

# Inhibition of Human Group IIA-Secreted Phospholipase A<sub>2</sub> and THP-1 Monocyte Recruitment by Maslinic Acid

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**Abstract** Maslinic acid is a natural pentacyclic triterpenoid which has anti-inflammatory properties. A recent study showed that secretory phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) may be a potential binding target of maslinic acid. The human group IIA (hGIIA)-sPLA<sub>2</sub> is found in human sera and their levels are correlated with severity of inflammation. This study aims to determine whether maslinic acid interacts with hGIIA-sPLA<sub>2</sub> and inhibits inflammatory response induced by this enzyme. It is shown that maslinic acid enhanced intrinsic fluorescence of hGIIA-sPLA<sub>2</sub> and inhibited its enzyme activity in a concentration-dependent manner. Molecular docking revealed that maslinic acid binds to calcium binding and interfacial phospholipid binding site, suggesting that it inhibit access of catalytic calcium ion for enzymatic reaction and block binding of the enzyme to membrane phospholipid. The hGIIA-sPLA<sub>2</sub> enzyme is also responsible in mediating monocyte recruitment and differentiation. Results showed that maslinic acid inhibit hGIIA-sPLA<sub>2</sub>-induced THP-1 cell differentiation and migration, and the effect observed is specific to hGIIA-sPLA<sub>2</sub> as cells treated with maslinic acid alone did not significantly

affect the number of adherent and migrated cells. Considering that hGIIA-sPLA<sub>2</sub> enzyme is known to hydrolyze glycerophospholipids present in lipoproteins and cell membranes, maslinic acid may bind and inhibit hGIIA-sPLA<sub>2</sub> enzymatic activity, thereby reduces the release of fatty acids and lysophospholipids which stimulates monocyte migration and differentiation. This study is the first to report on the molecular interaction between maslinic acid and inflammatory target hGIIA-sPLA<sub>2</sub> as well as its effect towards hGIIA-sPLA<sub>2</sub>-induced THP-1 monocyte adhesive and migratory capabilities, an important immune-inflammation process in atherosclerosis.

**Keywords** Phospholipase A<sub>2</sub> · Lipases · Immunology · Physiology · Inflammation · Physiology

## Abbreviations

hGIIA-sPLA <sub>2</sub>	Human Group IIA-secreted phospholipase A <sub>2</sub>
β-Py-C10-PG	1-Hexadecanoyl-2-(1-pyrenedecanoyl)-sn-glycero-3-phosphoglycerol Ammonium salt
PC	Phosphatidylcholine
NEFA	Non-esterified fatty acid
NF-κB	Nuclear factor-kappa B
PKC	Protein kinase C

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## Introduction

According to recent statistics from World Health Organization (WHO), it was shown that the mortality rate for people suffering from cardiovascular disease is higher than any other diseases [1]. Atherosclerosis is the principal cause for cardiovascular diseases such as myocardial infarction and

stroke. It is characterized by accumulation of lipids and cholesterol within the arterial walls [2]. Blood monocytes that are recruited to the vessel wall are the precursors of the lipid-laden macrophages that form fatty streaks—one of the earliest manifestations of atherosclerosis. In the last decade there have been a great deal of research on anti-inflammatory therapies being developed for clinical intervention of atherosclerosis. Maslinic acid (Pubchem CID: 73659) is a natural pentacyclic triterpenoid found in medicinal plants. The anti-inflammatory effect of maslinic acid has been confirmed in a number of studies, demonstrating that it reduces susceptibility of plasma and hepatocyte membranes to lipid peroxidation [3] and inhibits production of nitric oxide, tumor necrosis factor alpha, and cyclooxygenase-2 [4, 5]. In a study conducted by Moneriz *et al.* (2011), secreted phospholipase A<sub>2</sub> (sPLA<sub>2</sub>) was discovered as one of the novel putative targets of maslinic acid [6].

Mammalian sPLA<sub>2</sub> enzymes are classified based on their structural features into one conventional group consisting of Group I, II, V, X and otoconin-90, and two other atypical groups which include Group III and XII [7]. Individual sPLA<sub>2</sub> function as an enzyme targeting membrane phospholipids, including cellular membranes, non-cellular lipids such as surfactant and lipoproteins, foreign phospholipids in bacterial membranes and dietary phospholipids, or serving as a ligand for membrane-bound and soluble receptors [8]. So far, only human Group IIA (hGIIA)-sPLA<sub>2</sub> subtype is found in human sera in different physiological and pathological conditions [9, 10]. The levels of hGIIA-sPLA<sub>2</sub> in the circulatory system or inflammatory exudates corresponds to the degree of severity in the inflammatory diseases and tissue injuries [11, 12].

hGIIA-sPLA<sub>2</sub> was also detected in the vascular smooth muscle cell of the media and the intima layers as well as in the macrophage-rich region of atherosclerotic plaques. It has been reported that hGIIA-sPLA<sub>2</sub> induce monocyte differentiation and migration that leads to inflammatory reaction in atherosclerotic lesion [13]. Another research described that hGIIA-sPLA<sub>2</sub> plays a role in modifying the lipoproteins and release lipid mediators in the arterial walls [14]. The modification of lipoproteins also leads to the expression of chemokines as a result of inflammatory reaction which then induces the recruitment of monocyte towards the intima layer where differentiation of monocyte occurs [15].

Given that sPLA<sub>2</sub> are an emerging class of mediators for inflammation and it possess the capacity to hydrolyze lipoproteins and release lipid mediator from cellular membranes, this study sought to investigate the interaction between maslinic acid and hGIIA-sPLA<sub>2</sub>. The interaction was examined by monitoring changes in the enzyme's relative intrinsic fluorescence and its inhibitory effect on the enzyme hydrolytic activity. The anti-inflammatory effect

of maslinic acid targeting hGIIA-sPLA<sub>2</sub> was performed in THP-1 promonocytic cells by determining its effect in suppressing hGIIA-sPLA<sub>2</sub>-induced monocyte-macrophage differentiation and migration.

## Materials and Methods

Maslinic acid was purchased from Cayman Chemicals; Fatty-acid free BSA was from Sigma. hGIIA-sPLA<sub>2</sub> protein was expressed, purified, and quantified as described [16]. The fluorescent substrate for PLA<sub>2</sub> assay, 1-hexadecanoyl-2-(10-pyrenedecanoyl)-sn-glycero-3-phosphoglycerol, ammonium salt ( $\beta$ -py-C10-PG) was from Molecular Probes (Eugene).

## Cell Culture

THP-1 cells were cultured at 37 °C under a 5 % CO<sub>2</sub> atmosphere in RPMI 1640 (GIBCO) with 100 U/mL penicillin/streptomycin, 2 mM glutamine, 5 % fetal bovine serum and maintained at  $5 \times 10^5$  cells/mL.

## Fluorescence Spectroscopy

The relative intrinsic fluorescence intensity of hGIIA-sPLA<sub>2</sub> enzyme with and without maslinic acid was determined by fluorescence spectroscopy as described previously [17]. The hGIIA-sPLA<sub>2</sub> enzyme was dissolved in Phosphate Buffered Saline (PBS) buffer to a final concentration of 100  $\mu$ M. Maslinic acid solution (1 mM) was prepared in ethanol. A series of samples containing different amounts of maslinic acid and a constant amount of hGIIA-sPLA<sub>2</sub> were prepared in a 20:80 % ethanol: buffer mixture. The final concentrations of maslinic acid were 3.13, 6.25, 12.5, 25, 50, 100  $\mu$ M, while the hGIIA-sPLA<sub>2</sub> protein concentration was fixed at 100  $\mu$ M. Each dilution comprising 100  $\mu$ L was transferred to a black, flat-bottom 96-well microplate (Corning). Fluorescence was measured at 25 °C on a BMG FLUOstar OPTIMA Microplate Reader with Ex = 280 nm and Em = 340 nm.

## In Vitro Phospholipase Assay

PLA<sub>2</sub> activity was evaluated as previously described [18] with  $\beta$ -py-C10-PG used as a substrate. To each well of a 96-well microtiter plate, 97  $\mu$ L of solution A (27  $\mu$ M bovine serum albumin, 50 mM KCl, 1 mM CaCl<sub>2</sub>, 50 mM Tris-HCl, pH 8.0) and 3  $\mu$ L of maslinic acid or 3  $\mu$ L of DMSO only for negative control reactions were added. Solution B was delivered in 100- $\mu$ L portions to the wells and they have the same composition as Solution A and hGIIA-sPLA<sub>2</sub> enzyme (200 ng). Blank contain an additional 100- $\mu$ L

portion of Solution A minus the enzymes. The assay was initiated by adding 100  $\mu\text{L}$  of Solution C [4.2  $\mu\text{M}$  of  $\beta$ -py-C10-PG (molecular probes) vesicles in assay buffer] with a repeating micropipette to all wells. The fluorescence (excitation = 342 nm, emission = 395 nm) was read with the BMG FLUOstar OPTIMA Microplate Reader. The increase in fluorescence was continuously recorded for 1 min, and the enzyme activity was calculated based on the initial slopes of fluorescence *versus* time. Enzyme activity was normalized to the vehicle-treated controls.

### Molecular Docking

To understand the mechanism of action, inhibition of hGIIA-sPLA<sub>2</sub> by maslinic acid was studied *in silico* using computational methods to support the *in vitro* results. Maslinic acid was docked into the active site of hGIIA-sPLA<sub>2</sub> to determine their interaction. The crystal structure of hGIIA-sPLA<sub>2</sub> (PDB code: 3U8D) was retrieved from protein data bank ([www.rcsb.org](http://www.rcsb.org)). Protein structure is prepared and energy-minimized. Molecular docking is performed using CDOCKER program in Discovery Studio suite 4.0.

### THP-1 Cell Treatment

THP-1 cells ( $2 \times 10^5$  cells/mL) were seeded into a 24-well plate. The cells were either untreated (negative control), treated with 1  $\mu\text{g}/\text{mL}$  of hGIIA-sPLA<sub>2</sub> only (positive control) or treated with hGIIA-sPLA<sub>2</sub> in the presence of increasing concentrations of maslinic acid (5, 10, 20, and 50  $\mu\text{M}$ ). The cells were incubated in 5 % CO<sub>2</sub> incubator. After 24 h, the cell suspension were transferred to a microcentrifuge tube and spun at 14000 rpm for 10 min. The supernatant collected was kept at  $-20\text{ }^\circ\text{C}$  for the migration assay. The 24-well plate was then rinsed four times with PBS and the remaining adherent cells were used for adhesion assay.

### Adhesion Assay

The adherent cells were fixed with methanol for 15 min at room temperature. The fixed cells were then stained with Giemsa stain for 30 min and washed with PBS to remove excess stain. The plates were air dried and examined under the inverted microscope. The adhesion score was measured by counting the number of adhered THP-1 cells per six different microscopic fields under high power field.

### Migration Assay

The migration assay was performed in a six-well plate using tissue culture polycarbonate filter inserts (8  $\mu\text{m}$

pore, Corning, Costar, Cambridge, MA, USA). THP-1 cells ( $0.5 \times 10^6$  cells/mL) were resuspended in 1 mL of serum-free medium and loaded into the upper chamber of an insert. The lower chamber contained medium collected from treated cells (as indicated in THP-1 cell treatment). The cells were then incubated for 24 h in the 5 % CO<sub>2</sub> incubator. After the incubation period, the remaining cells in the upper chambers of the filter inserts were removed and washed with PBS. The migrated cells on the lower side of the filter insert were then fixed with 3.7 % of formaldehyde in PBS and permeabilized with methanol before Giemsa staining. The stained cells were counted in 6 different microscopic fields under a high power field.

### Statistical Analysis

Statistical analyses were conducted using SPSS (version 22). Results are expressed as means  $\pm$  SD for the number of independent experiments indicated. Statistical analysis involved the use of an ANOVA test, followed by a Tukey's test where several experimental groups were compared to the control group. A  $p < 0.05$  was considered statistically significant.

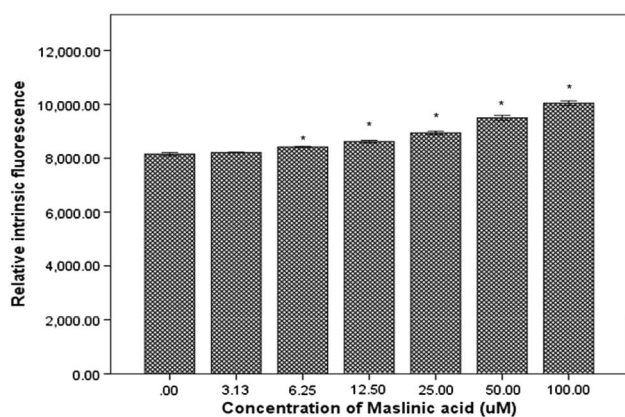
## Results

### Intrinsic Fluorescence Interaction of Maslinic Acid with hGIIA-sPLA<sub>2</sub> Enzyme

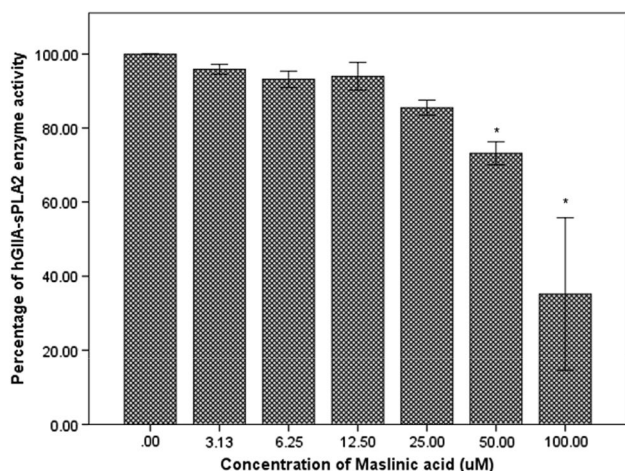
The change in intrinsic fluorescence reflects conformational change in protein due to substrate or ligand interaction. Thus it is expected that fluorescence measurement of hGIIA-sPLA<sub>2</sub> together with maslinic acid would provide information regarding the formation of enzyme-inhibitor complex. The results showed that maslinic acid enhanced intrinsic fluorescence of hGIIA-sPLA<sub>2</sub> (Fig. 1). Enhancement of relative fluorescence was observed with increasing concentration of maslinic acid (3.13–100  $\mu\text{M}$ ). The results indicated that maslinic acid may directly interact with hGIIA-sPLA<sub>2</sub> enzyme.

### Effect of Maslinic Acid on hGIIA-sPLA<sub>2</sub> Enzyme Activity

One important feature of the hGIIA-sPLA<sub>2</sub> enzyme is in hydrolyzing the *sn*-2 position of glycerophospholipid, producing arachidonic acid which is a precursor for eicosanoid components such as prostaglandins and leukotrienes. This enzyme has also been shown to hydrolyze phosphatidylcholine in lipoproteins to generate lysophosphatidylcholine and free fatty acids, thereby contributing to the accumulation of these lipid products at the atherosclerotic lesion site. It



**Fig. 1** Effect of maslinic acid on hGIIA-sPLA<sub>2</sub> intrinsic fluorescence. A series of samples containing different amounts of maslinic acid and a constant amount of hGIIA-sPLA<sub>2</sub> were prepared. The final concentrations of maslinic acid were 3.13, 6.25, 12.5, 25, 50 and 100 μM, while hGIIA-sPLA<sub>2</sub> protein concentration was fixed at 100 μM. Fluorescence was measured at 25 °C on a BMG FLUOstar OPTIMA Microplate Reader with *Ex* 280 nm and *Em* 340 nm. Values are expressed as means ± SD of three independent experiments. Asterisks represent  $p < 0.05$  compared to reactions containing hGIIA-sPLA<sub>2</sub> and ethanol only



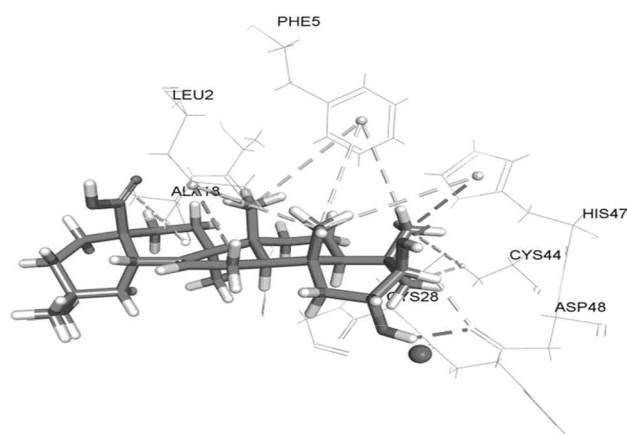
**Fig. 2** Effect of Maslinic acid on hGIIA-sPLA<sub>2</sub> enzymatic activity. The enzyme hGIIA-sPLA<sub>2</sub> was incubated with Py-PG-containing phospholipid vesicles (4.2 μM) in the presence of increasing concentrations of maslinic acid. Enzyme activity was determined according to the procedure described in the “Materials and Methods” section. Enzyme activity was normalized to negative control reactions containing hGIIA-sPLA<sub>2</sub> and β-py-C10-PG substrate only, and expressed as the mean percentage inhibition ± SD of three independent experiments. Asterisks represents  $p < 0.05$  compared to negative controls containing hGIIA-sPLA<sub>2</sub> and β-py-C10-PG substrate only

is hypothesized that reduction of the eicosanoids production may suppress inflammation. Maslinic acid inhibited hGIIA-sPLA<sub>2</sub> enzyme activity (as shown in Fig. 2) in a concentration-dependent manner. More than 60 % of inhibition ( $p < 0.05$ ) was achieved at 100 μM concentration. To

elucidate the kinetics of inhibition, hGIIA-sPLA<sub>2</sub> enzyme activity was determined in the presence of increasing substrate concentrations. Lineweaver–Burk double reciprocal plot (Supplementary Figure 1) showed that the  $V_{max}$  for hGIIA-sPLA<sub>2</sub> enzyme activity in the presence of maslinic acid was less than uninhibited enzyme and that both conditions (with maslinic acid and without inhibitor) have same  $K_m$ , indicating non-competitive inhibition.

### Molecular Interaction Between Maslinic Acid and hGIIA-sPLA<sub>2</sub> Enzyme

The crystal structure of hGIIA-sPLA<sub>2</sub> (3U8D) enzyme was obtained from PDB. The binding region of hGIIA-sPLA<sub>2</sub> consists of hydrophobic site with Leu2, Phe5, Ile9, Ala17, Ala18, Tyr21, Cys28, Cys44, and Phe98 while the catalytic site consists of hydrophilic residues His47 and Asp48 [19]. His47 and Asp48 are important hydrogen bond acceptors and calcium binding site [20]. The presence of His47 and Asp48 residue on the active site facilitates the hydrolysis mechanism by abstracting a proton from the water molecule then followed by nucleophilic attack on the *sn*-2 bond [21]. The docking interaction (Fig. 3) shows that maslinic acid binds to His47 and Asp48 residues via hydrogen bonding, suggesting that it inhibits the access of the catalytic calcium ion required for the enzymatic reaction. In addition, maslinic acid also formed hydrophobic interactions with hGIIA-sPLA<sub>2</sub> enzyme at Leu2, Phe5, Ala18, Cys44, which are the interfacial binding sites of the enzyme. The interfacial binding site of sPLA<sub>2</sub> enzyme represents the point of contact between the enzyme and the phospholipid bilayer substrate [22]. This indicates that apart from blocking the calcium binding site, maslinic acid may also interfere with



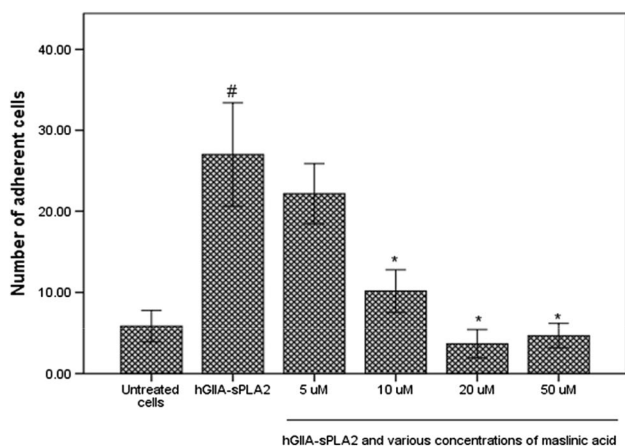
**Fig. 3** Model of hGIIA-sPLA<sub>2</sub> (PDB id 3U8D) with maslinic acid. Maslinic acid binds to His47 and Asp48 residues via hydrogen bonding, and also formed hydrophobic interactions with Leu2, Phe5, Ala18 and Cys44



enzyme binding to the membrane phospholipid, thereby inhibiting the enzymatic activity of hGIIA-sPLA<sub>2</sub>.

### Effect of Maslinic Acid on hGIIA-sPLA<sub>2</sub>-Induced THP-1 Cell Differentiation

To further elucidate the role of maslinic acid in regulating hGIIA-sPLA<sub>2</sub>-mediated inflammatory processes, an adhesion assay was performed in hGIIA-sPLA<sub>2</sub>-induced THP-1 cells. THP-1 is a human pro-monocytic cell line and it has been used as a model system for monocyte-macrophage differentiation. According to previous studies, promonocytic THP-1 cells are able to differentiate when stimulated with inflammatory activators. THP-1 cells in its differentiated state exhibit phenotypic modification such as adhering to culture plate surfaces as well as changes in its morphology [23]. Based on the result obtained in Fig. 4, hGIIA-sPLA<sub>2</sub> significantly induces THP-1 differentiation as indicated from the increased number of adherent cells. Treatment with maslinic acid significantly reduces the number of adherent cells at 10, 20 and 50 μM concentrations ( $p < 0.05$ ), indicating that maslinic acid has the capacity to suppress hGIIA-sPLA<sub>2</sub>-induced THP-1 cell differentiation. Considering that maslinic acid has been reported to inhibit cell proliferation and apoptosis through a mitochondrial-mediated pathway [24], a set of negative control cell treatments with maslinic acid alone was performed. Our results demonstrated that maslinic acid did not significantly affect the number of adherent cells (Supplementary



**Fig. 4** Effect of maslinic acid on hGIIA-sPLA<sub>2</sub>-induced THP-1 cell adhesion. THP-1 cells were untreated, treated with 1 μg/mL hGIIA-sPLA<sub>2</sub>, or treated with 1 μg/mL hGIIA-sPLA<sub>2</sub> in the presence of various concentrations of maslinic acid (5, 10, 20 and 50 μM). The cells were incubated at 37 °C for 24 h, and the number of adherent cells was determined as indicated in the “Materials and Methods” section. Each bar represents the mean number of adherent cells of three independent experiments ± SD;  $n = 3$ . #Signifies  $p < 0.05$  compared to untreated cells and Asterisks represent  $p < 0.05$  compared to cells treated with hGIIA-sPLA<sub>2</sub> only

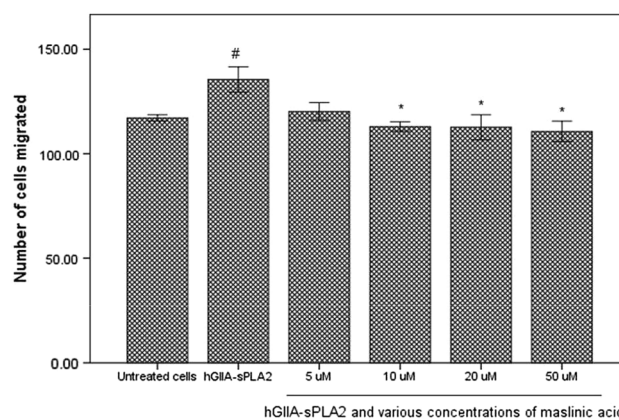
Figure 2). The basal level of differentiated cells (adhered) in the absence of hGIIA-sPLA<sub>2</sub> stimulation was relatively low and that maslinic acid treatment (5, 10, 20 and 50 μM) has no significant impact on the cellular process.

### Effect of Maslinic Acid on hGIIA-sPLA<sub>2</sub>-Induced THP-1 Cell Migration

To determine the migratory capacity of the cells in response to hGIIA-sPLA<sub>2</sub>, THP-1 cells were placed in the upper chambers of Transwell inserts. The lower chambers contained supernatants from non-stimulated cells, 24 h hGIIA-sPLA<sub>2</sub>-stimulated cells, or 24 h hGIIA-sPLA<sub>2</sub>-stimulated cells in the presence of various concentrations of maslinic acid. THP-1 cells showed migration in response to supernatants from hGIIA-sPLA<sub>2</sub>-stimulated cells. Based on the results obtained, maslinic acid significantly reduced the number of cells migrated in response to hGIIA-sPLA<sub>2</sub> treatment at the concentration of 10, 20 and 50 μM (Fig. 5) ( $p < 0.05$ ). The inhibitory effect was specific to hGIIA-sPLA<sub>2</sub> as cells treated with maslinic acid alone did not induce significant cell migration (Supplementary Figure 3).

## Discussion

Considering its mechanism of action in catalyzing hydrolysis of membrane phospholipids which contributes to eicosanoids production, it is hypothesized that sPLA<sub>2</sub> inhibitors may act as potential anti-inflammatory agents. Maslinic



**Fig. 5** Effect of maslinic acid on hGIIA-sPLA<sub>2</sub>-induced THP-1 cell migration. THP-1 cells were untreated, treated with 1 μg/mL hGIIA-sPLA<sub>2</sub>, or treated with 1 μg/mL hGIIA-sPLA<sub>2</sub> in the presence of various concentrations of maslinic acid (5, 10, 20 and 50 μM). The cells were incubated at 37 °C for 24 h, and the numbers of cells migrated were determined as indicated in “Materials and Methods”. Each bar represents mean number of migrated cells of three independent experiments ± SD;  $n = 3$ . #Signifies  $p < 0.05$  compared to untreated cells and \*represents  $p < 0.05$  compared to cells treated with hGIIA-sPLA<sub>2</sub> only

acid has been widely accepted as a natural compound with anti-inflammatory effects. Recent studies have elucidated its molecular mechanism and potential binding targets. Nevertheless, it is still unknown how maslinic acid regulates the inflammatory signaling pathways which contribute to the inhibition of iNOS/COX-2 activity and eicosanoid release. Given that sPLA<sub>2</sub>s are an emerging class of mediators for inflammation and it possess the capacity to hydrolyze lipoproteins and release lipid mediator from cellular membranes, this study sought to investigate the interaction between maslinic acid and hGIIA-sPLA<sub>2</sub>.

The results of this study demonstrated that maslinic acid enhances the intrinsic fluorescence of purified hGIIA-sPLA<sub>2</sub> enzyme, thus suggesting maslinic acid's direct interaction with the enzyme to form an enzyme-maslinic acid complex. In addition, maslinic acid inhibits hGIIA-sPLA<sub>2</sub> enzyme activity and the mode of inhibition is non-competitive. The inhibitory activity of maslinic acid observed is in agreement with other studies reporting the inhibition of ursolic acid, an ursane-type pentacyclic triterpene, on sPLA<sub>2</sub> enzymes isolated from snake venom and human inflammatory exudates [25]. The same study also showed that ursolic acid-mediated inhibition of hGIIA-sPLA<sub>2</sub> enzyme is non-competitive and irreversible. The molecular interaction between maslinic acid and hGIIA-sPLA<sub>2</sub> was further evaluated. We showed that maslinic acid forms a hydrogen bond with His47 and Asp48 amino acid residue. Both His47 and Asp48 are important hydrogen bond acceptors and calcium binding site which facilitates the hydrolysis mechanism by abstracting a proton from the water molecule then followed by nucleophilic attack on the *sn*-2 bond [20, 21]. In addition, maslinic acid also binds to Leu2, Phe5, Ala18, and Cys44 hydrophobic site. Specifically, Leu2 and Ala18 are interfacial residues of the hGIIA-sPLA<sub>2</sub> enzyme. According to Winget *et al.*, the interface is a flat surface which constitutes the active site of the PLA<sub>2</sub> enzyme where it “sits” on the membrane phospholipid before executing its enzymatic activity [22]. We suggest that maslinic acid blocks the access of catalytic calcium ion required for enzymatic reaction and inhibits the binding of hGIIA-sPLA<sub>2</sub> enzyme to the membrane phospholipid, thereby inhibiting the enzymatic activity of hGIIA-sPLA<sub>2</sub>.

Previous studies have established the immuno-modulatory function of hGIIA-sPLA<sub>2</sub> in mediating monocyte recruitment and differentiation [13]. Immune cells recruited to the atherosclerotic lesions amplify the inflammatory response in that area and contributes to plaque instability. Our results show that, upon treatment, maslinic acid significantly reduced hGIIA-sPLA<sub>2</sub>-induced THP-1 cell differentiation and migration. The effect observed is specific to hGIIA-sPLA<sub>2</sub> as cells treated with maslinic acid

alone did not significantly affect the number of adherent and migrated cells. The basal level of differentiated and migrated cells in the absence of hGIIA-sPLA<sub>2</sub> stimulation was relatively low and that maslinic acid treatment has no significant impact on the cellular process. Nevertheless, the molecular mechanism for the anti-inflammatory actions of maslinic acid in inhibiting hGIIA-sPLA<sub>2</sub>-induced cell differentiation and migration remains poorly understood. The hGIIA-sPLA<sub>2</sub> enzyme is known to hydrolyze glycerophospholipids from lipoproteins and cellular membranes, producing NEFA and lysophospholipids. The NEFA and lysophospholipids in turn induce monocyte recruitment to the lesional endothelial site. We hypothesized that maslinic acid binds and inhibits hGIIA-sPLA<sub>2</sub> enzymatic activity, thereby reduces the release of NEFA and lysophospholipids which stimulates monocyte migration and differentiation.

Some findings have demonstrated that hGIIA-sPLA<sub>2</sub>-induced cell migration was mediated by protein kinase C (PKC) pathway [26]. Our previous study showed that maslinic acid suppresses PKC  $\beta$ I,  $\delta$ , and  $\zeta$  activation and constitutive NF- $\kappa$ B activation in Raji B lymphoma cells [27]. Whether maslinic acid-mediated suppression of PKC/NF- $\kappa$ B activation is dependent on inhibition of hGIIA-sPLA<sub>2</sub> in THP-1 monocytes requires further investigation. Recent studies have also found that compounds that inhibit the interaction between hGIIA-sPLA<sub>2</sub> and integrin suppressed hGIIA-sPLA<sub>2</sub>-induced U937 human monocytic leukaemia cells adhesion and migration [28]. Subsequent research is also needed to establish the possibility of maslinic acid interfering with the hGIIA-sPLA<sub>2</sub> activity in a manner independent of their lipolytic enzymatic activity.

## Conclusion

Our findings provide insightful clues into the molecular interaction of maslinic acid and hGIIA-sPLA<sub>2</sub>. The results suggest that maslinic acid directly interacts with hGIIA-sPLA<sub>2</sub> and inhibit their enzyme activity by binding to the calcium binding and phospholipid interfacial site which blocks the enzyme catalytic reaction. Maslinic acid also inhibited hGIIA-sPLA<sub>2</sub>-induced THP-1 cell differentiation and migration. There are possibilities that maslinic acid targets hGIIA-sPLA<sub>2</sub> enzymatic activity itself and or acts as an inhibitor that blocks the direct binding of hGIIA-sPLA<sub>2</sub> to integrins. Thus, it is suggested that further study needs to be conducted in regards to the mechanism involved.

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## Compliance with Ethical Standards

**Conflicts of interest** The authors declare that there are no conflicts of interest.

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