PTPN11 mutations in the pathogenesis of pediatric myeloid neoplasms

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INTRODUCTION

Acute myeloid leukemia (AML) and other myeloid neoplasms, are associated with inappropriate activation or inactivation of genes in signaling pathways of myeloid cells. RAS/MAPK pathway genes undergoes the major signaling disruption in hematopoietic malignancies. For instances, *PTPN11* which is a positive regulator of cell survival and differentiation. *PTPN11* gene encodes cytoplasmic protein tyrosine phosphatase 2 (Shp-2) which contains two N-terminal SH2 domains, a catalytic PTP domain, a C-terminal tail with two tyrosine phosphorylation sites, and a proline-rich domain (Fig 1). Somatic mutations in *PTPN11* is found in myeloproliferative disorders (MD) and AML in different frequency distribution rates. The diagnostic variables to distinguish MD and AML should taken in account the variables, age, blast percentage, absence of *BCR-ABL1, PTPN11* mutations and the monosomy of chromosome 7 (mono 7). The distinction between MD and AML in childhood, is a challenge.

AIM

To identify genetic mutations in RAS/MAP kinase pathways, with emphasis in *PTPN11,* in order to distinguish somatic and germinal mutations.

MATERIAL AND METHODS

A series 388 myeloid neoplasm is subject of this study [AML (n, 342); JMML (n, 11); CML (n, 28) and MDS (n, 7)] referred for the Pediatric Hematology-Oncology Program (PHOP) between Jan/2010-May/2017. The identification of AML gene fusions (*RUNX1-RUNX1T1, CBFb-MYH11, PML-RARα*, *BCR-ABL1* and *KMT2A* rearrangements) for characterization of AMLs was performed using FISH, and/or RT-PCR, or multiplex RT-PCR techniques. Monosomy 7 were identified by FISH and exon 3 of the *PTPN11* gene was analyzed by Sanger sequencing (Fig 2). Frequency calculations and univariate analyzes were performed using the χ 2 and Fisher exact tests. Estimates of overall survival (OS) were performed using the Kaplan-Meier method and Log-Rank test.

RESULTS

Table 1. Demographic characteristics and classification of pediatric AML cases.

AML features	n⁺/n total (%)
Age groups (years)	
≤2	65/342 (19.0)
>2-10	130/342 (38.0)
>11	147/342 (43.0)
Sex	
Males	173/342 (50.6)
Females	169/342 (49.4)
Race	
Whites	142/342 (41,5)
Non-Whites	200/342 (58,5)
Geographic regions	
Northeast	107/342 (31.3)
Midwest	135/342 (39.5)
Southeast	84/342 (24.6)
South	16/342 (4.7)
WBC (x10⁹/l)	
=50	233/341 (68,3)
>50	108/341 (31,7)
* Morphological classification	
AML with minimal differentiation (M0)	19/342 (5.6)
AML without maturation (M1)	17/342 (5,0)
AML with maturation (M2)	43/342 (12.6)
Acute promyelocytic leukemia (M3)	76/342 (22.2)
Acute myelomonocytic leukemia (M4)	92/342 (26.9)
monoblastic/monocytic leukemia (M5)	50/342 (14.6)
Acute erythroid leukemia (M6)	5/342 (1.5)
Acute megakaryoblastic leukemia (M7)	25/342 (7.3)
Not Otherwise specified-NOS	15/342 (4.4)
Class alterations II**	
Presense	127/244 (52,0)
Absence	117/244 (48,0)

*According to the Franco-American-British group (FAB).
**Type II alterations include *RUNX1-RUNX1T1, CBFb-MYH11, PML-RARα*, *KMT2A* rearrangements, acute myeloid leukemia.; n⁺= número de casos positivos

Table 2. Clinical-laboratory characteristics of cases with mutation in *PTPN11*

	Myeloid neoplasms		
	JMML n(%)	CML n(%)	MDS n(%)
Age groups (years)			
≤2	3 (27.3)	1 (3.6)	1 (14.3
>2-10	5(45.5)	3 (10.7)	3 (42.9)
>11	3 (27.3)	24 (85.7)	3 (42.9)
Median (range)	46 (5-172)	177 (19-223)	27.0 (22-213)
Sex			
Males	6 (54,5)	15 (53.6)	6 (85.7)
Females	5 (45,5)	13 (46.4)	1 (14.3)
Race			
Whites	7 (63.6)	11 (39.3)	5 (71.4)
Non-Whites	4 (36.4)	17 (60.7)	
Geographic regions			
Northeast	4(27.3)	17(60.7)	2(28.6)
Midwest	3(36.4)	2(7.1)	2(28.6)
Southeast	2(18.2)	8(28.6)	2(28.6)
South	2(18.2)	1(3.6)	1(14.3)
WBC (x10⁹/l)			
≤50	4 (36.4)	5 (17.9)	5 (71.4)
>50	7 (63.6)	23 (82.1)	7 (28.6)
Median (range)	175 (0-540)	294 (4-300)	6.82 (4-590)
Total	11 (24.0)	28 (61.0)	7 (15.0)

JMML, juvenile myelomonocytic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndromes

Table 3. Distribution of the frequency of molecular changes in cases of pediatric AML according to age and gender

		Age groups (years)				Sex		
Molecular alterations [a]	Frequency n/total (%)	≤2 n (%)	>2-10 n (%)	≥11 n (%)	*p*	Males n (%)	Females n (%)	*p*
Class alterations II								
RUNX1-RUNX1T1								
POS	39/296 (13.1)	2 (5.1)	21 (53.8)	16 (41.0)	0.019	22 (56.4)	17 (43.6)	0.192
NEG	257/296 (86.8)	58 (22.6)	92 (35.8)	107 (41.6)		122 (47.5)	135 (52.5)	
CBFß-MYH11								
POS	17/292 (5.8)	2 (11.8)	6 (35.3)	9 (52.9)	0.525	5 (29.4)	12 (70.6)	0.087
NEG	275/292 (94.1)	58 (21.1)	105 (38.2)	112 (40.7)		136 (49.5)	139 (50.5)	
Rearranjos do *KMT2A*								
POS	39/225 (17.3)	23 (59.0)	9 (23.1)	7 (17.9)	< 0.0001	16 (41.0)	23 (59.0)	0.339
NEG	186/225 (82.6)	36 (19.4)	70 (37.6)	80 (43.0)		86 (46.2)	100 (53.8)	
PML-RARα								
POS	51/92 (55.4)	1 (2.0)	21 (41.2)	29 (56.9)	0.346	26 (51.0)	25 (4.0)	0.500
NEG	41/92 (44.6)	3 (7.3)	19 (46.3)	19 (46.3)		20 (48.8)	21 (51.2)	
Class alterations I								
FLT3 (ITD or TKD)								
MUT	60/269 (22.3)	1 (1.7)	21 (35.0)	38 (63.3)	<0.0001	29 (48.3)	31 (51.7)	0. 531
WT	209/269 (77.7)	51 (24.4)	86 (41.1)	72 (34.4)		100 (47.8)	109 (52.2)	
KRAS								
MUT	9/288 (3.1)	1 (11.1)	4 (44.4)	4 (44.4)	0.847	4 (44.4)	5 (55.6)	0.526
WT	279/288 (96.9)	52 (18.6)	111 (39.8)	116 (41.6)		137 (49.1)	142 (50.9)	
NRAS								
MUT	22/267 (8.2)	5 (22.7)	7 (31.8)	10 (45.5)	0.663	10 (45.5)	12 (54.5)	0.449
WT	245/267 (91.7)	42 (17.1)	100 (40.8)	103 (42.0)		121 (49.4)	124 (50.6)	
c-KIT								
MUT	15/193 (7,7)	2 (13.3)	9 (60.0)	4 (26.7)	0.225	9 (60.0)	6 (40.0)	0.174
WT	178/193 (92.2)	45 (25.3)	67 (37.6)	66 (37.1)		78 (43.8)	100 (56.2)	
PTPN11								
MUT	17/230 (7.4)	1 (5.9)	7 (41.2)	9 (52.9)	0.377	12 (70.6)	5 (29.4)	0.080
WT	213/230 (92.6)	39 (18.3)	87 (40.8)	87 (40.8)		106 (49.8)	107 (50.2)	

[a]The total number of cases analyzed reflects the availability of biological material for molecular tests; POS, positive; NEG, negative; MUT, mutated; WT, wild type; ITD, duplications in tandem; TKD, tyrosine kinase doman

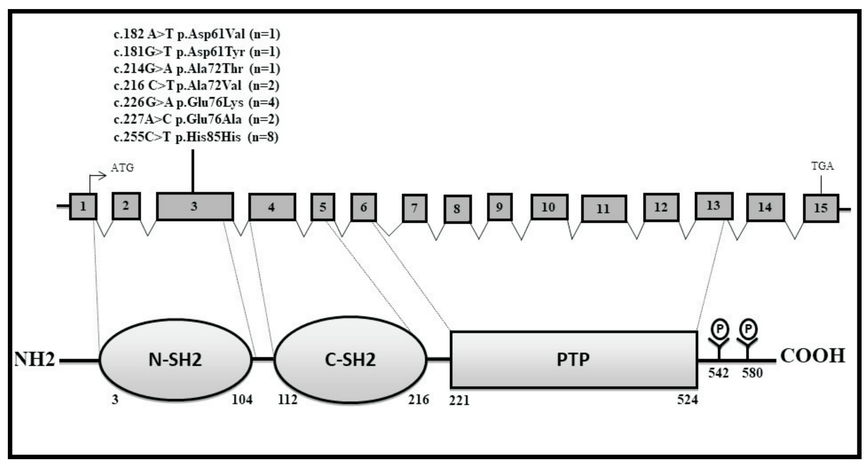

Figure 1. Structure of the the SHP-2 protein tyrosine phosphatase encoded by *PTPN11* gene and the mutations identified in exon 3. *PTPN11* has 15 exons: exons 2 and 3 encode the N-SH2 domain, exons 4 and 5, the C-SH2 domain, exons 6 to 13, domain tyrosine phosphatase (PTP), and exons 14 and 15 encode the carboxy-terminal region. Numbers of amino acid residues delineating various domains or corresponding to the phosphorylation sites are indicated in the figure. N, number of cases.

Table 4. Univariate analysis for overall survival parameters of AML cases

	Univariate analysis			
	N (N of events)	5-year pOS, (SE)	Median [a], (95% CI)	*p* [b]
Geographic regions of treatment [c]	206 (108)			0.001
Northeast	82 (45)	95.7 (2.9)	15.0 (7.3-22.6)	
South	10 (2)	77.1 (14.4)	e	
Southeast	47 (18)	54.6 (8.4)	e	
Midwest	67 (43)	8.8 (6.9)	6.0 (4.1-7.8)	
Age range (years) [c]	206 (108)			0.077
≤2	54 (24)	94.3 (2.8)	34.0 (10.4-57.5)	
>2-10	70 (35)	91.5 (3.1)	15,0 (7.9-22.0)	
≥11	82 (49)	27.9 (5.7)	11.8 (2.7-11.2)	
Race [c]	206 (108)			0.009
Whites	87 (40)	38.4 (8.2)	21,0 (0.0-46,7)	
Non-Whites	119 (68)	25.4 (5.1)	8.0 (3.8-12.1)	
Sex [c]	206 (108)			0.397
Males	106 (59)	28.3 (5.8)	13.0 (8.1-17.8)	
Females	100 (49)	36.3 (6.4)	16.0 (7.1-24.8)	
WBC (x10⁹/l) [c]	205 (108)			0.899
≤50	143 (72)	34.3 (5.1)	15.0 (7.8-22.1)	
>50	62 (36)	28.1 (7.4)	11.0 (6.2-15.7)	
Morphological classification [c]	206 (108)			0.457
AML with minimal differentiation (M0)	13 (5)	90.0 (9.5)	e	
AML without maturation (M1)	12 (4)	89.5 (5.0)	15.0 (0.0-63.0)	
AML with maturation (M2)	38 (22)	34.6 (8.6)	30.0 (0.6-15.3)	
Acute myelomonocytic leukemia (M4)	69 (39)	75.0 (6.9)	8.0 (3.3-18.6)	
monoblastic/monocytic leukemia (M5)	42 (20)	40.2 (9.2)	11.0 (7.5-22.4)	
Acute erythroid leukemia (M6)	5 (3)	94.7 (5.1)	15.0 (0.0-49.5)	
Acute megakaryoblastic leukemia (M7)	19 (12)	24.7 (11.4)	23.0 (0.5-5.42)	
Not Otherwise specified-NOS	8 (3)	44.4 (22.2)	3.0 (0.0-114.5)	
Type II mutations [d]				
RUNX1-RUNX1T1 [c]	30 (18)	23.8 (10.0)	11.4 (0.0-23.8)	0.585
CBFß-MYH11 [c]	13 (4)	67.1 (13.5)	e	0.063
KMT2A rearrangements [c]	31 (18)	17.6 (10.4)	10.6 (0.0- 21.7)	0.192
Type I mutations [c, d]				
FLT3	28 (17)	95.4 (1.8)	11.2 (7.1-15.2)	0.837
KRAS	7 (4)	98.2 (1.0)	16.6 (8.2-25.0)	0.788
NRAS	17 (6)	57.4 (13.4)	e	0.156
c-*KIT*	13 (5)	53.7 (0.9)	e	0.585
PTPN11	14 (12)	0.0 (0.0)	3.0 (0.0-6.4)	0.001
Concomitant type I mutations [c]	67 (36)			0.090
Single mutation	60 (30)	38.2 (7.4)	11.2 (5.78-16.6)	
More than one mutation	7 (6)	82.4 (5.1)	2.3 (0.0-5.78)	

[a] Median survival in months. [b] p values from log-rank test indicate whether the differences are significant between the subgroups. [c] Excluding acute promyelocytic leukemia subtype. [d] Analysis performed between groups positive and negative for the molecular alteration. e Median not reached. CI, confidence interval; N, number; pOS, the probability of overall survival; SE, Standard error

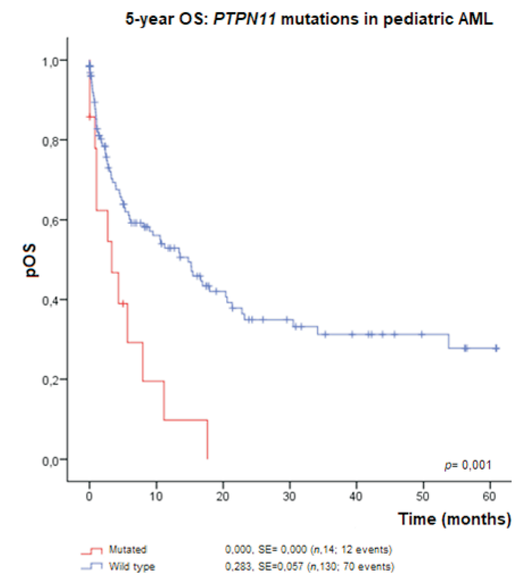

Figure 2. Survival analysis of the pediatric AML cases with mutated *PTPN11*. Kaplan-Meier estimates for the probability of overall survival (pOS) for *PTPN11* mutations. p values were calculated using log-rank test. SE, standard error.

PARTIAL CONCLUSION

Mutations in *PTPN11* had a significant impact on the overall survival in AML cohort indicating that these alterations should be better characterized.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA