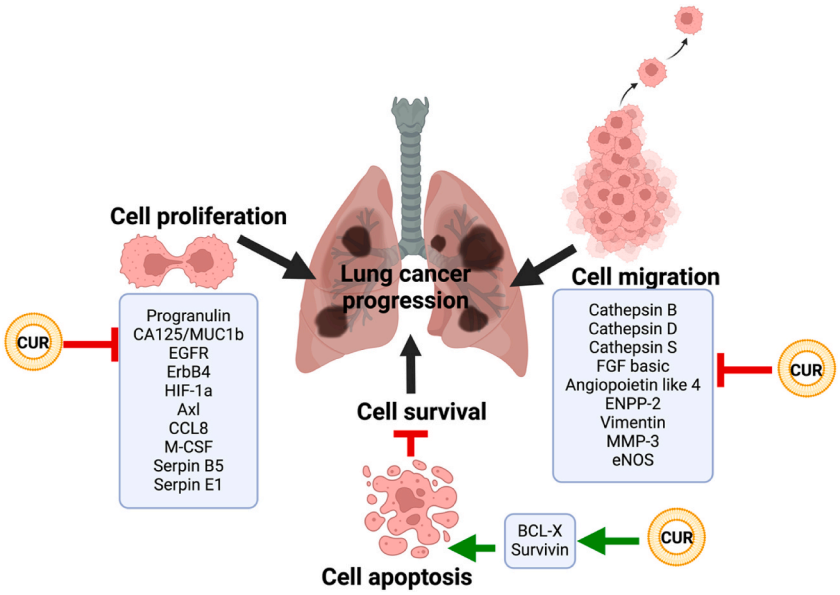

Fig. 7. Findings of proteins contributing to cell proliferation, cell migration and cell apoptosis in lung cancer model A549 post treatment with liposomal curcumin.

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