# Glycosylphosphatidilinoditol-specific phospholipase D expression in bone marrow mononuclear cells derived from acute leukemia patients☆

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**Abstract**

**BACKGROUND:** The correlation of gycosylphosphatidilinoditol-specific phospholipase D (GPI-PLD) activity, mRNA expression to leukemia type, hepatosplenomegaly and/or lymphadenopathy has been rarely reported.

**OBJECTIVE:** To explore the correlation of GPI-PLD expression to leukemia type and hepatosplenomegaly and/or lymphadenopathy of acute myeloid leukemia (AML) patients.

**METHODS:** Fresh bone marrow specimens were obtained from 43 newly diagnosed AML patients, 28 acute lymphocytic leukemia (ALL) patients, and 21 normal persons. Bone marrow mononuclear cells were harvested by density gradient centrifugation. GPI-anchored human placent alkaline phosphatase was used as substrate. GPI-PLD activity was determined bytriton-X114 phase partitioning procedure. GPI-PLD mRNA expression was detected by semi-quantitative RT-PCR. The relationship of GPI-PLD activity, mRNA expression and leukemia type, hepatosplenomegaly and/or lymphadenopathy was analyzed.

**RESULTS AND CONCLUSION:** Compared with control group, GPI-PLD activity and mRNA expression in bone marrow mononuclear cells were significantly higher in AML group ($P < 0.01$), while they were significantly lower in the ALL group ($P < 0.01$). Of 43 patients with AML patients, 13 patients had hepatosplenomegaly and/or lymphadenopathy. The GPI-PLD activity (%) and mRNA expression were significantly higher in AML patients without hepatosplenomegaly and lymphadenopathy than those patients with hepatosplenomegaly and/or lymphadenopathy ($P < 0.05$). These results demonstrated that GPI-PLD activity alteration is consistent with GPI-PLD mRNA expression in AML patients, and the expression levels correlate to leukemia type and hepatosplenomegaly and/or lymphadenopathy of AML patients.

## INTRODUCTION

Leukemia is a malignant disorder originating from hematopoietic tissue, which is characterized by unrestricted proliferation of leukemic blast cells in bone marrow and other hematopoietic tissues. In addition, these blasts may invade most tissues or organs. It is well known that hepatosplenomegaly and lymphadenopathy are more common signs in acute lymphocytic leukemia (ALL) patients than in acute myeloid leukemia (AML) patients. Extramedullary infiltration of leukemic blast cells is one of the main factors that cause leukemia relapse and death. But the mechanism of leukemic extremedullary infiltration is still not full understood.

Glycosylphosphatidylinositol specific phospholipase D (GPI-PLD) is a specific phospholipase that splits GPI-anchored proteins on cell membrane[1]. GPI-PLD can be excreted from many organs or cells[2] such as bone marrow stromal cells and hematopoietic cells. Some proteins expressed on leukemic blast cells are anchored on the surface of cell plasma membrane by binding with GPI anchor, which are defined as GPI-anchored proteins. Some of these proteins such as CD24 are adhesion molecules that play important roles in the process of tumor cells adhesion and migration[3-6]. But it is unclear if GPI-PLD takes part in extramedullary infiltration of leukemic blast cells and correlates to acute leukemia type. In order to explore the significance of GPI-PLD for acute leukemia, this study was designed to examine the GPI-PLD activity and mRNA expression in bone marrow mononuclear cells separated from AML, ALL and normal plasma, and to analyze the correlation of GPI-PLD expression to leukemia type and hepatosplenomegaly and/or lymphadenopathy of acute AML patients.

## MATERIALS AND METHODS

### Design

A cytological *in vitro* observation.

### Time and setting

This study was performed at the Laboratory of Hematology, Xiangya Hospital between January 2004 and December 2005.

### Subjects

Acute leukemia was diagnosed primarily according to morphological criteria formulated by French-American-British classification. Fresh bone marrow specimens were obtained from 43 newly diagnosed AML patients (M1: 3; M2: 21; M3: 12; M4: 2; M5: 5)[ 23 males and 20 females, 38.4(15–69) years of age], 28 ALL patients[16 males, 12 females, 35.1(11–60)years of age], and 21 normal persons[8 males, 13 females, 33.0 (21–63)years of age ]. For the leukemic specimens, the leukemic blast cells in bone marrow were more than 30%. The above mentioned bone marrow specimens were provided by Department of Hematology, Xiangya Hospital. Written informed consent regarding laboratory examinations was obtained from each subject and relatives.

**Reagents**

Human placent alkaline phosphatase (P-ALP)was kindly provided by professor Tang Jian-hua from Biochemistry Department, Xiangya School of Medicine, Central South University in China. Reverse transcription kit was purchased from Promaga Corporation, USA. Primers were synthesized by Shanghai Bioasia Corporation, China. Tag polymerase was purchased from Jingmei Corporation, China, and Trizol was from Invitrogen Corporation, USA.

**Methods**

***Bone marrow mononuclear cells isolation and GPI-PLD activity assay***

Under sterile condition, 2–5 mL bone marrow was taken, and anticoagulated with heparin. Following PBS dilution, lymphocyte isolation medium was added to obtain bone marrow mononuclear cells by density gradient centrifugation. For all specimens, the percentage of leukemic blast cells in bone marrow mononuclear cells was more than 80%. GPI-PLD activity assay was performed as previously described method[7]. According to preliminary test, 0.16% Nonidet P40 (NP40) was used as detergent. The mixture of substrate, cells, and buffer was incubated at 37 ℃ for 90 minutes.

***Semi-quantitative RT-PCR analysis***

Total RNA isolation and reverse transcription were performed as manufacturer's instructions. The resulting preparation was quantified by spectrophotometry, and RNA integrity was verified by 1.5% agarose gel electrophoresis.

PCR was performed with 5 μL 10× buffer, 5 μL 25 mmol/L $MgCl_2$, 1 μL 10 mmol/L dNTP, 10 pmol GPI-PLD, 10 pmol glyceraldehydes-3-phosphate-dehydrogenase (GAPDH), 2.5 U Tag polymerase, 5 μL complementary deoxyribonucleic acid, and 31.5 μL $ddH_2O$. The tubes were heated on an according to the following protocol: one cycle of denaturation at 94 ℃ for 2 minutes; 35 cycles of denaturation at 94 ℃ for 1 minute, annealing at 59 ℃ for 1 minute, extension at 72 ℃ for 1 minute; and a final extension at 72 ℃ for 7 minutes. The sequences of GPI-PLD specific primers[8] were 5'-GAG GTT CAC CGT GTG GCC TTT C-3'and 5'-GCC ATAT CAT CCA GTC CTC CAA G-3', with an amplified product of 705 bp. The sequences of GAPDH primers were 5'-AAG AAG ATG CGG CTT GAC TGT CGA GCC ACA T-3'and 5'-TCT CAT GGT TCA CAC CCA TGA CGA ACA TG-3', with an amplified product of 457 bp.

The PCR products were separated by 1.5% agarose gel electrophoresis. The gels were photographed under UV transillumination and scanned using an Eagle Eye Still Video System/Stratagene Eagle Sight (version 3.1). The intensity of the DNA bands was quantified using Metamorph Imaging System (Version 4.5, Universal Imaging Corp. USA). The ratio was calculated by absorbace (GPI-PLD/GAPDH).

**Statistical analysis**

All experimental data were expressed as Mean ± SD. Significant differences between different leukemia groups were determined using One-Way ANOVA. (SPSS 11.5 for windows). $P < 0.05$ was considered statistically significant.

## RESULTS

**GPI-PLD activity and mRNA expression in different types of leukemia**

GPI-PLD activity and mRNA expression could be dateable in all specimens. Compared with the control group, GPI-PLD activity (%) and mRNA expression in bone marrow mononuclear cells were significantly higher in the AML group ($P < 0.01$), but they were significantly lower in the ALL group ($P < 0.01$) (Table 1, Figure 1).

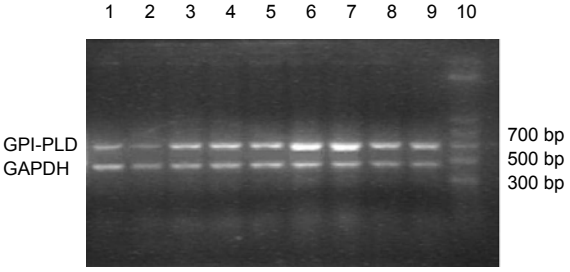
Table 1 Results of GPI-PLD activity and mRNA expression in different groups ($\bar{x}\pm s$)

| Group | *n* | Activity (%) | mRNA (Absorbance) |
|---|---|---|---|
| AML | 43 | 46.16±7.17[a] | 1.84±0.28[a] |
| AML with hepatosplenomegaly and/or lymphadenopathy | 13 | 41.15±5.82[ab] | 1.61±0.18[ab] |
| AML without hepatosplenomegaly and lymphadenopathy | 30 | 48.34±6.66[a] | 1.93±0.26[a] |
| ALL | 28 | 24.55±6.50[a] | 0.88±0.19[a] |
| Control | 21 | 35.42±5.35 | 1.25±0.22 |

GPI-PLD: glycosylphosphatidylinositol specific phospholipase D; AML: acute myeloid leukemia; ALL: acute lymphocytic leukemia; [a]$P < 0.01$, *vs*. control group; [b]$P < 0.05$, *vs*. AML without hepatosplenomegaly and lymphadenopathy

**Correlation of GPI-PLD to hepatosplenomegaly and/or lymphadenopathy in AML patients**

Of 43 AML patients, 13 had hepatosplenomegaly and/or lymphadenopathy. The GPI-PLD activity (%) and mRNA expression were significantly higher in AML patients without hepatosplenomegaly and lymphadenopathy than those patients with hepatosplenomegaly and/or lymphadenopathy (Table 1 and Figure 1).




Lanes 1, 2: ALL; lanes 3–5: control; lanes 6, 7: AML without hepatosplenomegaly and lymphadenopathy; lanes 8, 9: AML with hepatosplenomegaly and/or lymphadenopathy; lane 10: marker; GPI-PLD: glycosylphosphatidylinositol specific phospholipase D; GAPDH: glyceraldehyde phosphate dehydrogenase; AML: acute myeloid leukemia; ALL: acute lymphocytic leukemia

Figure 1 GPI-PLD mRNA expression

## DISCUSSION

There is substantial evidence for the release of GPI-anchored proteins by intracellular GPI-PLD[9-10]. Some GPI-anchored proteins such as CD87 and CD24 expressed on hematopoietic tumor cells are also cell adhesion molecules that facilitate tumor cell extramedullary infiltration[5, 11-13]. There are some studies indicate that GPI-PLD has effects on the adhesion and migration ability of tumor cells by regulating GPI-anchored protein on tumor cells[14-16]. GPI-PLD expression or activity may have effect on the metastasis of hematopoietic tumor cells. It is necessary to investigate whether GPI-PLD correlates to leukemia type and leukemic extramedullary infiltration.

In order to study the correlation of GPI-PLD to the leukemia type and leukemic extramedullary infiltration, the mRNA expression and activity of GPI-PLD in bone marrow mononuclear cells isolated from 71 leukemia patients and 21 normal persons were detected by semi-quantitative RT-PCR , using GPI-anchored human PLAP as substrate and triton-X114 for partitioning, respectively.

The alteration of GPI-PLD mRNA expression and activity in different groups are consistent, which excludes the possibility that the GPI-PLD mRNA that was measured due to absorption from plasma other than synthesis by themselves. The present results revealed that GPI-PLD mRNA expression and activity in bone marrow mononuclear cells isolated from ALL patients, who are easy to suffer from extramedullary infiltration, were lower than those from AML patients. Interestingly, GPI-PLD mRNA expression and activity in bone marrow mononuclear cells isolated from AML patients with hepatosplenomegaly and lymphadenopathy were also lower than those from AML without hepatosplenomegaly and lymphadenopathy. It has been well known that ALL patients are easier to suffer from extramedullary infiltration than AML patients. So we suspect that GPI-PLD correlates to leukemic extramedullary infiltration, because the expression of adhesion molecules, such as CD24 and CD87, on leukemic cell membrane is consistent with GPI-PLD activity, which facilitates the adhesion and migration of leukemic cells. The GPI-PLD mRNA expression and activity are obviously lower in ALL patients than in AML patients. It is worthy to further study whether GPI-PLD can be used as a supplementary marker of leukemia type.

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# 急性白血病骨髓单个核细胞糖基化磷脂酰肌醇特异性磷脂酶 D 的表达☆

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**摘要**

**背景**：据作者查新检索，国内外有关急性白血病骨髓单个核细胞糖基化磷脂酰肌醇特异性磷脂酶 D 活性及 mRNA 表达与白血病类型、患者肝脾淋巴结肿大关系的报道罕见。

**目的**：探讨糖基化磷脂酰肌醇特异性磷脂酶 D 的表达与急性白血病类型、急性髓系白血病患者肝脾淋巴结肿大的关系。

**方法**：急性髓系白血病组骨髓标本 43 例，急性淋巴细胞白血病组骨髓标本 28 例，以 21 例健康志愿者作为正常对照组。密度梯度离心法获得骨髓单个核细胞，用 GPI 锚定的胎盘碱性磷酸酶作为底物，Triton-X114 分相法检测糖基化磷脂酰肌醇特异性磷脂酶 D 的活性，半定量 RT-PCR 法检测糖基化磷脂酰肌醇特异性磷脂酶 D mRNA 的表达，并分析糖基化磷脂酰肌醇特异性磷脂酶 D 活性和 mRNA 表达水平与急性白血病类型、急性髓系白血病患者肝脾淋巴结肿大的关系。

**结果与结论**：与正常对照组比较，急性髓系白血病组骨髓单个核细胞糖基化磷脂酰肌醇特异性磷脂酶 D 活性和 mRNA 表达均明显升高($P < 0.01$)；急性淋巴细胞白血病组骨髓单个核细胞糖基化磷脂酰肌醇特异性磷脂酶 D 活性和 mRNA 表达均明显降低($P < 0.01$)。在 43 例急性髓系白血病患者中，13 例患者检查发现有肝脾淋巴结肿大，30 例患者检查无肝脾淋巴结肿大，无肝脾淋巴结肿大标本骨髓单个核细胞糖基化磷脂酰肌醇特异性磷脂酶 D 活性和 mRNA 表达均明显高于有肝脾淋巴结肿大标本($P < 0.05$)。结果证实急性白血病骨髓单个核细胞糖基化磷脂酰肌醇特异性磷脂酶 D 的活性与 mRNA 变化一致，其表达水平与急性白血病类型及急性髓系白血病患者有无肝脾和/或淋巴结肿大有关。

**关键词**：白血病；糖基化磷脂酰肌醇特异性磷脂酶 D；髓外浸润；骨髓单个核细胞；干细胞
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## 骨髓间充质干细胞的分离与培养：本刊中文部

1　改良小鼠骨髓间充质干细胞培养方法及长效荧光标记的可行性
王美华(解放军军事医学科学院附属医院消化内科，北京市　100071)
国家自然科学基金青年基金(30800277)
推荐理由：目前实验室以小鼠为干细胞研究的动物体内研究模型既经济又便利，可控性操作性强。但小鼠骨髓间充质干细胞培养，扩增，传代，保持干细胞活性困难，实验通过贴壁筛选和Percoll分离法，优化干细胞培养液血清和换液方式，培养扩增小鼠骨髓间充质干细胞。参照以往人和猕猴骨髓间充质干细胞培养经验，对小鼠骨髓间充质干细胞培养血清选择Hyclone的顶级胎牛血清，控制血清为培养液的10%。用LG-DMEM培养液冲出骨髓细胞后，滤去骨渣和小肌肉碎块。然后加在相对密度1.082的percoll分离液上，以1.5×$10^6$/$cm^2$浓度接种75 $cm^2$培养瓶。此方法可方便，快捷的获得纯度较高的间充质干细胞并保持其活性，为干细胞动物应用实验，扫清障碍。
另外，目前干细胞因其本身增殖传代的特点，干细胞体内示踪实验中常用的各种染剂，病毒转染，磁素标记等方法，荧光保持时间短，而稳定基因转染又会影响干细胞活性，因此不能满足长期体内观察实验需要。而实验中所用的长效DiI染剂，本身用于神经干细胞染色观察，可持续数月，经验证其标记骨髓间充质干细胞后，经多次传代，仍有较高荧光效果，可以很好解决干细胞示踪实验困难，见2009年45期8929页。

2　密度梯度离心与贴壁筛选法体外分离培养胎儿骨髓间充质干细胞
刘斌(中山大学附属第三医院脊柱外科，广东省广州市　510630)
广东省科技计划项目(2007B031001004，2005B34001003)

3　比较不同传代人骨髓间充质干细胞的生物学特性：推荐第3~6代细胞可用于临床
胡炜(新疆维吾尔自治区中医院脊柱二科，新疆维吾尔自治区乌鲁木齐市　830000)
北京市优秀人才项目(2006ID0900300332)

4　脐带血清和成人自体血清体外培养人骨髓间充质干细胞的比较
旷文勇(湖南省儿童医院血液内科，湖南省长沙市　410007)
湖南省科技厅重点支持项目(06SK2007)

5　人骨髓间充质干细胞的分离培养及BrdU标记鉴定
张向荣(南昌大学第一附属医院眼科，江西省南昌市　330006)
江西省科技厅重大科技招标项目(200604)；江西省卫生厅重大科技招标项目(200502)；江西省自然科学基金资助项目(0540056)

6　不同体积分数富血小板血浆对犬骨髓间充质干细胞增殖的影响
钟达(中南大学湘雅医院骨科，中南大学骨科研究所，湖南省长沙市　410008)
湖南省科技厅科技计划资助(06sk3029-2)

7　建立大鼠骨髓间充质干细胞稳定分离培养体系与鉴定
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8　全骨髓贴壁法分离培养大鼠骨髓间充质干细胞及其诱导分化
蔡鹏(福建医科大学附属协和医院泌尿外科，福建省福州市　350001)
福建省自然科学基金资助项目(C0710018)；国家自然科学基金(30500254，30700906)；教育部博士点基金(20070001784)

9　周期性压力培养对兔骨髓间充质干细胞增殖的影响
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10　成人与胎儿骨髓间充质干细胞生物学性状的比较
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