Pharmacological Reports 71 (2019) 167–174

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Short communication

Antitumor effect of chondroitin AC lyase (*Ps*PL8A) from *Pedobacter saltans* on melanoma and fibrosarcoma cell lines by *in vitro* analysis

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ARTICLE INFO

Article history:
Received 10 August 2017
Received in revised form 3 September 2018
Accepted 19 October 2018
Available online 25 October 2018

Keywords:
Chondroitin AC lyase
MTT
Apoptosis
Melanoma
Fibrosarcoma

ABSTRACT

Background: PGs are involved in cellular communication and cancer biology. The role of CS in melanoma and fibrosarcoma cell lines was explored by using chondroitin AC lyase (*Ps*PL8A).
Methods: The proliferation of mouse fibroblast L929, human melanoma (SK-Mel 28) and fibrosarcoma (HT-1080) cell lines after treatment with chondroitin AC lyase (*Ps*PL8A) was studied by MTT assay. The mode of cell death was studied by Annexin-V FITC using flow cytometry and fluorescence microscopy. The alteration in mitochondrial cell potential was studied by JC-1 dye using fluorescence microscopy and flow cytometry.
Results: Treatment of L929 cells with *Ps*PL8A imparts no cytotoxicity and showed no alteration in proliferation with nearly 95–98% cell viability. An overall 58% and 59% inhibition of SK-Mel 28 and HT-1080 cell proliferation was observed with 1.3 μM of *Ps*PL8A after 24 h of incubation. The *Ps*PL8A (1.3 μM) treated SK-Mel 28 and HT-1080 cells showed significant green fluorescence with annexin-V FITC under fluorescence microscopy and 56.6% and 35.5% apoptosis, respectively by flow cytometry analysis. The results of fluorescence microscopy and flow cytometry of SK-Mel 28 and HT-1080 upon treatment with *Ps*PL8A (1.3 μM) for 24 h, gave green fluorescence due to dissipation of mitochondrial potential with JC-1 dye.
Conclusions: Chondroitin AC lyase (*Ps*PL8A) displayed anti-tumor potential against human melanoma SK-Mel 28 and fibrosarcoma HT-1080 cell lines, while the mouse fibroblast L929 cells were unaffected.

Introduction

Glycosaminoglycans (GAGs) are the natural heteropolysaccharides that are present in mammalian tissue [1]. Proteoglycans (PGs) are glycoconjugates consisting of protein and polysaccharide GAGs. PGs are generally found on the cell surface, extracellular matrix (ECM) and in basement membranes of animal tissues [2]. Chondroitin sulphate (CS) are the GAG chains that bound to serine residue of the protein core linked through a tetrasaccharide linkage region consisting of xylose-galactose-galactose-glucuronic acid and forming PG [3]. CS is involved in various biological functions including cell migration, proliferation, microbial recognition, adhesion, pathogenesis, cell-matrix interactions, chemokine and cytokine activation etc. [4]. GAGs are highly charged linear chains of polysaccharides. In the recent years, cell biology studies have revealed that the altered structure of glycosaminoglycans in several diseases indicate their importance as biomarkers for disease diagnosis and progression, as well as pharmacological targets [1,5]. Chondroitin sulphate proteoglycans (CSPGs) are able to regulate key cellular processes, including proliferation, apoptosis, migration, adhesion and invasion. Versican and decorin are the major CSPGs and are over-expressed in the stroma of a wide variety of malignant tumors, including osteosarcoma, testicular tumors, breast, pancreatic and colon cancer [6,7].

The enzymes capable of degrading GAGs have been studied in order to understand the structure of GAGs and harness their therapeutic effects by manipulating cell signaling, differentiation, migration and adhesion [8,9]. Chondroitin sulphate lyases have been reported to exhibit the functions such as the inhibition of cell proliferation, differentiation and migration and some possible functions such as antitumor, pathogenic infection control, wound repair and neuro-generation. [8,10,11]. PGs are involved in cellular communication and cancer biology. Metastasis of tumors involves a complex sequence of events, called as the “metastatic cascade” [12]. Chondroitin sulphate chains as a part of PGs have important role in the process of tumor growth and metastasis [12–14]. Chondroitin sulphate proteoglycan 4 (CSPG4) was identified as a

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https://doi.org/10.1016/j.pharep.2018.10.009
1734-1140/

highly immunogenic tumor antigen on the surface of melanoma cells and is associated with melanoma tumor formation and its progression [15]. CSPG4 promotes tumor growth by associating with cell surface receptor proteins and receptor tyrosine kinase (RTK) and extracellular signal-regulated kinase (ERK) 1 and 2 pathways [15]. Chondroitin AC lyase (*Ps*PL8A) from *Flavobacterium heparinum* inhibited melanoma (SK-Mel2) invasion and proliferation as well as endothelial proliferation and angiogenesis [14]. Recruitment of CS lyase for meddling with cancer cell surface receptors can offer exciting therapeutic possibilities. These observations have prompted the researchers to explore the possible role of chondroitin sulphate lyases in understanding the cancer biology. *Ps*PL8A degrades the CS chains and hence can be potentially utilized to treat and control the progression of melanoma and fibrosarcoma. In the present study the effect of *Ps*PL8A on human melanoma and fibrosarcoma cell lines were studied. The effect of *Ps*PL8A was explored on mouse fibroblast (L929) cells, melanoma (SK-Mel-28) and fibrosarcoma (HT-1080) cell lines. The mode of cell death and changes in mitochondrial membrane potential was studied after treatment of cancer cells with *Ps*PL8A.

Material and methods

Chemicals and reagents

Dulbecco's Modified Eagle Medium (DMEM) low glucose medium, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and Mitochondrial staining kit were procured from Sigma-Aldrich, USA. Fetal bovine serum (FBS), 50 μg/ml streptomycin and 50 IU/ml penicillin were purchased from Gibco, USA. Phosphate buffer saline (PBS) and Trypsin-EDTA solution were purchased from Hi-Media Pvt. Ltd., India. FITC (Fluorescein isothiocyanate)-Annexin V and Propidium iodide (PI) cell apoptosis kit were purchased from Invitrogen, Ltd. UK.

Expression and purification of Ps*PL8A*

The gene encoding *Ps*PL8A cloned in pET28a(+) and expressed in *E. coli* BL21 (DE3) cells as described earlier [16] was used in the present study. The *Ps*PL8A was purified by immobilized metal ion affinity chromatography and dialysed in Tris-HCl buffer, pH 7.2. The purity of protein was checked by running a 10.5% (w/v) SDS gel. *Ps*PL8A was filtered through a 0.22 μm filter (Millipore, USA) prior to use in all experiments.

Mammalian cell culture and maintenance of cell lines

The mouse fibroblast cell line (L929), Human melanoma (SK-Mel28) and Fibrosarcoma HT-1080 were procured from National Centre for Cell Science (NCCS), Pune, India. All cell lines were cultured in DMEM low glucose medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (Gibco, USA), 50 μg/ml streptomycin and 50 IU/ml penicillin (Gibco, USA) incubated at 37 °C under 5% CO_2. The cells were grown in T-25 and T-75 flasks at 37 °C under 5% CO_2 in incubator. After the cells reached confluent stage, they were washed several times with 1XPBS (pH 7.4) and harvested with 0.25% (v/v) trypsin-EDTA solution for further experiments.

In vitro *cell proliferation assay of L929, SK-Mel28 and HT-1080 cells with* Ps*PL8A*

The effect of *Ps*PL8A enzyme on L929, SK-Mel28 and HT-1080 cells viability was analysed by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [17]. The cells were seeded at a density of 2×10^4 cells/well in 96 well plates which were incubated at 37 °C under 5% CO_2, overnight for cell adherence. After the incubation, media was completely removed and the L929, SK-Mel 28 and HT-1080 cells were gently washed with 1x PBS (pH 7.4). After the PBS wash cells were exposed to different concentrations varying between (0.0013 μM -1.3 μM) of the *Ps*PL8A enzyme dissolved in serum-free DMEM medium (incomplete). The incomplete DMEM medium without *Ps*PL8A enzyme was used as a negative control. The plates were incubated at 37 °C in 5% CO_2 for 12 h and 24 h. MTT assay was carried out after 12 h and 24 h by removing the medium and washing the wells with 200 μl of 1x PBS pH 7.4. A 100 μl of MTT (0.5 mg/ml) was added to each well and plate was incubated at 37 °C in 5% CO_2 for 4 h. After the incubation, MTT was removed from the wells and the formazan formed was dissolved by adding 100 μl of dimethyl sulfoxide. The absorbance at 570 nm (A_{570}) was monitored by a 96-well microplate reader (Tecan, Infinite 200 Pro, Switzerland). The cell viability (%) was calculated [18].

$$\text{Viability (\%)} = (N_t/N_c) \text{ X } 100$$

Where, N_t is the absorbance of treated cells and N_c is the absorbance of untreated cells.

Mode of cell death using Annexin-FITC assay by fluorescence microscopy

The mode of cell death of SK-Mel 28 and HT-1080 cells after *Ps*PL8A enzyme treatment was analysed by staining the cells with Annexin-V-FITC and Propidium Iodide (PI) staining kit from Invitrogen, Ltd. UK. The cells were seeded at the density of 2×10^6 cells in a 24 well cell culture plate. After overnight incubation, the cells were treated with *Ps*PL8A enzyme at a concentration of 1.3 μM and incubated at 37 °C in 5% CO_2 incubator for 24 h. The cells in only incomplete DMEM medium were used as control. After treatment, the cells were washed with cold 1x PBS solution and incubated with 1x Annexin-binding buffer, pH 7.4. The cells were treated with PI (100 μg/ml) and Annexin-V-FITC dye at the concentration as mentioned by manufacturer (Invitrogen, Ltd. UK) for 15 min at 25 °C and kept in dark. The stain was removed and 400 μl of 1x Annexin-binding buffer was added and cells were observed under fluorescence microscope (Nikon, TS2R, Japan) with UV excitation/emission at 494/518 nm and 535/617 nm for annexin V-FITC and PI, respectively. The cells showing green fluorescence were considered as apoptotic and red cells were dead or necrotic.

Apoptosis analysis of SK-Mel 28 and HT-1080 cell lines by flow cytometry

Analysis of apoptotic/necrotic features by flow cytometry was performed using an Annexin V-fluorescein isothiocyanate (FITC) apoptosis kit from Invitrogen, Ltd., UK. The untreated and *Ps*PL8A enzyme (1.3 μM) treated SK-Mel 28 and HT-1080 cells at a cell density of 2×10^6 cells/ml were used. The staining with Annexin V-FITC and PI was performed according to the instructions given by the manufacturer as mentioned in Section in Mode of cell death using Annexin-FITC assay by Fluorescence Microscopy. The cells were analyzed for apoptosis/necrosis by flow cytometery. The fluorescence of the stained cells was detected with a flow cytometer (BD bioscience, BD FACSCalibur, USA) and the results were analyzed by using CellQuest software.

Mitochondrial cell potential analysis of SK-Mel 28 and HT-1080 cells by fluorescence microscopy

The dissipation of the mitochondrial electrochemical potential gradient (Dy) is known as an early event in apoptosis. Mitochondrial cell potential analysis was carried out by using mitochondrial electrochemical gradient kit from Sigma Aldrich, USA. The JC-1

staining solution was prepared as per manufacturer instruction (Sigma Aldrich, USA). The growth medium was aspirated from the flask and the cells were overlaid with the 400 μl working solution of JC-1 dye. The cells were incubated for 20 min at 37 °C in a humidified atmosphere containing 5% CO_2. The staining solution was aspirated and the cells were washed three times with ice cold 1X JC-1 buffer and then twice by DMEM complete medium. The cells were overlaid with the growth medium and were observed under fluorescence microscope (Nikon, TS2R, Japan) under 20X magnification. The cells treated with 0.1 μM valinomycin were used as positive control.

Mitochondrial cell potential analysis of SK-Mel 28 and HT-1080 cells by flow cytometry

Mitochondrial cell potential analysis for HT-1080 and SK-Mel28 was performed by flow cytometry using JC-1 mitochondrial staining kit from Sigma-Aldrich, USA. SK-Mel 28 and HT-1080 cells were seeded at density of 2×10^6 cells/ml. The untreated and *Ps*PL8A enzyme (1.3 μM) treated cells were used for analysis. The cells were stained with JC1 dye according to the instructions provided by the kit manufacturer (Sigma-Aldrich, USA). The fluorescence of the stained cells was detected with a flow cytometer (BD bioscience, BD FACSCalibur, USA) in FL-1 (Green) and FL-2 (Red) channels and the results were analysed by using Flow Jo software.

Statistical analysis

All experiments were performed in triplicates (n = 3). The results were presented as mean of three determinations ± SD (standard deviation).

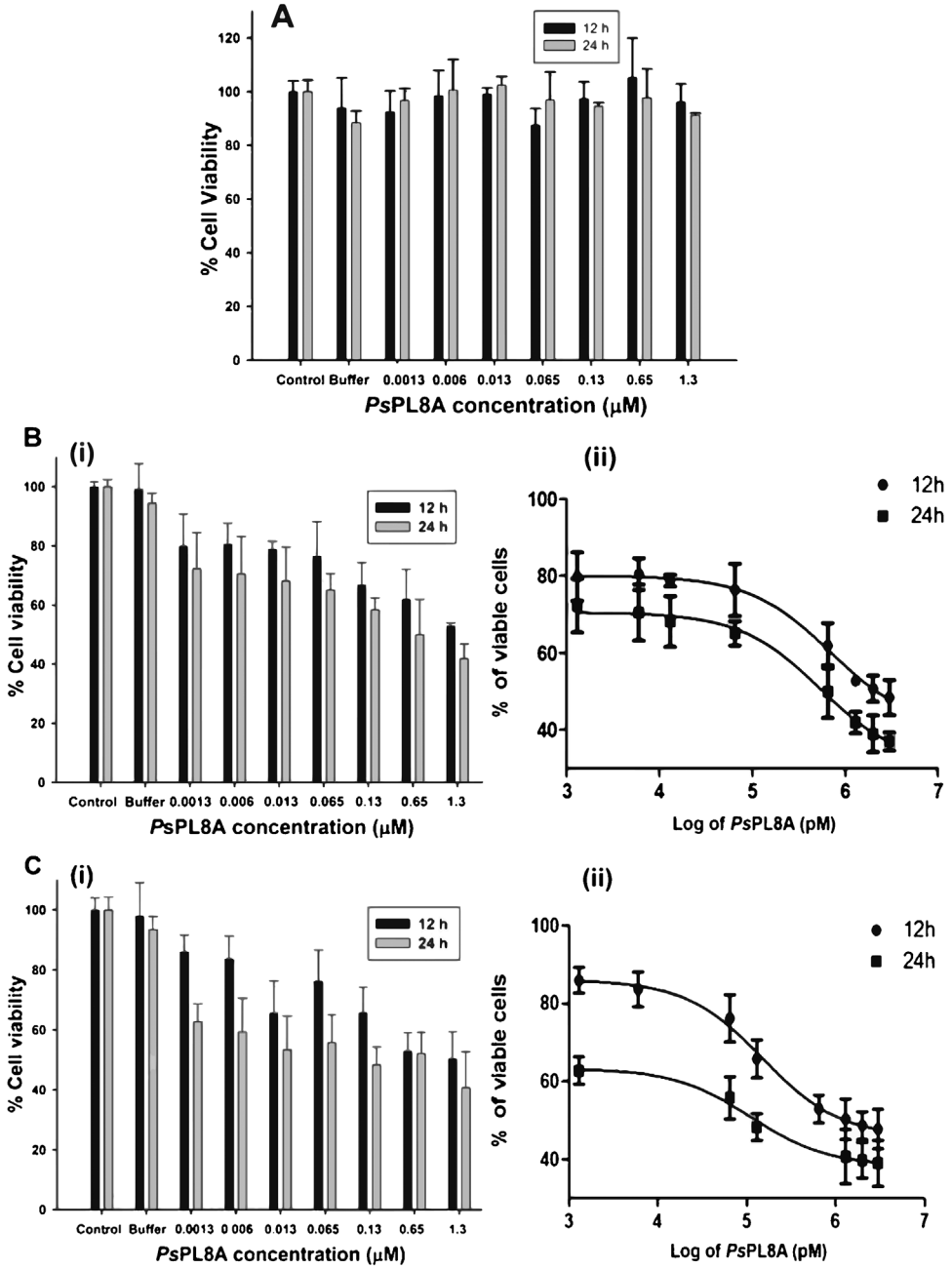

Fig. 1. *In vitro* cell proliferation assay (MTT) with varying concentration (0.0013–1.3 μM) of *Ps*PL8A. (A) Normal mouse fibrobast L929 cells, (B) Human melanoma SK-Mel 28 cells (i) MTT assay, (ii) IC_{50} graph (C) Human fibrosarcoma HT-1080 cells (i) MTT assay, (ii) IC_{50} graph.

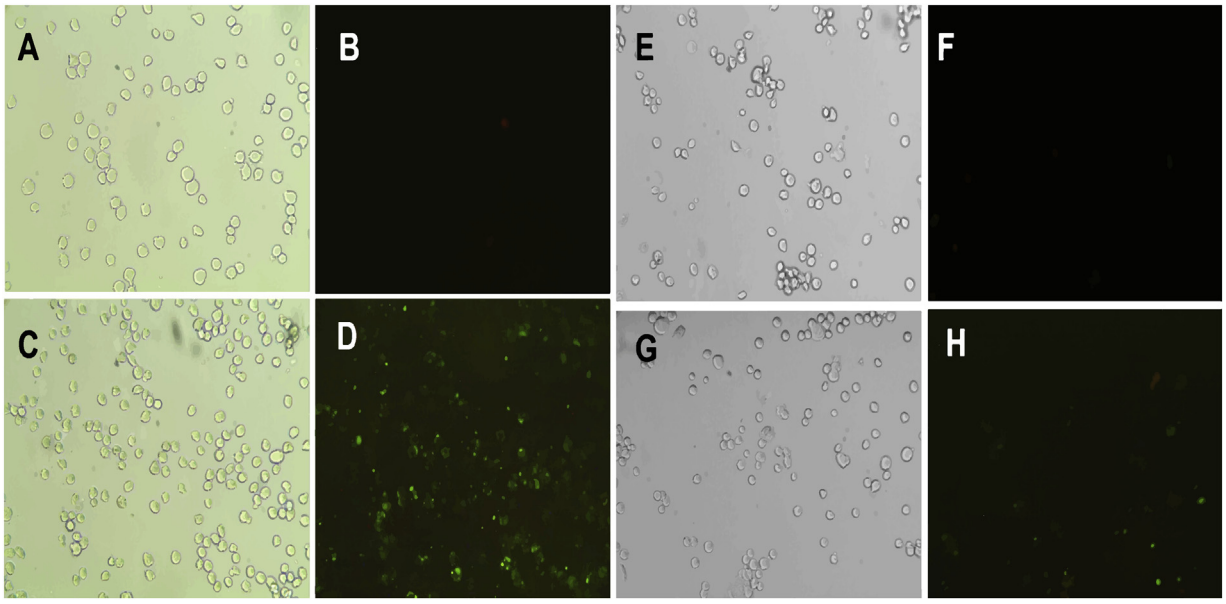

Fig. 2. Apoptosis analysis of SK Mel-28 cells by fluorescence (20X magnification). The untretaed cells under (A) Phase contrast microscope and (B) fluorescence microscope. The *Ps*PL8A enzyme (1.3 μM) treated SK-Mel 28 cells under (C) Phase contrast microscope (D) fluorescence microscope. Apoptosis analysis of HT-1080 cells by fluorescence (20X magnification). The untreated cells under (E) Phase contrast microscope and (F) fluorescence microscope. Treated HT-1080 cells treated with *Ps*PL8A enzyme (1.3 μM) under (G) Phase contrast microscope (H) fluorescence microscope.

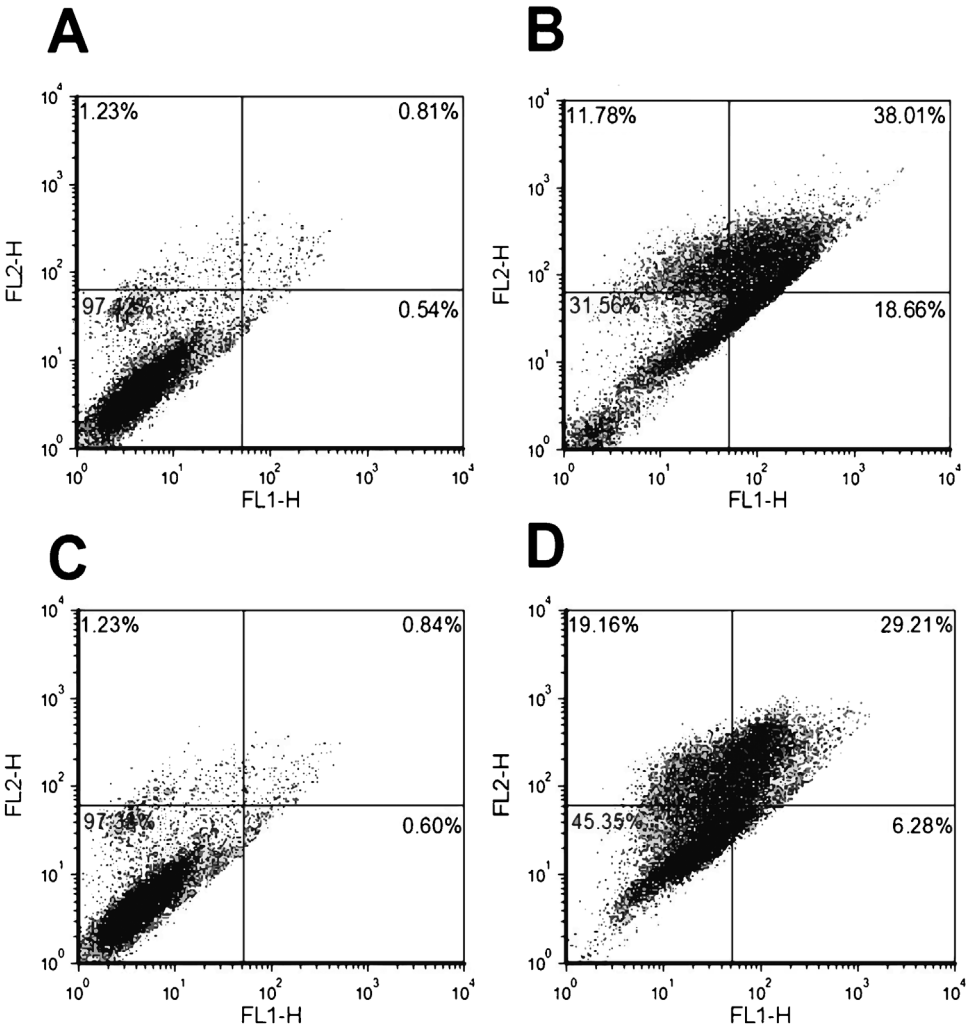

Fig. 3. Apoptosis analysis of SK Mel-28 cells (A) untreated cells (B) *Ps*PL8A enzyme (1.3 μM) treated cells and of HT-1080 cells (C) untreated cells (D) *Ps*PL8A enzyme (1.3 μM) treated cells by flow cytometry.

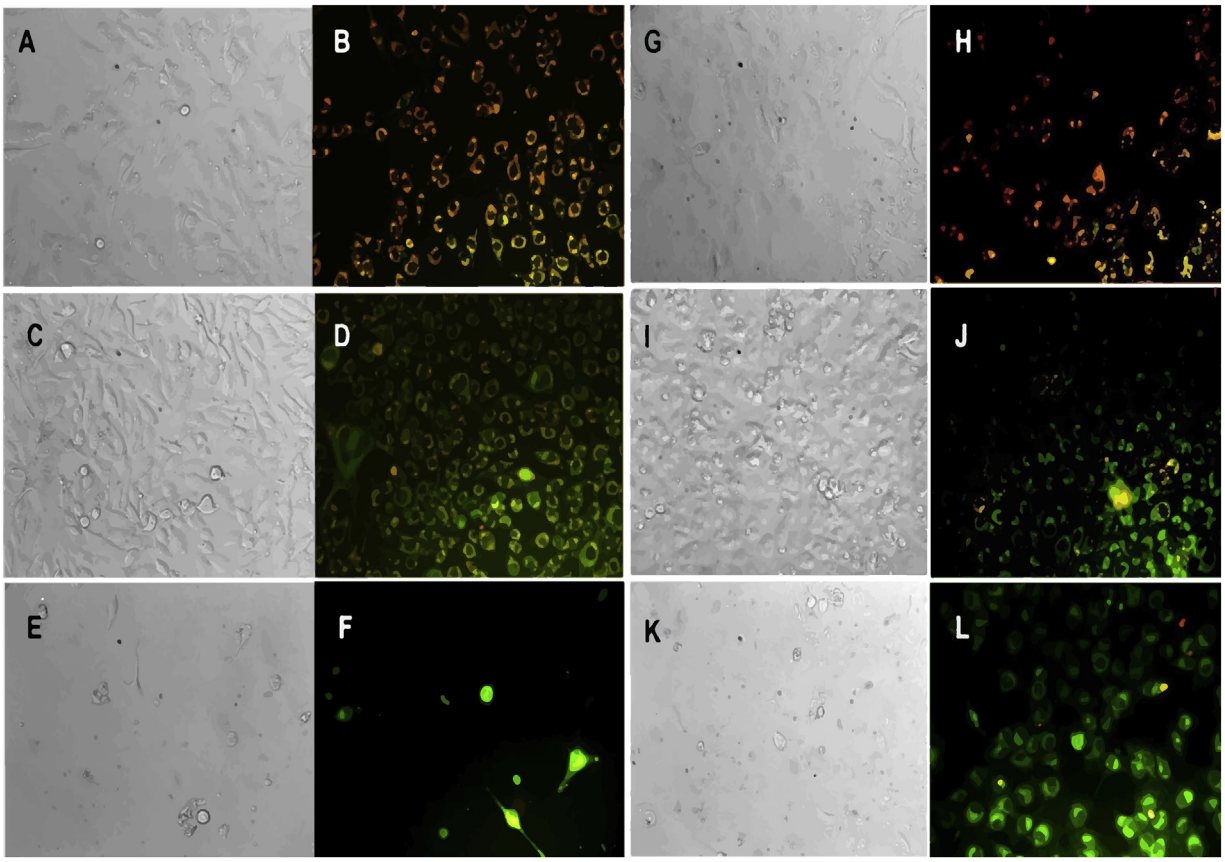

Fig. 4. Mitochondrial cell potential analysis of untreated SK-Mel 28 cells (A) Phase contrast microscope (B) fluorescence microscope. SK-Mel 28 cells treated with 1.3 μM *Ps*PL8A enzyme under (C) Phase contrast microscope (D) fluorescence microscope. Positive control of SK-Mel 28 cells treated with 0.1 μM Valinomycin under (E) Phase contrast microscope (F) under fluorescence microscope. Untreated HT-1080 cells (G) Phase contrast microscope (H) fluorescence microscope. HT-1080 cells treated with 1.3 μM *Ps*PL8A enzyme under (I) Phase contrast microscope (J) fluorescence microscope. Positive control of HT-1080 cells treated with 0.1 μM Valinomycin under (K) Phase contrast microscope (L) under fluorescence microscope.

Result and discussion

In vitro *cell proliferation assay of L929, SK-Mel28 and HT-1080 cells treated with* Ps*PL8A*

The effect of *Ps*PL8A in the concentration range, 0.0013 μM–1.3 μM was studied on L929 cells, SK-Mel 28 and HT-1080 cells. The L929 cells treated with *Ps*PL8A showed no significant change in the proliferation of the cells displaying 95–98% cell viability (Fig. 1A). *Ps*PL8A imparts no cytotoxicity to the normal fibroblast cells with increase in time as well as concentration. The proliferation of SK-Mel 28 and HT-1080 cell lines were studied after treatment with *Ps*PL8A (0.0013 μM–1.3 μM). An overall 58% and 59% inhibition of SK-Mel 28 [Fig. 1B(i)] and HT-1080 [Fig. 1C(i)] cells proliferation, respectively was observed with 1.3 μM of *Ps*PL8A after 24 h of incubation. The IC_{50} values were calculated using GraphPad Prism software. The IC_{50} value for SK-Mel 28 cell lines at 12 h and 24 h were 0.68 μM and 0.54 μM, respectively (Fig. 1B(ii)]. While, for HT-1080 cells the IC_{50} value at 12 h and 24 h were 0.15 μM and 0.11 μM, respectively [Fig. 1C(ii)]. *Ps*PL8A and chondroitin B lyase inhibited the proliferation of SK-Mel 2 human melanoma cells [12]. *Ps*PL8A from *Flavobacterium heparinum* inhibited melanoma invasion and proliferation as well as endothelial proliferation and angiogenesis [12]. Melanoma cells expresses CS proteoglycan 4 (CSPG4). CSPG4 is the transmembrane protein which traverses cell membrane and modulates integrin function and enhances growth factor receptor-regulated pathways including extracellular signal-regulated protein kinases (ERK) 1,2 [15].

Apoptosis analysis of SK-Mel 28 and HT-1080 cell lines by fluorescence microscopy

Apoptosis is the mode of cell death that is distinguished from necrosis by characteristics morphological and biochemical changes. These changes include compaction and fragmentation of the nuclear chromatin, shrinkage of the cytoplasm and loss of membrane asymmetry. The apoptotic/necrotic mode of cell death was analysed by staining the untreated and *Ps*PL8A treated SK-Mel 28 and HT-1080 cells with Annexin V-FITC and PI. The untreated SK-Mel 28 (Fig. 2A and B) and HT-1080 (Fig. 2E and F) cells showed no or faint green fluorescence after staining, demonstrating the viability of cells. The *Ps*PL8A (1.3 μM) treated SK-Mel 28 (Fig. 2C and D) and HT-1080 (Fig. 2G and H) cells showed strong green fluorescence of cell membrane with Annexin-V FITC displaying membrane blebbing. The Annexin-V FITC binds strongly in the *Ps*PL8A treated cells due to the loss of integrity of cellular membrane confirming the presence of apoptotic bodies after the *Ps*PL8A treatment. The dead cells showed both the membrane staining by Annexin-V as well as nuclear staining by propidium iodide.

Apoptosis analysis of SK-Mel 28 and HT-1080 cell lines by flow cytometry

The mode of cell death after *Ps*PL8A (1.3 μM) treatment was also studied by flow cytometric analysis of SK-Mel 28 and HT-1080 cells after staining with annexin V-FITC and PI. The untreated

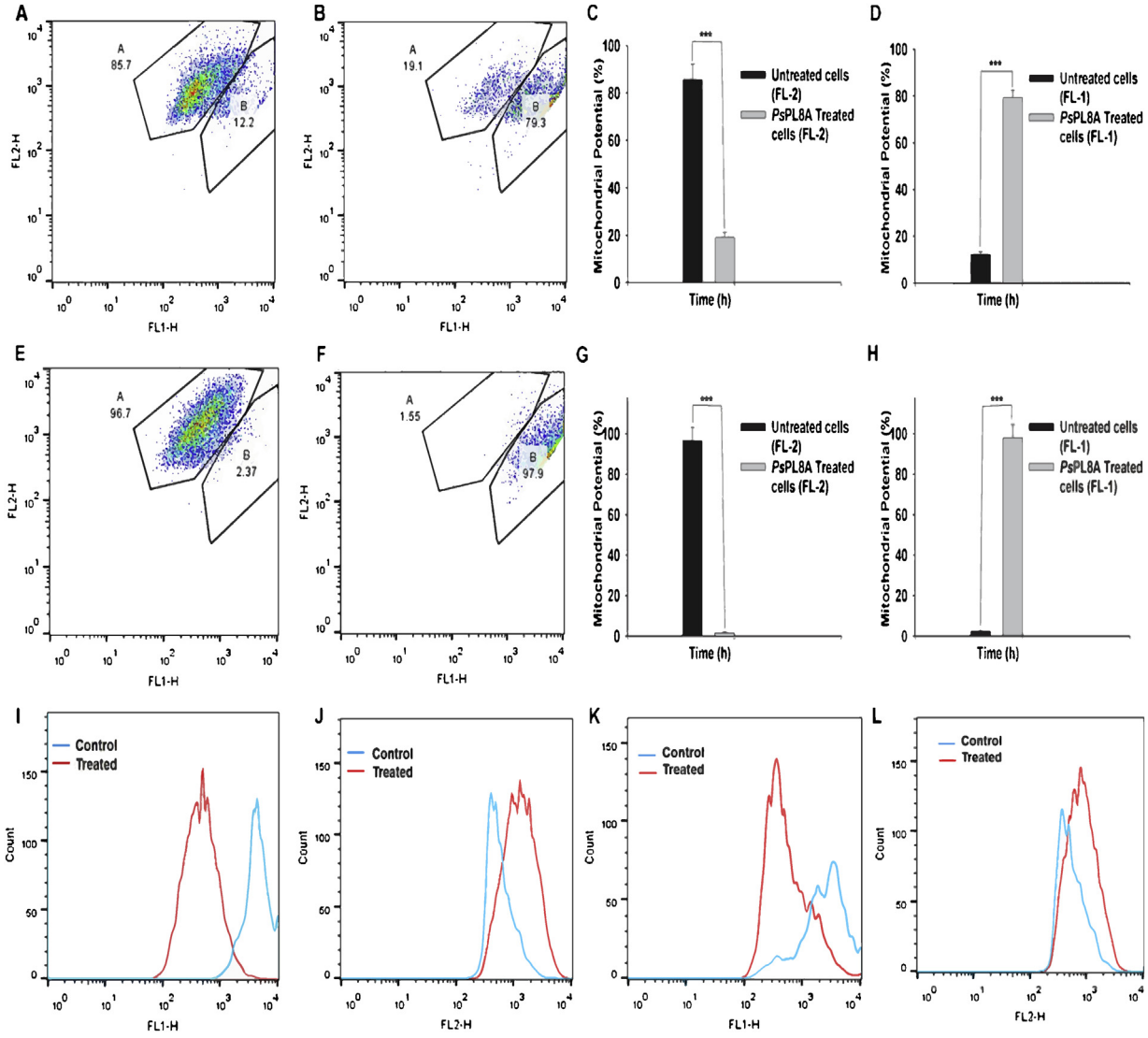

Fig. 5. Flow cytometric analysis of mitochondrial cell potential of SK-Mel 28 cells (A) Untreated cells (B) Cells treated with *Ps*PL8A enzyme (1.3 μM) for 24 h. (C) Bar graph for FL-2 channel for untreated and treated SK-Mel 28 cells, (D) Bar graph for FL-1 channel for untreated and treated SK-Mel 28 cells. Flow cytometric analysis of mitochondrial cell potential of HT-1080 cells (E) Untreated cells (F) cells treated with *Ps*PL8A enzyme (1.3 μM) for 24 h, (G) Bar graph for FL-2 channel for untreated and treated HT-1080 cells, (H) Bar graph for FL-1 channel for untreated and treated HT-1080 cells, Histogram analysis of SK-Mel 28 cells (I) FL-1 histogram (J) FL-2 histogram and HT-1080 cells (K) FL-1 histogram (L) FL-2 histogram. The Bar graph represents means ± SD of three independent experiments using unpaired *t*-test in GraphPad Prism 5 statistical software. The differences were considered to be significant and represented in bar graph with letters $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.0001$ (***) and ns = non-significant *p* value.

(negative control) SK-Mel 28 (Fig. 3A) and HT-1080 cells (Fig. 3C) did not show staining with Annexin V-FITC/PI and hence the live cell population was present in the lower left quadrant. SK-Mel 28 (Fig. 3B) and HT-1080 cells (Fig. 3D) treated with *Ps*PL8A (1.3 μM) for 24 h showed Annexin V-FITC/PI staining, indicating cell death majorly by apoptosis. The flow cytometry analysis showed that *Ps*PL8A treated SK-Mel 28, results in overall 56.6% apoptosis with 38.01% cells in late apoptotic stage, 18.66% in the early apoptotic stage and 11.78% necrotic cells (Fig. 3B). *Ps*PL8A treated HT-1080 cells displayed overall 35.49% apoptosis with 29.21% cell in late apoptotic stage, 6.28% in early apoptotic stage and 19% cells in necrotic stage (Fig. 3D). The results of annexin-V FITC and PI staining by both microscopic analysis and flow cytometry suggested that *Ps*PL8A treatment induce the cell death in human melanoma and fibrosarcoma cells by apoptosis. Apoptosis of SK-Mel 2 cells by *Ps*PL8A from *Flavobacterium heparinum* was also reported in a previous study [12].

Analysis of mitochondrial cell potential of SK-Mel 28 and HT-1080 cells after treatment with Ps*PL8A by fluorescence microscopy*

The $\Delta\psi_m$ is a key indicator of cell health or injury. The dissipation of mitochondrial potential is one of the early event in apoptosis of cells. Any event that dissipates the mitochondrial membrane potential prevents the accumulation of the JC-1 dye in the mitochondria and thus, dye is dispersed throughout the entire cell leading to a shift from red (J-aggregates) to green fluorescence (JC-1 monomers) [19]. The effect of *Ps*PL8A treatment on SK-Mel 28 and HT-1080 was studied using JC-1 dye. The untreated SK-Mel 28 (Fig. 4A and B) and HT-1080 cells (Fig. 4G and H) were used as negative controls which gave red

fluorescence with JC-1 dye. This indicated the maintenance of mitochondrial potential gradient among untreated cells. The SK-Mel 28 (Fig. 4C and D) and HT-1080 cells (Fig. 4I and J) upon treatment with *Ps*PL8A (1.3 μM) for 24 h, gave green fluorescence due to dissipation of mitochondrial potential with JC-1 dye. The positive control of SK-Mel 28 and HT-1080 cells treated with valinomycin was kept, where it dissipates the mitochondrial electrochemical potential by permeabilizing the mitochondrial membrane for K^+ ions. The SK-Mel 28 (Fig. 4E and F) and HT-1080 cells (Fig. 4K and L) treated with 0.1 μM of valinomycin showed green fluorescence.

Analysis of mitochondrial cell potential analysis of SK-Mel 28 and HT-1080 cells after treatment with Ps*PL8A by flow cytometry*

The mitochondrial cell potential of untreated and *Ps*PL8A treated cells was also analysed by flow cytometry (Fig. 5). The untreated SK-Mel (Fig. 5A) and HT-1080 cells (Fig. 5E) gave strong red signal due to the presence of JC-1 aggregates. The treatment of *Ps*PL8A enzyme showed a shift towards green fluorescence for 79% population of SK-Mel 28 cells (Fig. 5B) and 98% for HT-1080 cells (Fig. 5F). The FL-1 (Fig. 5I) and FL-2 (Fig. 5J) histogram analysis of SK-Mel 28 gave FL-1 mean/FL-2 mean ratio for normal cells as 0.84 and for treated cells as 4.50. Similarly, FL-1 (Fig. 5K) and FL-2 (Fig. 5L) histogram analysis of HT-1080 gave FL-1 mean/FL-2 ratio for normal cells as 0.38 and treated cells 6.68. Fig. 5C and D and G and H represents bar graph for statistical analysis of FL-2 and FL-1 channels for SK-Mel28 cells and HT-1080 cells, respectively. The results of JC-1 dye staining suggested that *Ps*PL8A treatment causes collapse of the mitochondrial membrane potential and eventually apoptosis in human melanoma and fibrosarcoma cells. This disruption of mitochondrial potential by *Ps*PL8A treatment signifies the early event leading to cell death by apoptosis. The data of mitochondrial cell potential further contended the Annexin-V FITC results for apoptosis mode of cell death.

CS affects the cell proliferation of melanoma predominantly by their relative abundance and their interaction with the various cell receptors [12,15]. Previous studies suggest that various CSPGs regulate basic fibroblast growth factor, hepatocyte growth factor and interferon- γ with their respective receptors [12,20]. Cluster of differentiation 44 (CD44) and CSPG4 are the two cell surface antigens having associated CSPGs that are involved in tumor growth and invasion of melanoma [15,21]. CSPG4 is also associated with the progression of other cancers including oligodendrocytomas, gliomas, triple-negative breast carcinomas and squamous cell carcinoma [15]. Removing cellular and extracellular chondroitin sulfate can also reduce the cell activation through signalling pathways, which occurs during adhesion [12]. The present study gave an insight to explore the possible role of *Ps*PL8A in understanding the cancer biology. *Ps*PL8A degrading the CS chains can be potentially utilized to treat and control the progression of melanoma and fibrosarcoma.

Conclusion

The anti-proliferative effect of *Ps*PL8A on SK-Mel 28 and HT-1080 cell lines were determined in the present study. The treatment of mouse fibroblast L929 cell lines with *Ps*PL8A imparts no cytotoxicity. The SK-Mel 28 and HT-1080 cell lines treated with *Ps*PL8A showed inhibition of cell proliferation. Mode of cell death studied by Annexin-V FITC using flow cytometry and fluorescence microscopy concluded the cell death by apoptosis. The SK-Mel 28 and HT-1080 cells treated with *Ps*PL8A stained with JC-1 dye and analysed by fluorescence microscopy and flow cytometry displayed green fluorescence indicating dissipation of mitochondrial potential and confirming apoptosis. *Ps*PL8A inhibiting the melanoma and fibrosarcoma cell proliferation and leading their apoptotic mode of cell death prospects it as a potential agent for therapeutic approach for cancer treatment.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The research work and AR was supported by Council of Scientific and Industrial Research (CSIR) project no. 37(1672)/16/EMR-II to AG.

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