CD44 EXPRESSION IN T CELL ACUTE LYMPHOBLASTIC LEUKEMIA AND ACUTE MYELOID LEUKEMIA AND ITS ASSOCIATION WITH RAS AND NOTCH1 PATHWAYS

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BACKGROUND

CD44 is an adhesion glycoprotein widely expressed in hematopoietic cells. As a hematopoietic stem cell (HSC) marker, CD44 assists the homing and anchorage of HSC to their niche. In T-cell development, the loss of CD44 expression marks the commitment to the T-cell fate and it is suggested that CD44 directs precursors from the bone marrow to the thymus. CD44 was identified as a target of the RAS pathway, as well as a direct NOTCH1 transcriptional target. In murine models, CD44 expression was associated with tumor progression, organ infiltration and influencing survival. In human T-cell acute lymphoblastic leukemia (T-ALL), the relevance of CD44 is still unknown. In early T-cell precursor-ALL (ETP-ALL) the transcriptional profile was similar to minimally differentiated acute myeloid leukemia (AML). In AML, expression of CD44 variant proteins has been associated with poor prognosis. We have investigated the cellular expression of CD44 in pediatric T-ALL and AML in order to identify its association with leukemia maturational subtypes and with mutations in RAS and NOTCH1 pathways.

MATERIAL AND METHODS

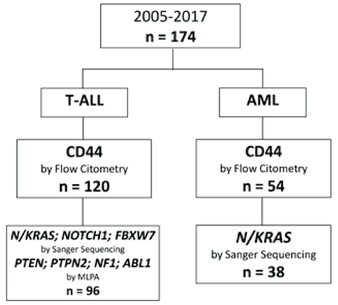

Figure 1: Methodology flowchart of T-cell Acute Lymphoid Leukemia and Acute Myeloid Leukemia cases. MLPA – Multiplex Ligation Probe Amplification

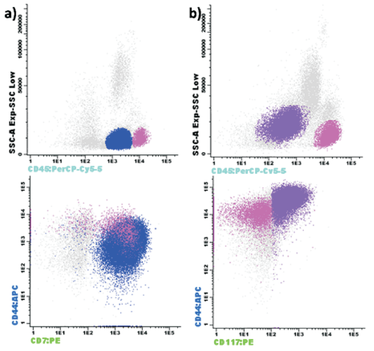

Figure 2: CD44 expression evaluated by multiparameter flow cytometry in **a)** T-cell Acute Lymphoid Leukemia, where the lymphoblasts are depicted in blue and the lymphocytes in pink and **b)** Acute Myeloid Leukemia, where the myeloblasts are in violet.

The Fisher's exact test or chi-square test were used to evaluate the distribution of categorical variables, whereas Mann-Whitney (two groups) or Kruskal Wallis (more than two groups) tests were used to evaluate the distribution of non parametric continuous variables. t-test and one-way ANOVA were used for parametric variables. p values of < 0.05 were considered statistically significant.

RESULTS

There was no association between variation of CD44 expression and T-ALL clinical findings; AML have a higher CD44 expression (MFI:105.8) than T-ALL (MFI:42.5), and ETP-ALL, p<0.0001 **(Figure 3)**. T-ALL cases with a more immature phenotype (ETP and pre-T subtypes) had a lower CD44 expression (MFI:29.6) than the more mature subtypes (cortical and mature T) (MFI:57.7) (p=0.01) **(Figure 4)**. AML with CD7 positivity had a lower CD44 expression (MFI:28.2) than AML $CD7^{neg}$ (MFI:175.5) (p=0.03) **(Figure 5)**. The frequency of genetic alterations in T-ALL cases was 47.2% *NOTCH1*^{mut}, 17.9% *FBXW7*^{mut}, 12.4% *N/KRAS*^{mut}, 6.1% *NUP214-ABL1*^{amp}, 16.2% *PTEN*^{del}, 7.1% *NF1*^{del} and 3% *PTPN2*^{del}.

Cases with low CD44 expression were more frequently *NOTCH1/FBXW7*^{mut}, than cases with high CD44 expression (p = 0.01) **(Table 1)**. There was no significant difference in CD44 expression between *N/KRAS*^{mut} and *N/KRAS*^{WT}, and in *PTEN*^{del}, *NUP214-ABL1*^{amp}, *NF1*^{del} and *PTPN2*^{del} **(Figure 6)**. In AML, there was also no significant difference in CD44 expression between *N/KRAS*^{mut} (MFI:161.9) and *N/KRAS*^{WT} (MFI:175.5) **(Figure 7)**.

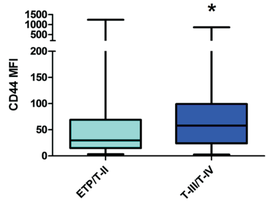

Figure 4: CD44 expression in immature subtypes (ETP and pre-T-ALL) and mature subtypes (cortical T and mature T-ALL). ETP/T-II, n = 38; T-III/T-IV, n = 73. p = 0.01 (Mann Whitney). Box Plot with horizontal lines representing the minimum, maximum, quartiles and median CD44 Median Fluorescence Intensity (MFI) values. ETP – Early T cell Precursor; T-II – pre-T; T-III – cortical T; T-IV – mature T. * p = 0.01

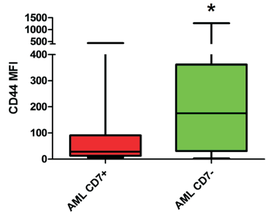

Figure 5: CD44 expression in AML cases with or without CD7 positivity. AML CD7+, n = 12; AML CD7-, n = 35. p = 0.03 (Mann Whitney). Box Plot with horizontal lines representing the minimum, maximum, quartiles and median CD44 Median Fluorescence Intensity (MFI) values. AML – Acute Myeloid Leukemia ** p = 0.005

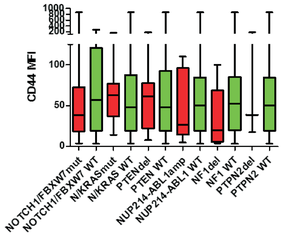

Figure 6: CD44 expression in T-ALL cases with or without NOTCH1/FBXW7 mutations (mut n = 61; WT n = 42; p = 0.2), *N/KRAS* mutations (mut n = 12; WT n = 90; p = 0.5), PTEN deletion (del n = 16; WT n = 80; p = 0.9), NUP214-ABL1 amplifications (amp n = 5; WT n = 91; p = 0.7), NF1 deletions (del n = 7; WT n = 89; p = 0.1) and PTPN2 deletions (del n = 3; WT n = 93; p = 0.9) (Mann Whitney). Box Plot with horizontal lines representing the minimum, maximum, quartiles and median CD44 Median Fluorescence Intensity (MFI) values.

Table 1: Genetic alterations in T-ALL according to CD44 expression

	Total	CD44 MFI High	CD44 MFI Low	p
NOTCH1/FBXW7				
Mutated	61 (33.7%)	5 (8.2%)	56 (91.8%)	0.01
WT	42 (66.3%)	12 (28.6%)	30 (71.4%)	
N/KRAS				
Mutated	12 (11.8%)	1 (8.3%)	11 (91.7%)	0.69
WT	90 (88.2%)	16 (17.8%)	74 (82.2%)	
NUP214-ABL1				
Amplification	5 (5.2%)	1 (20%)	4 (80%)	1
WT	91 (94.8%)	15 (16.5%)	76 (83.5%)	
PTEN				
Deletion	16 (16.7%)	2 (12.5%)	14 (87.5%)	1
WT	80 (83.3%)	14 (17.5%)	66 (82.5%)	
NF1				
Deletion	7 (7.3%)	0 (0%)	7 (100%)	0.6
WT	89 (92.7%)	16 (18%)	73 (82%)	
PTPN2				
Deletion	3 (3.1 %)	1 (33.3%)	2 (66.7%)	0.43
WT	93 (96.9%)	15 (16.1%)	78 (83.9%)	
Total	**103**	**17 (16.5%)**	**86 (83.5%)**	

MFI - Median Fluorescence Intensity

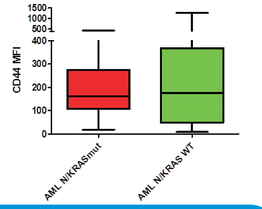

Figure 7: CD44 expression in AML cases with or without N/KRAS mutations (mut n = 8; WT n = 29; p = 0.8) (Mann Whitney). Box Plot with horizontal lines representing the minimum, maximum, quartiles and median CD44 Median Fluorescence Intensity (MFI) values. AML – Acute Myeloid Leukemia

CONCLUSION

CD44 is under expressed in T-ALL when compared with AML. *N/KRAS* mutations, deletions in *PTEN*, *NF1* and *PTPN2* and amplification of *NUP214-ABL1* do not seem be associated with different expression of CD44 in pediatric T-ALL. Cases with *NOTCH1/FBXW7* mutations had a lower expression of CD44.

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