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ORIGINAL ARTICLE

PIP4K2A and *PIP4K2C* transcript levels are associated with cytogenetic risk and survival outcomes in acute myeloid leukemia

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Abstract

Phosphoinositide signaling pathway orchestrates primordial molecular and cellular functions in both healthy and pathologic conditions. Phosphatidylinositol-5-phosphate 4-kinase type 2 lipid kinase (PIP4K2) family, which compromises PIP4K2A, PIP4K2B and PIP4K2C, has drawn the attention in human cancers. Particularly in hematological malignancies, PIP4K2A was already described as an essential protein for a malignant phenotype, although the clinical and biological impact of PIP4K2B and PIP4K2C proteins have not being explored in the same extent. In the present study, we investigated the impact on clinical outcomes and gene network of *PIP4K2A, PIP4K2B* and *PIP4K2C* mRNA transcripts in acute myeloid leukemia (AML) patients included in The Cancer Genome Atlas (2013) study. Our results indicate that *PIP4K2A* and *PIP4K2C,* but not *PIP4K2B*, mRNA levels were significantly reduced in AML patients assigned to the favorable risk group ($p < 0.05$) and low levels of *PIP4K2A* and *PIP4K2C* positively affect clinical outcomes of AML patients ($p < 0.05$). Gene set enrichment analyses indicate that the expression of *PIP4K2* genes is associated with biological process such as signal transduction, metabolism of RNA and genomic instability related-gene sets. In summary, our study provides additional evidence of the involvement of members of the PIP4K2 family, in particular PIP4K2A and PIP4K2C, in AML.

Keywords Phosphatidylinositol-5-phosphate 4-kinase type 2 lipid kinase, PIP4K2A, PIP4K2C, TCGA, Acute myeloid leukemia.

Introduction

The phosphoinositide signaling pathway orchestrates primordial molecular and cellular functions in both healthy and pathologic conditions, including cell proliferation, survival, apoptosis, and cell motility [1,2]. Phosphatidylinositol-5-phosphate 4-kinase type 2 (PIP4K2) is a lipid kinase family that contains three members, PIP4K2A, PIP4K2B and PIP4K2C (also known as type II PIP4Kα, PIP4Kβ and PIP4Kγ or PIP5K2A, PIP5K2B and PIP5K2C, respectively). These proteins phosphorylate the phosphatidylinositol (PtdIns)5P at the position four of the inositol ring producing PtdIns4,5P_2, which is important for phosphoinositide signal transduction pathways [3,4]. The encoded PIP4K2A is widely expressed in primary healthy blood cells [5] and leukemia cell lines [6,7], and potentially involved in erythroid differentiation [6,8]. In addition, PIP4K2A was identified as an essential protein for proliferation,

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clonogenicity, and survival in leukemia cell lines and primary acute myeloid leukemia (AML) blasts [9], although the functions of PIP4K2B and PIP4K2C in hematological malignancies remain to be explored.

Recently, McElligott et al. [10] have demonstrated promising data on the efficacy of selective inhibitors of PIP4K2 in preclinical models of hematological cancers, which may boost clinical trials in this field of research. Although an extensive knowledge of AML landscape has gained in the last two decades, new biomarkers remain useful and necessary to refine the current WHO 2016 classification and indicate potential therapeutic targets. Here, we set out to investigate the clinical impact of *PIP4K2A, PIP4K2B* and *PIP4K2C* mRNA transcript levels on outcomes of AML patients included in The Cancer Genome Atlas (TCGA) (2013) study. In addition, we used Gene Set Enrichment Analysis (GSEA) to obtain novel insights about the *PIP4K2* gene expression networks in AML.

Material and methods

The cancer genome atlas (TCGA) data

PIP4K2A, PIP4K2B and *PIP4K2C* mRNA expression data from RNAseq analyzes of 173 AML patients included in the TCGA (2013) study [11] were obtained through the cBioPortal for cancer genomics (http://www.cbioportal.org) [12,13]. The mRNA transcript data were expressed as *z*-score and were dichotomized according to the median values. TCGA AML patients' characteristics are described in Table 1. The association analysis between *PIP4K2A, PIP4K2B*, and *PIP4K2C* expression and most recurrent mutations (frequency $> 5\%$, except *PML, RARA* and *KMT2A* mutations) was also performed in TCGA AML cohort.

Amazonia! data

PIP4K2A (probe 212829_at), *PIP4K2B* (probe 1553048_a_at) and *PIP4K2C* (probe 218942_at) mRNA expression data from AML samples ($n=584$) deriving from the *Amazonia!* database 2008 were directly obtained from the public access data portal (http://amazonia.transcriptome.eu) [14]. For this analysis, AML samples with expression values measured by Affymetrix Human Genome U133 plus 2.0 arrays and risk-stratified by the European Leukemia Net group (ELN) [15] recommendations were included. Data sets were cross-referenced using tumor-specific identification numbers.

Gene Set Enrichment Analysis (GSEA) for *PIP4K2s* biological pathways in AML

All genes from the RNAseq of TCGA AML cohort were preranked according to their differential expression (fold change) comparing tumors with high and low expression of *PIP4K2A, PIP4K2B,* and *PIP4K2C,* using their median expression rate as the cutoff. GSEA was performed using GSEA v.4.0 [16] and the Reactome database (https://reactome.org/). Enrichment scores (ES) were calculated based on Kolmogorov-Smirnov statistic, tested for significance using 1000 permutations, and normalized (NES) to take into account the size of each gene set. As suggested by GSEA, a false discovery rate (FDR) cutoff of 25% (FDR *q*-value < 0.25) was used [16].

Statistical analyzes

Statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, Inc., San. Diego, CA, USA) or SPSS statistics for Windows, version 21.0 (SPSS, Chicago, IL, USA). Mann–Whitney test or Kruskal–Wallis test and Dunn post-hoc tests were used for measured factors. Fisher's-exact test and chi-square test were used for categorical factors. Patients were dichotomized according to the median value of *PIP4K2A, PIP4K2B* and *PIP4K2C* relative gene expression. Kaplan-Meier method and log-rank tests were used to estimate survival outcomes. Univariable and multivariable proportional hazards regression analyses were performed for potential prognostic factors for overall survival (OS) and disease-free survival (DFS). OS was defined from time of sampling to date of death or last seen. DFS was defined as the time for relapse/progression after complete remission or last seen. Factors that presented *p*-values ≤ 0.10 in univariate analyzes were included in multivariate analyze. The level of significance was set at $p < 0.05$.

Results

PIP4K2A and *PIP4K2C* transcript levels are reduced in favorable-risk group and impact survival outcomes in AML patients

In TCGA AML cohort ($n=173$), *PIP4K2A* and *PIP4K2C* mRNA levels were significantly reduced in AML patients assigned to the favorable-risk group ($p < 0.05$) (Fig. 1(A)). *PIP4K2B* expression was similar among AML patients stratified by cytogenetic risk. In agreement, high expression of *PIP4K2A* and *PIP4K2C*, but not *PIP4K2B*, were associated with intermediate and adverse cytogenetic risk groups of AML patients (both $p < 0.0001$; Table 1 and Fig. 1(B)). Using microarray data from *Amazonia!* [14], same results were found in an independent AML cohort ($n=528$) (Supplementary Fig. 1), corroborating the association between high *PIP4K2A* and *PIP4K2C* expressions with unfavorable cytogenetic risk in AML.

In univariate proportional hazards regression analyses, low *PIP4K2A* and *PIP4K2C* expression was associated with better OS and DFS in AML patients ($p < 0.05$, Table 2). *PIP4K2B* mRNA levels did not affect clinical outcomes of AML patients (all $p > 0.05$, Table 2). Kaplan Meier and long-rank analysis corroborate these findings (Fig. 2). In the multivariate proportional hazards regression analysis correcting for known cofounders, age, white blood cell count, and cytogenetic risk were independent predictors for DFS and OS (all $p < 0.05$). However, *PIP4K2A* and *PIP4K2C* transcript levels did not retained their clinical significance after adjustment (Table 2).

Next, we investigate whether the combination of high and/or low expression of *PIP4K2A* and *PIP4K2C* genes could refine the identification of risk groups in AML. Using proportional hazards regression analysis (Table 3), and Kaplan

Table 1 Acute myeloid leukemia patients' characteristics from TCGA AML[a] study and their association with *PIP4K2A, PIP4K2B* and *PIP4K2C* gene expression[b].

Factors	Total	*PIP4K2A* expression		*p*-value[d]	*PIP4K2B* expression		*p*-value[d]	*PIP4K2C* expression		*p*-value[d]
		Low	High		Low	High		Low	High	
AML patients	173	87	86		87	86		87	86	
Gender				0.287			1.00			1.00
Male/Female	92/81	50/37	42/44		46/41	46/40		46/41	46/40	
Age (years), median (range):	58 (18–88)	59 (18–81)	58 (21–88)	0.693	61 (22–82)	56 (18–88)	0.061	57 (21–88)	60 (18–81)	0.458
Bone marrow blasts (%), median (range)	72 (30–100)	75 (30–100)	71 (30–97)	**0.032**	70 (30–98)	76 (33–100)	0.051	72 (33–100)	74 (30–99)	0.834
White blood cell count × 10^9/L	17 (0.4–297.4)	29.4 (0.4–297.4)	14.7 (0.6 −223.8)	0.347	22.2 (0.7–223.8)	11.8 (0.4–297.4)	0.136	17 (0.4–223.8)	17.9 (0.06–297.4)	0.306
Cytogenetic risk[c]				**0.002**			0.922			**<0.0001**
Favorable	32	25	7		17	15		27	5	
Intermediate	104	45	59		51	53		46	58	
Adverse	34	16	18		17	17		12	22	
No growth	3	1	2		2	1		2	1	

Abbreviations: TCGA, The Cancer Genome Atlas; AML, acute myeloid leukemia.

[a] The clinical and laboratorial data of TCGA AML cohort were obtained from cBioPortal for Cancer Genomics (http://www.cbioportal.org).

[b] Gene expression values were dichotomized by median.

[c] Karyotype findings included favorable: t(8;21) ($n=7$), inv(16) ($n=7$) and t(15;17) ($n=12$); intermediate: normal ($n=79$), trisomy 8 ($n=6$), trisomy 14 ($n=2$), trisomy 21 ($n=2$), t(9;22) ($n=3$) and others non recurrent abnormalities ($n=12$), and adverse: complex kayotype ($n=7$), −5/del(5q) ($n=13$), −7 ($n=11$), *KMT2A* rearranged ($n=4$) and others abnormalities ($n=2$).

[d] For statistical analyzes, Mann–Whitney test was used for measured factors, and Fisher's exact test or Chi-squared test was used for categorical factors. Statistically significant difference is highlighted in bold.

Table 2 Univariate and multivariate analysis for overall survival and disease-free survival of acute myeloid leukemia patients from TCGA cohort according to clinical and laboratorial characteristics and *PIP4K2A, PIP4K2B* and *PIP4K2C* expression.

Factors	Overall survival						Disease free survival[c]					
	Univariate			Multivariate			Univariate			Multivariate		
	HR[a]	(95% C.I.)	*p*[c]	HR[a]	(95% C.I.)	*p*[c]	HR[a]	(95% C.I.)	*p*[c]	HR[a]	(95% C.I.)	*p*[c]
Gender Male *vs.* female	0.97	0.68–1.40	0.85	–	–	–	0.99	0.64–1.52	0.974	–	–	–
Age at diagnosis[b]	1.04	1.03–1.06	**<0.0001**	1.04	1.03–1.06	**<0.0001**	1.01	0.99–1.02	0.054	1.01	1.001–1.03	**0.047**
White blood cell count[b]	1.004	1.0008–1.008	**0.02**	1.007	1.003–1.01	**0.001**	1.006	1.00–1.01	**0.007**	1.006	1.001–1.03	**0.004**
Cytogenetics risk[c] Adverse *vs.* intermediate *vs.* favorable	1.97	1.47–2.64	**<0.0001**	1.81	1.30–2.51	**<0.0001**	1.7	1.22 – 2.37	**0.002**	1.49	1.006–2.19	**0.047**
PIP4K2A expression[d] High *vs.* low expression	1.50	1.03–2.17	**0.03**	1.37	0.93–2.01	0.11	1.64	1.06–2.53	**0.025**	1.37	0.86–2.18	0.176
PIP4K2B expression[d] High *vs.* low expression	0.81	0.56–1.18	0.28	–	–	–	0.84	0.55–1.3	0.457	–	–	–
PIP4K2C expression[d] High *vs.* low expression	1.46	1.01–2.11	**0.04**	1.14	0.77–1.68	0.49	1.62	1.05–2.49	**0.027**	1.26	0.79–2.02	0.321

Abbreviations: TCGA, The Cancer Genome Atlas.

Statistically significant difference is highlighted in bold.

[a] Hazard ratios (HR) >1 indicate that increasing values for continuous variable or the first factor for categorical variable has the poorer outcome.

[b] Factors were analyzed as continuous variable.

[c] Missing values were excluded in the calculation of *p*-values.

[d] Factors were dichotomized by median.

Table 3 Univariate and multivariate analysis for overall survival and disease-free survival of acute myeloid leukemia patients from TCGA cohort according to clinical and laboratorial characteristics and *PIP4K2A*/*PIP4K2C* expression.

Factors	Overall survival						Disease free survival[c]					
	Univariate			Multivariate			Univariate			Multivariate		
	HR[a]	(95% C.I.)	*p*[c]	HR[a]	(95% C.I.)	*p*[c]	HR[a]	(95% C.I.)	*p*[c]	HR[a]	(95% C.I.)	*p*[c]
Gender Male *vs.* female	0.96	0.66–1.39	0.847	–	–	–	0.99	0.64–1.52	0.974	–	–	–
Age at diagnosis[b]	1.04	1.02–1.05	>**0.001**	1.04	1.02–1.06	>**0.001**	1.01	0.99–1.02	0.054	1.01	1.002–1.03	**0.047**
White blood cell count[b]	1.004	1.00–1.008	**0.017**	1.006	1.002–1.01	**0.002**	1.006	1.001–1.01	**0.007**	1.006	1.002–1.01	**0.004**
Cytogenetics risk[c] Adverse *vs.* intermediate *vs.* favorable	1.98	1.47–2.66	>**0.001**	1.78	1.28–2.49	**0.001**	1.7	1.22–2.37	**0.002**	1.49	1.01–2.19	**0.039**
PIP4K2A/PIP4K2C expression[d] High/High *vs.* High/Low *vs.* Low/High *vs.* Low/Low	1.23	1.06–1.44	**0.008**	1.18	1.02–1.39	**0.038**	1.28	1.07–1.53	**0.007**	1.49	0.99–1.44	0.057

Abbreviations: TCGA, The Cancer Genome Atlas.

Statistically significant difference is highlighted in bold.

[a] Hazard ratios (HR) >1 indicate that increasing values for continuous variable or the first factor for categorical variable has the poorer outcome.

[b] Factors were analyzed as continuous variable.

[c] Missing values were excluded in the calculation of *p*-values.

[d] *PIP4K2A* and *PIP4K2C* were dichotomized by median.

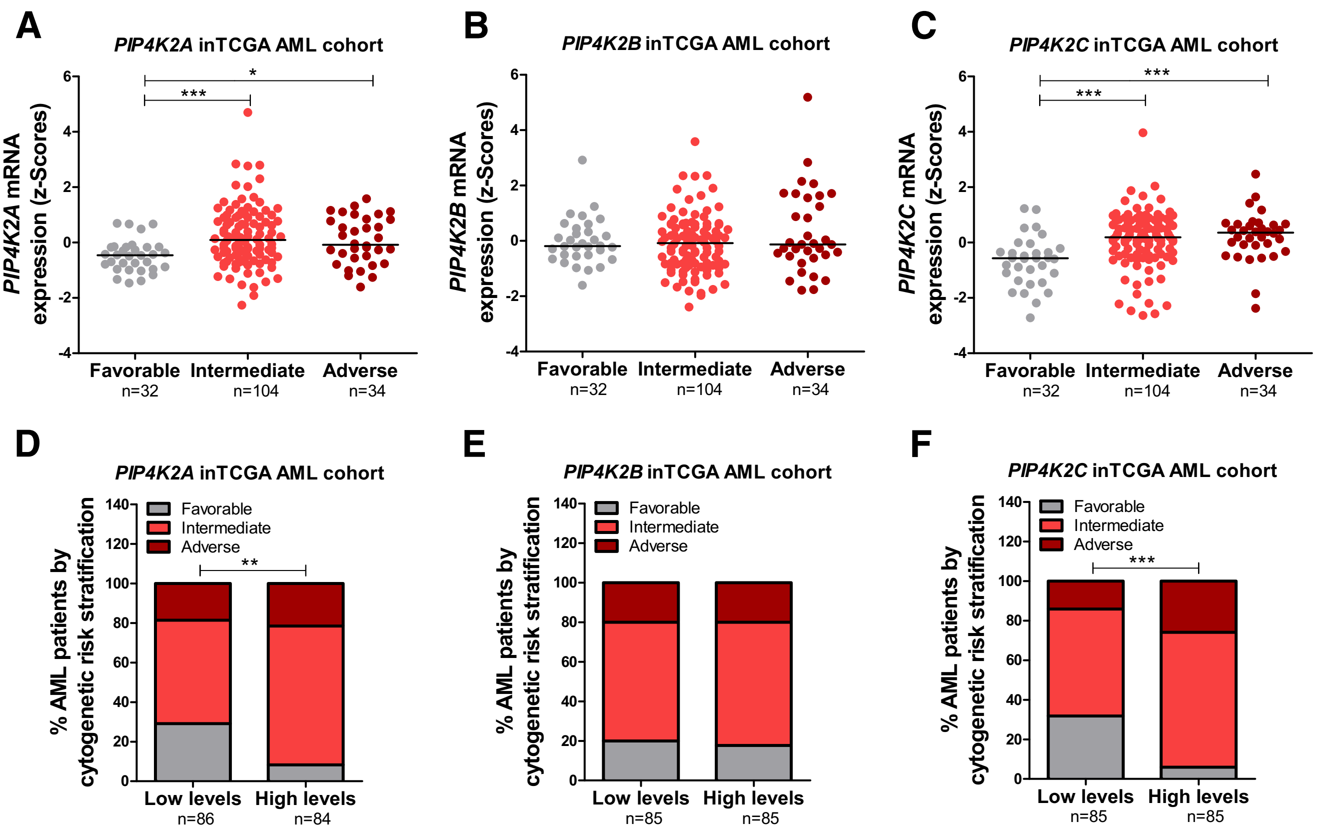


Fig. 1 *PIP4K2A* and *PIP4K2C* are highly expressed in intermediate and adverse cytogenetic risk of acute myeloid leukemia patients. *PIP4K2A* (A), *PIP4K2B* (B) and *PIP4K2C* (C) mRNA levels were compared among acute myeloid patients (AML) stratified by cytogenetic risk. The "*y*" axis represents the *PIP4K2* mRNA levels and is expressed as *z*-score. Horizontal lines represent the median. Numbers of subjects for each group are indicated. $^{*}p < 0.05$, $^{***}p < 0.0001$; Kruskal–Wallis test and Dunn post-hoc test. Association analysis of *PIP4K2A* (D), *PIP4K2B* (E) and *PIP4K2C* (F) mRNA levels and AML cytogenetic risk. The "*y*" axis represents the percentage of AML patients by cytogenetic risk stratification. Gene expression values were dichotomized by the median value. The number of AML patients for each group is indicated. $^{**}p < 0.001$, $^{***}p < 0.0001$; chi-square test.

Meier and log-rank analysis (Fig. 3), we observed that AML patients with low *PIP4K2A* and low *PIP4K2C* presents better survival outcomes than AML patients with high *PIP4K2A* and/or *PIP4K2C*. In multivariate analyses, the stratification in four groups according to the median of *PIP4K2A* and *PIP4K2C* expression was an independent predictor of poor OS (Table 3).

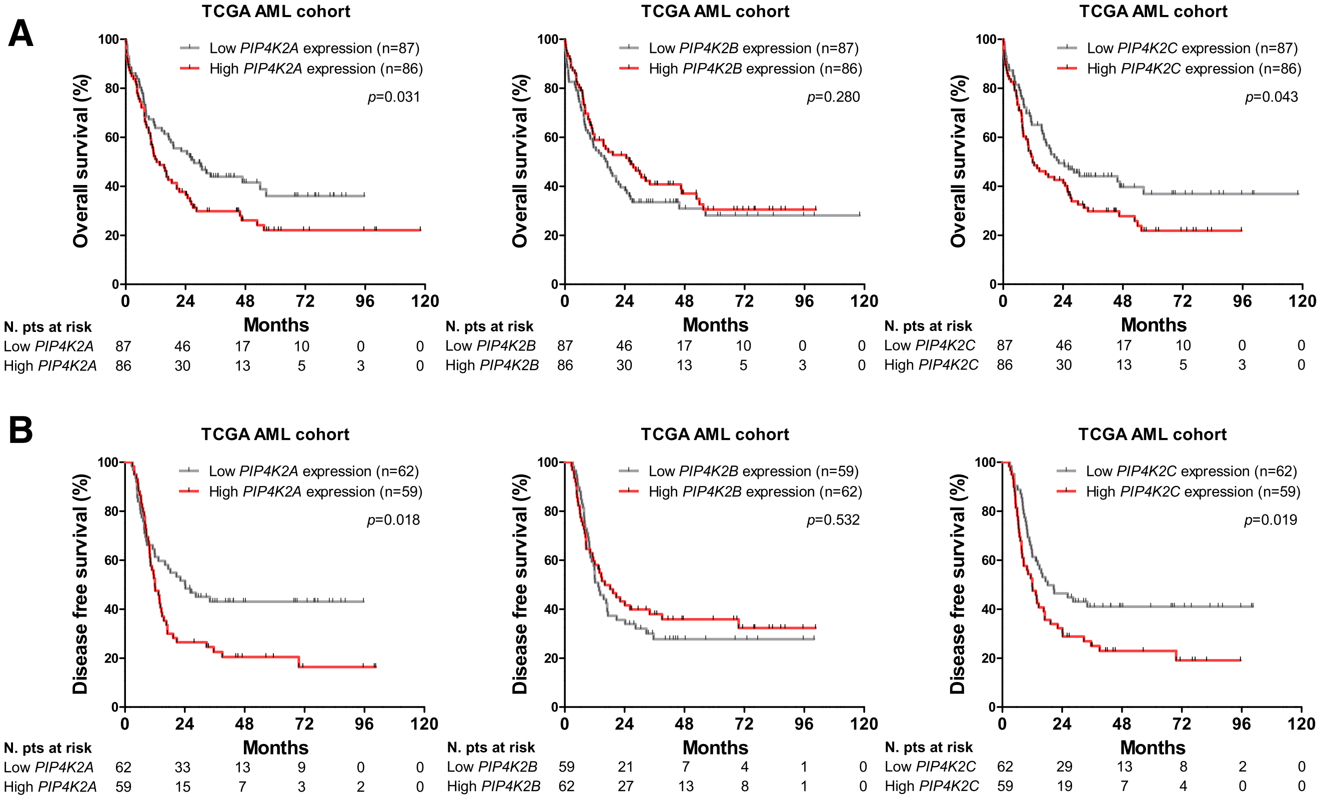
Regarding recurrent mutations in AML, *PIP4K2A* high expression was associated with higher frequency of *DNMT3A* mutations, *PIP4K2B* high expression was associated with reduced frequency of *NPM1* mutations, and *PIP4K2C* high expression was associated with high frequency of *NPM1* and *TP53* mutations, and low frequency of *RUNX1* mutations (all $p < 0.05$, Table 4).

GSEA analysis adds novel insights on PIP4K2 signaling network in AML

We performed GSEA to evaluate the association between *PIP4K2* genes expression and relevant biological pathways in AML. Multiple biological pathways were enriched in samples with high *PIP4K2A* expression, especially those related to the immune system and signal transduction. Among these pathways, we highlighted the generation of second messenger molecules, which is a well-defined function of PIP4K2s, and the CTNNB1 and ERK signaling cascades, which have been shown to modulate the leukemia phenotype (Fig. 4(A), Supplementary Table 1). On the other hand, high *PIP4K2B* was negatively enriched by multiple biological pathways, some of than opposite to *PIP4K2A*, including immune system-related gene sets and CTNNB1- and ERK-related pathways (Fig. 4(B), Supplementary Table 2). Notably, tumors with low *PIP4K2C* expression showed an enrichment of pathways involved in the metabolism of RNA (an emergent pathway related with hematological neoplasm), cell cycle (especially involved in cell cycle checkpoints and chromosomal stability) and DNA repair (Fig. 4(C), Supplementary Table 3). These findings become interesting if we consider the association between cytogenetic abnormalities and high *PIP4K2C* expression levels in AML, suggesting that the high *PIP4K2C* expression may be associated with genomic instability network.

Discussion

In the present study, we found that high *PIP4K2A* and *PIP4K2C* expression were associated with cytogenetics find-



A

N. pts at risk	0	24	48	72	96	120
Low *PIP4K2A*	87	46	17	10	0	0
High *PIP4K2A*	86	30	13	5	3	0

N. pts at risk	0	24	48	72	96	120
Low *PIP4K2B*	87	46	17	10	0	0
High *PIP4K2B*	86	30	13	5	3	0

N. pts at risk	0	24	48	72	96	120
Low *PIP4K2C*	87	46	17	10	0	0
High *PIP4K2C*	86	30	13	5	3	0

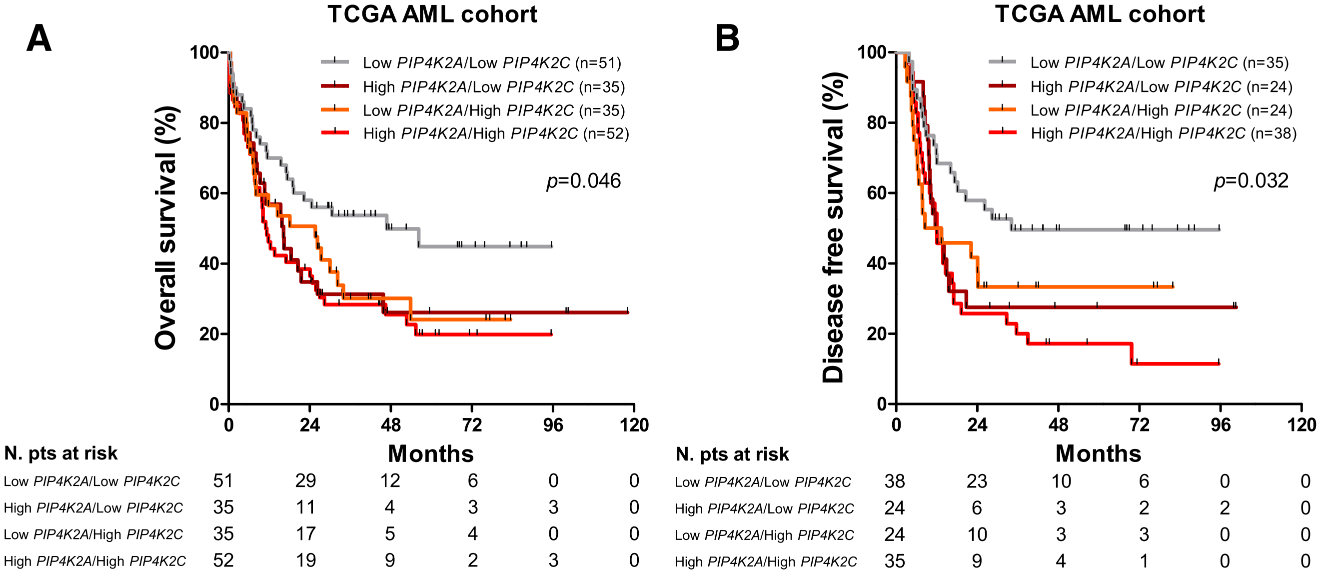
B

N. pts at risk	0	24	48	72	96	120
Low *PIP4K2A*	62	33	13	9	0	0
High *PIP4K2A*	59	15	7	3	2	0

N. pts at risk	0	24	48	72	96	120
Low *PIP4K2B*	59	21	7	4	1	0
High *PIP4K2B*	62	27	13	8	1	0

N. pts at risk	0	24	48	72	96	120
Low *PIP4K2C*	62	29	13	8	2	0
High *PIP4K2C*	59	19	7	4	0	0

Fig. 2 High *PIP4K2A* and *PIP4K2C* negatively impact survival outcome of acute myeloid leukemia (AML) patients from The Cancer Genome Atlas (TCGA) AML study. Kaplan–Meier analyzes of disease-free survival (DFS) (A) and overall survival (OS) (B) according to *PIP4K2A, PIP4K2B* or *PIP4K2C* mRNA levels. Patients were dichotomized according to the median value of gene expression for survival analyzes. In TCGA AML cohort, patients with high *PIP4K2A* expression had a significantly shorter DFS and OS than patients with low *PIP4K2A* expression. AML patients with high *PIP4K2C* expression had a significantly shorter OS than patients with low *PIP4K2C* expression. *PIP4K2B* did not impact DFS and OS of TCGA AML cohort. Number (N.) of patients (pts) at risk and *p* values are indicated; log-rank test.



A

N. pts at risk	0	24	48	72	96	120
Low *PIP4K2A*/Low *PIP4K2C*	51	29	12	6	0	0
High *PIP4K2A*/Low *PIP4K2C*	35	11	4	3	3	0
Low *PIP4K2A*/High *PIP4K2C*	35	17	5	4	0	0
High *PIP4K2A*/High *PIP4K2C*	52	19	9	2	3	0

B

N. pts at risk	0	24	48	72	96	120
Low *PIP4K2A*/Low *PIP4K2C*	38	23	10	6	0	0
High *PIP4K2A*/Low *PIP4K2C*	24	6	3	2	2	0
Low *PIP4K2A*/High *PIP4K2C*	24	10	3	3	0	0
High *PIP4K2A*/High *PIP4K2C*	35	9	4	1	0	0

Fig. 3 Acute myeloid leukemia (AML) patients from The Cancer Genome Atlas (TCGA) AML study with low *PIP4K2A*/low *PIP4K2C* present better survival outcomes. Kaplan–Meier analyzes of disease-free survival (DFS) (A) and overall survival (OS) (B) according to *PIP4K2A* plus *PIP4K2C* expression, which dichotomized according to the median value. In TCGA AML cohort, patients with low *PIP4K2A* plus low *PIP4K2C* expression had better DFS and OS than patients with high *PIP4K2A* and/or *PIP4K2C* expression. Number (N.) of patients (pts) at risk and *p* values are indicated; log-rank test.

Table 4 Association between *PIP4K2A, PIP4K2B* and *PIP4K2C* gene expressions and recurrent mutations in acute myeloid leukemia patients from TCGA AML[a] study.

Genes	AML patients	*PIP4K2A* expression			*PIP4K2B* expression			*PIP4K2C* expression		
	(*n* = 173)	Low (*n* = 86)	High (*n* = 87)	*p*-value[b]	Low (*n* = 86)	High (*n* = 87)	*p*-value[b]	Low (*n* = 86)	High (*n* = 87)	*p*-value[b]
FLT3, mutated (%)	49 (28)	27 (31)	22 (25)	0.40	27 (31)	22 (25)	0.40	23 (28)	26 (30)	0.73
NPM1, mutated (%)	48 (28)	23 (27)	25 (29)	0.86	30 (35)	18 (21)	**0.04**	14 (16)	34 (39)	**0.001**
DNMT3A, mutated (%)	42 (24)	12 (14)	30 (34)	**0.002**	23 (27)	19 (22)	0.48	15 (17)	27 (31)	0.05
RUNX1, mutated (%)	24 (14)	10 (12)	14 (16)	0.51	10 (12)	14 (16)	0.51	20 (23)	4 (5)	**0.0004**
IDH2, mutated (%)	17 (10)	9 (10)	8 (9)	0.80	7 (8)	10 (11)	0.61	8 (9)	9 (10)	1.00
IDH1, mutated (%)	16 (9)	10 (11)	6 (7)	0.30	4 (5)	12 (14)	0.06	6 (7)	10 (11)	0.43
TET2, mutated (%)	15 (9)	7 (8)	8 (9)	1.00	6 (7)	9 (10)	0.59	7 (8)	8 (9)	1.00
TP53, mutated (%)	14 (8)	5 (6)	9 (10)	0.40	10 (12)	4 (5)	0.10	3 (4)	11 (13)	**0.04**
CEBPA, mutated (%)	13 (8)	3 (4)	10 (11)	0.08	6 (7)	7 (8)	1.00	8 (9)	5 (6)	0.40
NRAS, mutated (%)	12 (7)	3 (4)	9 (10)	0.13	8 (9)	4 (5)	0.24	4 (5)	8 (9)	0.37
WT1, mutated (%)	10 (6)	6 (7)	4 (5)	0.53	6 (7)	4 (5)	0.53	8 (9)	2 (3)	0.06

Abbreviations: TCGA, The Cancer Genome Atlas; AML, acute myeloid leukemia.
Gene expression values were dichotomized by median.

[a] The clinical and laboratorial data of TCGA AML cohort were obtained from cBioPortal for Cancer Genomics (http://www.cbioportal.org).

[b] Fisher's exact test. Statistically significant difference is highlighted in bold.

ings at presentation and negatively influenced survival outcomes of AML patients. Although we have previous demonstrated that *PIP4K2A* mRNA expression did not differ between normal and AML bone marrow samples [7], the current study indicated that levels of *PIP4K2A* among AML patients appear to be important for prognostication and provide initial evidence of the participation of *PIP4K2C* in hematologic malignancies.

Some recurrent mutations in AML were associated with the *PIP4K2* expression, including *DNMT3A, NPM1, RUNX1*, and *TP53* mutations. The relevance of *DNMT3A* mutations in the development of hematological malignancies has been reported [17–19]. In AML, *DNMT3A* mutations have been associated with a worse prognosis [20–22]. *RUNX1* mutations may have paradoxical roles in AML: when associated with the *RUNX1-RUNX1T1* rearrangement, it is associated with good prognosis, however when present in patients with noncomplex karyotype, it negatively impacts on clinical outcomes [23–25]. The interpretation of *NPM1* mutations in AML is also complex since the interaction with other mutations (*e.g. FLT3* and *DNMT3A* mutations) alters its prognostic value [23,26–30]. Thus, the interpretation of our results in the light of *NPM1* mutations isolated is not informative. On the other hand, *TP53* mutations are strongly associated with poor response to chemotherapy, cytogenetic abnormalities, and worse prognosis in AML [23,31–33]. In the present study, the high *PIP4K2A* and *PIP4K2C* expression was associated with increased frequency of *DNMT3A* and *TP53* mutations, respectively, which corroborates that the high expression of both *PIP4K2* genes are related to AML groups with higher genetic risk. However, the association between the high *PIP4K2C* expression and low frequency of *RUNX1* mutations may be related to the fact that 30% of these mutations observed in TGCA AML cohort are linked to *RUNX1-RUNX1T1*, which is a group of good prognosis in AML.

PIP4K2 proteins appear to play important physiological functions during normal development and in regulation of oxidative stress signaling [34–36]. Although the PIP4K2 isoforms exhibit similar biochemical function, there are differences in potency of enzyme activity, expression between different tissues, and cellular sublocalization [3,37]. Regarding the functions of these proteins in cancer phenotype, the general knowledge is still under construction. PIP4K2A silencing did not modulate the malignant phenotype of HEL (AML) and Namalwa (acute lymphoblastic leukemia) cell lines [7], but reduced the cell viability of K562 (chronic myeloid leukemia) cell line [6]. On the other hand, Jude et al. [9] using the targeted knockdown screen for phosphoinositide modulator-related genes, identified that PIP4K2A play important functions, which are in part dependent of CDKN1A, for survival, proliferation, clonogenicity and leukemia-initiating potential in primary human AML cells and murine MLL-AF9 AML model. In addition, PIP4K2A plus PIP4K2B inhibition only influenced *TP53* mutated/null models in solid tumors [36]. These results suggest that different signaling backgrounds and cellular types may be modulated by the PIP4K2A functions. However, the biological

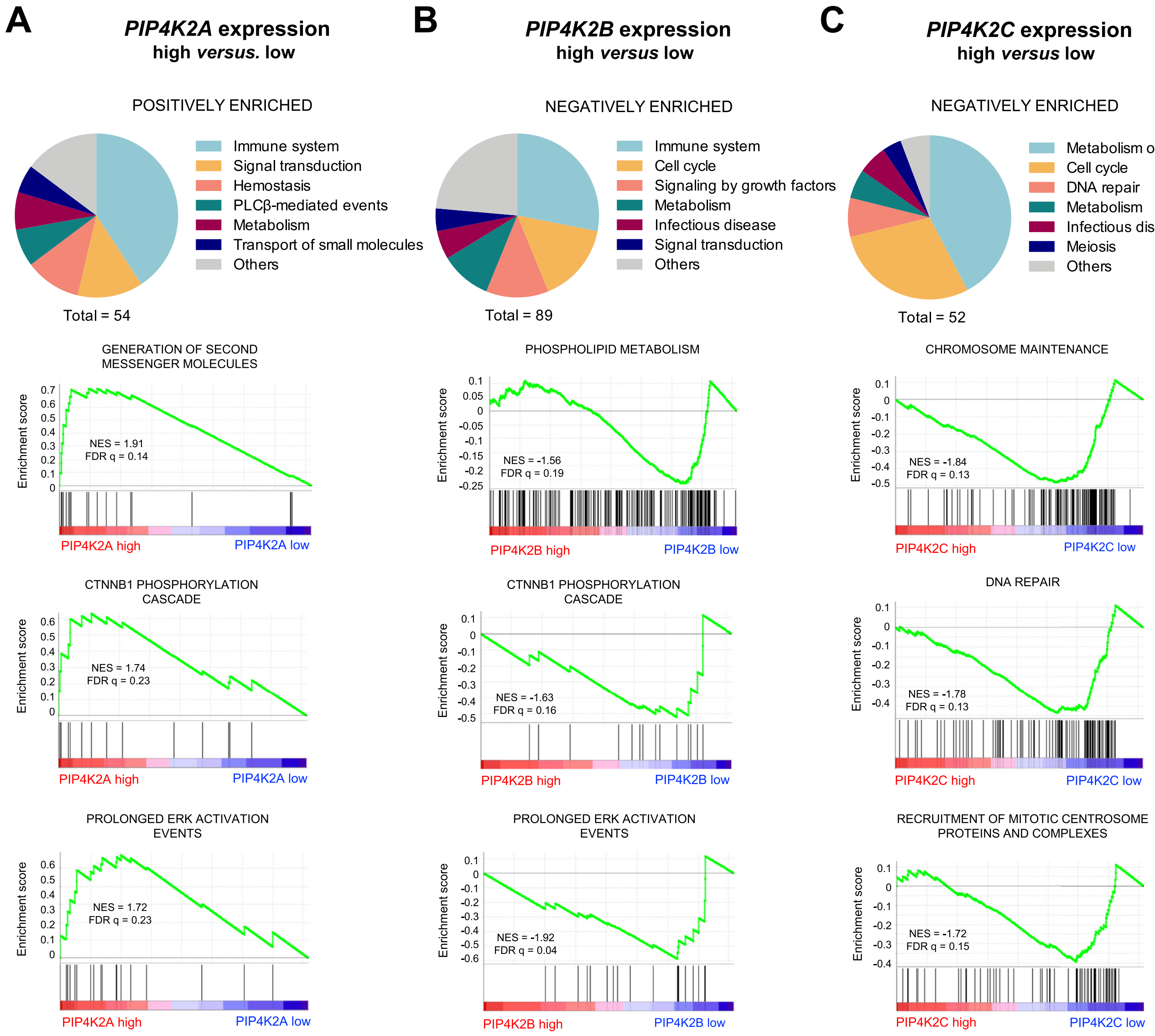


Fig. 4 Gene Set Enrichment Analysis (GSEA) adds novel insights into PIP4K2s biological pathways in acute myeloid leukemia. (*Upper panel*) pie charts illustrate the ontologies of significantly enriched pathways (FDR adjust p-value $>$ 0.25) comparing acute myeloid leukemia (AML) patients with high *versus* low expression of *PIP4K2A* (A), *PIP4K2B* (B) and *PIP4K2C* (C). (*Lower panels*) Representative GSEA plots reflecting the degree to which the expression of a gene set is up- or down-regulated in AML patients with high *versus* low expression of *PIP4K2A* (A), *PIP4K2B* (B) and *PIP4K2C* (C). The top portion of the plot shows the running enrichment scores (ES) for the gene set. The point with the maximum deviation from zero is defined as the ES for the gene set. The leading edge subset (the subset of genes with the most significant contribution to the ES) is shown as vertical bars accumulated prior to the peak score, for positive ES, or subsequent to the peak score, for negative ES. FDR-adjusted p-values and enrichment scores normalized for gene set size (NES) were indicated in each plot.

significance of PIP4K2B and PIP4K2C proteins remains to be explored.

Our functional analysis of PIP4K2 signaling networks by GSEA, indicated that high *PIP4K2A* expression is associated with an enrichment of signal transduction cascades involved in leukemia phenotype, including CTNNB1- and ERK-related pathways. Aberrant β-catenin (CTNNB1) expression associated with activation of Wnt signaling has been reported in primary AML samples, which was related to clonal growth capacity and survival outcomes [38]. In AML mice models, Wnt signaling is important for propagation/acceleration of the disease and to the transformation of leukemic stem cells [39]. Similarly, it is well established that ERK signaling pathway activation contributes to a malignant phenotype, aggressiveness and chemotherapy resistance in leukemia [40].

In the present study, *PIP4K2C* expression negatively correlated with the expression of genes involved in the metabolism of RNA and genomic instability by GSEA analysis. The recurrence of mutations in genes involved in the machinery of RNA processing and metabolism has drawn attention to this cellular process in leukemogenesis and has been a field of intense study, but the clinical and biological significance of these alterations still under construction [41–44]. On the other hand, the functional significance of reduced expression of genes involved in DNA repair and chromosomal maintenance is clearer in the context of hematological malignancies. Alterations in these processes result in accumulation of mutations, aneuploidy, clonal evolution, and development and progression of the disease, being markers of poor prognosis [23,45]. The association between high *PIP4K2C* expression and increased frequency of *TP53* mutations corroborates that this gene may be involved in genetic instability.

Although in our data indicate that combined analysis of the expression of *PIP4K2A* and *PIP4K2C* displayed a prognostic value in presented study, it is important to note that recent findings indicate that PIP4K2 have a therapeutic potential in hematological malignancies. McElligott et al. [10] reported that PIP4K2 selective inhibitors inhibit *in vitro* cellular growth of leukemia cell lines and induce regression of AML xenograft tumors *in vivo*. Taken together, these results open a new round of investigation for PIP4K2 in leukemia.

In conclusion, reduced *PIP4K2A* and *PIP4K2C,* but not *PIP4K2B*, mRNA levels were associated with favorable cytogenetic findings and positively impacted the clinical outcomes of AML patients. Our study provides additional evidence of the involvement of members of the PIP4K2 family (PIP4K2A and PIP4K2C) in AML.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Declarations of interest

None.

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Author contributions

K.L. conception and design, data analysis and interpretation, and manuscript writing. J.L.C-S., G.S.K. and D.A.P-M data analysis and interpretation, and manuscript editing. F.T., P.A.C.M.F. and R.P.M. conception, data interpretation and manuscript editing. A.R.L-A data analysis and interpretation, manuscript writing and final approval of manuscript. JAM-N conception and design, data analysis and interpretation, manuscript writing and final approval of manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.04.002.

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