Lysophosphatidylcholine attenuates melanoma cell adhesion and migration dependent on the degree of fatty acid saturation

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Introduction

Metastatic dissemination of tumor cells is the most destructive complication in cancer diseases. During metastasis, tumor cells invade the blood, interact with blood components like platelets and leukocytes, arrest at the endothelial surface of distant organs, and finally transmigrate through the endothelium to proliferate in the tissue matrix. Since adhesion receptors are crucially involved in all these steps, the blockade of adhesion receptors appears to be a promising strategy to interfere in this fatal cascade.

Recently, we have observed antimetastatic effects of empty liposomes consisting of saturated phospholipids (PLs) in orthotopic pancreatic cancer nude mouse models [1]. Further studies confirmed saturated lysophosphatidylcholine (sLysoPC) as the active agent, which probably arises in high local concentrations in the tumor tissues as a degradation product from the saturated PLs of the liposomes based on their passive tumor accumulation due to their enhanced permeability and retention effect (EPR) [2, 3].

sLysoPC concentration of 450 µmol/L, which is only slightly higher than normal plasma levels (~ 300 µmol/L), induced morphological changes, reduced cell adhesion of murine B16.F10 melanoma cells in vitro, and led to a pronounced reduction of their metastatic lung invasion in vivo [4, 5]. The reduced capacity of sLysoPC-treated B16.F10 cells for receptor-mediated cell contact formation (e.g., P-selectin-mediated platelet binding, and endothelial contacts the integrin via VLA-4), appears to be pivotal for the diminished metastatic like lung invasion [5].

Nonetheless, the molecular mode of action of sLysoPC leading to reduced adhesion receptor activity and alleviated metastatic burden in mice remains elusive. However, a deregulation of tumor membrane properties, most likely due to a rigidification, appears probable. To follow this hypothesis and obtain first insights, the present study compares a saturated and an unsaturated LysoPC derivative in their impact on receptor-mediated adhesion and migration of human MV3 melanoma cells.

Material and methods

Cell culture and LysoPC supplementation

MV3 melanoma cells were cultivated in RPMI 1640 medium (supplemented with 10% fetal calf serum). The morphological cell appearance was microscopically checked, and cells were monthly tested for absence of mycoplasms. LysoPC solutions (450 µmol/L) were prepared by adding the respective amount of LysoPC plus 20 mg/mL bovine serum albumin (BSA, Sigma-Aldrich, Taufkirchen, Germany) to RPMI 1640 medium. LysoPC (1-Octadecanoyl-2-hydroxy-sn-glycero-3-phosphocholine, (sLysoPC C18:0) or 1-oleoyl-2-hydroxy-sn-glycero-3-phosphocholine, (LysoPC C18:1), were purchased from Avanti Polar Lipids, Alabaster, AL, USA.

Flow chamber assay

The VLA-4-mediated interaction of MV3 cells with VCAM-1 Fc-chimera under
physiological flow conditions was analyzed on glass slides coated with VCAM-1 Fc-chimera in a parallel plate flow chamber, as described [5]. Video sequences of the flow chamber were captured, and number of adhering cells was determined by the Imago-quant Multi-Track-AVI-2 software (Medi-quant GmbH, Aschau, Germany).

Cell migration

MV3 cells (1×10⁴) were seeded into each well of a 24-well plate (Greiner Bio-One, Frickenhausen, Germany) previously coated with 10 µg/mL fibronectin (Roche Diagnostics, Mannheim, Germany) and incubated with LysoPC. After 72 hours, a scratch wound was induced into the confluent cell monolayer by a pipette tip. Wound healing was observed for 12 hours at 37 °C, and the speed of migration was determined by linear regression.

Fluorescence anisotropy

Membrane rigidity was determined by measurement of N,N,N-Trimethyl-4-(6-phenyl-1,3,5-hexatrien-1-yl)phenylammonium p-toluenesulfonate (TMA-DPH, Sigma-Aldrich, Germany) fluorescence anisotropy using a polarstar galaxy plate reader (BMG labtech, Ortenberg, Germany). Briefly, cells were detached using trypsin/EDTA, suspended in PBS (1×10⁶ cells/mL), and used at a fluorophore ratio of 0.5 µmol/L TMA-DPH for 1×10⁵ MV3 cells [6]. Fluorescence anisotropy was detected at 430 nm after excitation at 355 nm.

Results

In previous studies, we could demonstrate a pronounced sLysoPC-uptake by melanoma cells accompanied by reduced VLA-4 integrin function. To investigate whether the LysoPC effect occurs dominantly via a rigidification of the cell membrane, we compared the effects of a saturated, thus rigid LysoPC (LysoPC C18:0), with an unsaturated LysoPC (LysoPC C18:1) on an integrin-mediated 2D cell migration. As indicated in Figure 1A, an incubation of MV3 melanoma cells, with 450 µmol/L sLysoPC C18:0 with a 2% BSA containing cell culture medium, for 72 hours revealed a strong reduction of migration, while LysoPC C18:1 only had a slight inhibitory effect on cell migration. 2% BSA alone, as associated material of LysoPC treatments, had no effect.

These results were corroborated by cell adhesion experiments to VCAM-1 under physiological flow conditions. Here, VLA-4-mediated cellular adhesion of Mn²⁺ stimulated cells was reduced under the influence of sLysoPC C18:0 to levels as low as unstimulated cells, whereas LysoPC C18:1 induced only a slight reduction, and BSA even increased adhesion (Figure 1B).

To obtain a first insight as to whether these two LysoPC species induce different effects on cell membrane rigidity, fluore-
ence anisotropy was measured and indicated an increased membrane rigidity of MV3 cells treated with sLysoPC C18:0 compared to the unsaturated LysoPC C18:1 cells (Figure 1C).

**Conclusion**

These data give a first indication that saturated LysoPC induces a rigidification of tumor cell membranes. One can assume that a change in membrane rigidity will seriously affect membrane compartmentalization and thus, several signaling pathways. On that basis, the function of integrins in mediating cell migration and intercellular adhesion, which is of crucial importance for melanoma metastasis, seems also to be affected [7].

This might be a link to the antimetastatic effects of LysoPC shown in different mice models [5]. However, the underlying cellular mechanisms of integrin activation require further investigations.

**References**


