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Aberrant DNA methylation of a cell cycle regulatory pathway composed of P73, P15 and P57KIP2 is a rare event in children with acute lymphocytic leukemia

Andreia A. Canalli [a], Hui Yang [a], Sima Jeha [b], Koyu Hoshino [a], Blanca Sanchez-Gonzalez [a], Mark Brandt [a], Sherry Pierce [a], Hagop Kantarjian [a], Jean-Pierre Issa [a], Guillermo Garcia-Manero [a,*]

[a] *Department of Leukemia, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA*
[b] *Department of Pediatrics, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA*

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Abstract

Aberrant DNA methylation of multiple promoter associated CpG islands is a frequent phenomenon in acute lymphocytic leukemia (ALL). Recently, methylation of a cell cycle control pathway composed of P73, P15 and P57KIP2 has been shown to confer poor prognosis to adult patients with ALL. Using bisulfite PCR methods, we have explored the prevalence of methylation of this pathway in a cohort of children with ALL ($N = 20$), and compared these results with those observed in a group of adult patients ($N = 53$). P73 was methylated in 4 (20%) pediatric patients, P15 in 3 (15%), and P57KIP2 in 2 (10%). These compared to 14 (26%), $p = 0.5$, 16 (30%), $p = 0.04$ and 20 (37%), $p = 0.04$, respectively in adult patients. Methylation of two or more genes was not observed in any pediatric patient, but in 15 (28%) adult patients ($p = 0.003$). Poor survival of adult patients was associated with methylation of ≥ 2 genes ($p = 0.003$). These results indicate that differences in DNA methylation of specific molecular pathways may contribute to the prognostic differences known to occur between pediatric and adult patients with ALL.

Keywords: Acute lymphocytic leukaemia; DNA methylation; P73; P15; P57KIP2

1. Introduction

At the present time no clear explanation exists for the disparity in prognosis observed between pediatric and adult patients with acute lymphocytic leukemia (ALL) [1]. Although poor-risk genetic alterations, such as the presence of the Philadelphia (Ph) chromosome, are more frequent in adult patients [1], when pediatric and adult patients with Ph chromosome positive ALL are compared, prognosis still is worse in older patients [2], suggesting that other abnormalities, not yet identified, play a role in these differences.

Aberrant DNA methylation of multiple promoter associated CpG islands (DNA methylation) is frequently observed in both adult and pediatric patients with ALL [3–5]. Because of the potential redundant function of proteins involved in the transition from G_1 to S phase of the cell cycle [6,7], and the observation that several of these genes are the target of aberrant methylation in ALL [3,8], we had previously investigated the impact of methylation of a triad of cell cycle regulatory genes, including *P73*, a *p53*-like gene, and the cyclin-dependent kinase inhibitors P15 and P57KIP2, in adult ALL [9]. Aberrant methylation of two or more genes of this triad occurred in close to 25% of patients with Ph negative disease and was associated with survival similar to that of pa-

* Corresponding author. Present address: Department of Leukemia, University of Texas MD Anderson Cancer Center, Unit 428, 1515 Holcombe Blvd., P.O. Box 301402, Houston, TX 77230-1402, USA. Tel.: +1 713 745 3428; fax: +1 713 794 4297.
E-mail address: ggarciam@mdanderson.org (G. Garcia-Manero).

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tients with Ph positive disease [9], the most prevalent adverse molecular feature in adult ALL.

In view of the relevance of methylation of the P73-P15-P57KIP2 pathway in adult patients, we have studied the frequency of methylation of this pathway in a cohort of pediatric patients, and compared them with a group of adult patients previously studied by us [9], results of which are shown here.

2. Material and methods

2.1. Patient characteristics

Patient characteristics are summarized in Table 1. Twenty pediatric patients were analyzed. In 7 (35%) patients cytogenetic analysis was not performed because of insufficient cell yield, and in 2 (10%) it was not performed. All pediatric patients had been treated on Children Cooperative Group Protocols CCG 1882, 1922 and 1952, depending on their risk, at MD Anderson Cancer Center (MDACC). Two pediatric patients died: one during induction therapy but in complete remission (CR), the other with t(4;11) (q23; q21) disease required two courses of induction chemotherapy to achieve CR, and died at relapse. Both patients are considered as induction failures (Table 1). Adult patients had been previously reported [9]. As none of the pediatric cases was known to be Ph positive, and because our previous studies had shown a distinct methylation profile in patients with Ph positive disease [3], we excluded adult patients with the Ph abnormality from this analysis. In all, 53 adult patients were studied. All adult patients had been treated at MDACC with the hyperC-VAD chemotherapy program [10]. Median disease free survival (DFS) and overall survival (OS) of the pediatric group has not been reached, and were 120 and 155 weeks for the adults respectively ($p = 0.003$). All samples were collected following institutional guidelines.

2.2. DNA extraction, bisulfite modification and methylation analysis

Methods of DNA extraction, bisulfite modification and methylation analysis have been described elsewhere [9]. Pediatric samples consisted of paraffin embedded bone marrow biopsy specimens, and adult samples of bone marrow aspi-

Table 1
Patient characteristics

Patient characteristic	Adults ($N = 53$)		Pediatrics ($N = 20$)	
	N	%	*N*	%
Median age in years (range)	38 (15–78)		4 (4–14.6)	
Male sex	34	64	13	65
Median % marrow blasts (range)	87 (22–99)		91 (42–98)	
Median WBC $\times 10^9$/L (range)	14.1 (0.9–669.6)		9.0 (1.4–152)	
Median HgB g/dL (range)	8.4 (4.1–15.8)		7.7 (4.4–14.0)	
Median Plt count $\times 10^9$/L (range)	52 (11–302)		90.5 (5–367)	
Cytogenetics				
Diploid	16	30	5	25
Hypodiploid	2	4	0	0
Hyperdiploid	4	8	4	20
Pseudodiploid	8	15	1	5
t(v;11q23)	0	0	1	5
t(12;21)	0	0	0	0
t(1;19)	1	2	0	0
Insufficient metaphases	8	15	7	35
Others	14	26	0	0
Not done	0	0	2	10
Immunophenotype				
Calla	27	51	13	65
T/calla	3	6	2	10
Null	5	9	2	10
Pre-B	4	8	1	5
ND	3	6	1	5
T-cell	6	11	1	5
B-cell	4	8	0	0
T/pre-B	1	2	0	0
Complete remission	49	93	18	90
Relapse	26	53	1	5
Disease free survival (weeks)	120		401+	
Overall survival (weeks)	155		406+	

T/calla is sIg negative, CD10+, and 2 T cell markers. Null sIg negative, T cell markers negative and CD 10, 19 and 20 also negative. T/pre-B means 2 T cells markers and positive of either CD19 or CD20.