Inhibition of phosphatidylcholine-specific phospholipase C induces down-regulation of CXCR4 overexpression and reduction of 1H-MRS-detected PCho in human lymphoblastoid cells

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Introduction
New pharmacological therapies are based on selective molecular targeting of mechanisms able to deregulate pathways of cellular proliferation, survival and apoptosis and activate angiogenesis and metastasis.

Recent evidence indicates an important role for CXCL12 (a CXC chemokine) and its receptor (CXCR4) in the metastatic homing of tumor cells (Ottaiano A et al. Cancer Immunol Immunother. 2005).

MRS is a powerful approach to detect metabolic alterations of phosphatidylcholine metabolism (PC) associated with tumor cell growth and progression and to investigate the underlying molecular mechanisms. In particular, it has been shown that the inhibition of some PC-cycle enzymes could represent molecular targets for new anticancer therapy (Glunde K. Mol Pharm. 2006; Iorio E et al. Cancer Res. 2010).

Purpose of this study was to investigate alterations of CXCR4 expression in a human T-lymphoblastoid cell line (CEM) exposed to a selective inhibitor of the PC-specific phospholipase C (PC-plc). In fact, PC-PLC activation/deactivation status has been reported to modulate the expression of membrane receptors and proteins crucial for specific cell functions, such as HER2 in breast cancer and CD16 in Natural Killer cells (Paris L et al. Breast Cancer Res. 2010; Cecchetti S et al. Eur J Immunol. 2007).

Methods: For MRS experiments CEM cells were grown in complete medium and incubated in the absence (CTRL) or presence of the PC-PLC inhibitor tricyclodecan-9-yl-potassium xanthate (D609, 25 μg/ml) for 24 h. 1H MRS experiments were performed on ethanolic cell extracts on a Bruker Avance 400 spectrometer using a 1H-X multinuclear inverse probehead. Flow cytometry analyses were performed on a FACScan apparatus (BD Biosciences) using Alexa fluor 488 conjugated secondary Abs.

Results
1) Cytofluorimetric analyses performed on unfixed, viable cells, showed overexpression of both CXCR4 and PC-PLC on the outer plasma membrane of CEM cells. Western blot analyses of anti-CXCR4 or anti-PC-PLC immunoprecipitates (IP) blotted with the mutual antibodies (anti-PC-PLC or anti-CXCR4, respectively) showed for the first time the existence of a physical interaction between PC-PLC and the CXCR4 receptor (Fig. 1).

2) Cell exposure to the PC-PLC inhibitor D609 induced a strong down-modulation of CXCR4 (up to 40-50% from 3h to 24h of treatment) from the plasma membrane, suggesting a linkage between PC catabolism and the CXCR4/CXCL12 axis (Fig. 2).

3) MRS analyses on aqueous extracts showed a significant decrease (by about 42 %) in the intracellular PCho content of D609-treated CEM cells compared with the untreated controls (Fig. 3).

Conclusions and future directions
- Inhibition of the investigated CXC receptor-chemokine axis is currently being investigated as a possible therapeutic strategy for anticancer treatment. The clinical development for these targeted therapies requires identification of pharmacodynamic markers of treatment effectiveness.
- The results of this study showed for the first time the existence of a physical interaction between the phosphatidylcholine-cycle enzyme PC-PLC and CXCR4 on the plasma membrane of a T-lymphoblastoid cell line (CEM).
- Specific inhibition of PC-PLC activity induced down-modulation of CXCR4 from the plasma membrane of CEM cells.
- 1H MRS profiling of cell extracts showed that a decrease in the PCho signal may act in these cells as a significant marker of simultaneous PC-PLC inhibition and CXCR4 downmodulation.
- Further clarification of these molecular mechanisms may contribute to the development of non invasive MRS approaches to monitor the effects of the CXCR4-CXCL12 axis in these and in other tumor cells.

Fig 1.  Fig. 2  Fig. 3

PCho