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Clonogenic acute myelogenous leukemia cells are heterogeneous with regard to regulation of differentiation and effect of epigenetic pharmacological targeting

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Abstract

Differentiation-inducing therapy with the DNA-methylation inhibitor Decitabine (5′-aza-deoxycytidine) and histone deacetylase (HDAC) inhibitors are now considered in acute myelogenous leukemia (AML). We investigated the in vitro effects of Decitabine and two structurally unrelated HDAC inhibitors (Sodium 4-phenyl butyrate, Tricostatin A) on clonogenic AML cells. Based on morphological criteria we identified four major colony types: (i) non-erythroid colonies, (ii) erythroid colonies that were detected only for a subset of patients and could be further sub classified into mature and immature forms, and (iii) intermediate colonies. Erythroid differentiation was associated with low CD34 expression. The colonies showed differences in morphology, viability, cell cycle distribution and expression of differentiation markers. Both Decitabine and the two HDAC inhibitors altered AML cell expression of differentiation markers, whereas the drugs did not have any major influence on cell cycle distribution. However, the pharmacological effects differed between the four colony subsets, and differences were also detected between the two HDAC inhibitors. We conclude that clonogenic AML cells can be classified into well-defined subsets based on their differentiation, and these subsets differ in their biological characteristics as well as their response to pharmacological targeting of epigenetic regulation.

Keywords: Acute myelogenous leukemia; Differentiation; Colony formation; Histone deacetylase inhibitors; DNA methylation inhibitor

1. Introduction

Acute myelogenous leukemia (AML) is characterized by bone marrow accumulation of immature leukemia cells [1–3]. The overall disease-free survival is less than 50% even for patients below 60 years of age who can receive the most intensive therapy with allotransplantation or high-dose cytarabine [1]. However, the majority of the patients are above this age and are treated with less intensive chemotherapy or only supportive therapy [1]. New therapeutic approaches are therefore needed both for the young patients with resistant disease and for the elderly patients who cannot receive the most intensive treatment due to a high risk of treatment-related mortality.

Epigenetic targeting is now suggested as a possible therapeutic strategy in AML [2–5]. Histone deacetylase (HDAC) inhibitors as well as the methylation-inhibitory agent Decitabine induce cell cycle arrest, differentiation and apoptosis in human AML [4–7]. A major part of these previous studies has been performed on leukemia cell lines or by investigating the total AML cell population and examination of the total population will mainly reflect the characteristics of the majority of more mature cells within the hierarchically organized AML cell population [8,9]. Thus, it is not known whether these pharmacological agents have similar effects on more immature AML progenitors. In our present study

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we therefore characterized the effects of epigenetic targeting on AML cell differentiation for the minority of clonogenic AML cells.

HDAC or methylation inhibition is expected to have a relatively low risk of serious toxicity, and both approaches are now considered for clinical use in AML patients [4–7]. An important cause of death from AML is leukemia relapse derived from chemoresistant residual AML stem cells/progenitors [1]. In our present study we therefore characterized the effects of epigenetic pharmacological targeting on clonogenic AML cells, a small leukemia cell subset that is more closely related to the leukemic stem cells than the non-proliferating majority of the hierarchically organized AML clone.

Table 1
Clinical and biological characteristics of 47 AML patients grouped according to the CD34 expression of their primary leukemia cells

Patient	Sex	Age	FAB	Colonies per 50,000 cells seeded			Membrane molecule expression (%)						Cytogenetics	Flt3
				nERY + IM	ERY	ERY/nERY + IM	CD34	HLA-DR	CD33	CD13	CD14	CD15		
1	M	69	M1	170	96	0.56	99	84	90	100	nt	nt	inv(16)	wt
2	F	59	M2	2	0	0	99	78	66	97	1	4	−7	wt
3	M	65	M2	0	0	0	99	99	6	89	1	2	Multiple	wt
4	M	74	M5	58	0	0	98	81	100	98	2	8	Normal	ITD
5	F	56	M1	1	1	1.00	98	65	67	95	1	10	Normal	wt
6	M	83	M1	16	0	0	97	98	21	13	1	nt	nt	wt
7	M	69	M1	93	0	0	97	97	99	99	nt	45	nt	wt
8	F	80	M2	29	0	0	96	96	87	98	3	14	Multiple	wt
9	M	43	M5	16	0	0	95	57	98	94	7	64	inv(16)	D835
10	F	64	M1	9	20	2.22	95	97	89	98	1	3	Multiple	ITD
11	F	48	M0	58	0	0	92	4	97	2	1	11	Extra 21	nt
12	F	44	M1	13	0	0	89	100	83	83	1	12	del(7)	ITD
13	F	55	M0	39	4	0.10	87	94	5	94	1	nt	Normal	ITD
14	M	72	M1	84	0	0	84	91	60	85	2	34	Plus-8	wt
15	F	75	M4	54	0	0	76	67	75	98	9	55	Normal	ITD
16	M	63	M1	0	0	0	68	92	41	82	32	nt	Normal	wt
17	F	58	M2	72	23	0.32	56	78	98	93	<1	nt	Normal	ITD
18	M	29	M4	19	0	0	55	73	96	92	5	24	Normal	ITD
19	M	65	M2	176	0	0	54	53	56	13	86	14	nt	wt
20	F	63	M4	26	93	3.58	51	91	100	61	1	26	Normal	ITD
21	M	32	M3	29	42	1.44	41	4	98	72	3	10	t(15;17)	ITD
22	F	41	M2	54	22	0.41	31	42	nt	nt	nt	nt	Normal	ITD
23	F	74	M2	194	30	0.15	30	nt	64	41	3	8	nt	wt
24	F	75	M1	112	0	0	23	66	55	43	11	2	nt	ITD
25	M	65	M1	11	0	0	20	96	96	96	1	7	Normal	ITD
26	F	78	M0	20	6	0.30	19	60	95	98	76	23	Multiple	wt
27	M	81	M4	42	0	0	17	49	97	32	47	82	Plus-11	ITD
28	M	56	M4	26	0	0	14	78	99	74	1	42	Normal	ITD
29	F	45	M4	35	2	0.06	7	7	12	71	50	87	Normal	D835
30	F	56	M2	329	0	0	2	49	91	83	1	13	nt	wt
31	M	48	M5	26	4	0.15	2	99	98	98	98	71	Normal	ITD
32	M	74	M4	25	4	0.16	2	2	92	31	1	3	nt	ITD
33	M	61	M4	42	2	0.05	1	77	100	20	18	89	Normal	ITD
34	M	64	M1	82	3	0.04	1	2	97	28	1	3	nt	ITD
35	F	40	M5	0	0	–	1	93	100	3	4	90	t(9;11), +8	wt
36	M	79	M4	85	0	0	1	34	71	17	1	54	nt	ITD
37	M	75	M1	3	2	0.40	1	51	68	79	8	62	Multiple	wt
38	F	45	M4	12	12	1.00	1	1	98	90	1	24	Normal	wt
39	F	36	M5	117	150	1.28	1	95	98	29	9	100	t(9;11)	wt
40	M	82	M5	136	0	0	1	46	98	19	20	20	−Y	wt
41	F	61	M5	11	0	0	1	96	99	91	82	99	Normal	wt
42	M	72	M5	27	45	1.70	1	63	99	31	40	69	Normal	wt
43	M	53	M4	24	24	1.00	1	97	100	81	92	88	Normal	wt
44	F	61	M5	4	4	1.00	1	94	98	5	45	95	Multiple	wt
45	F	46	M5	50	0	0	1	95	99	42	37	99	Normal	nt
46	F	81	M2	6	4	0.67	1	2	92	31	1	3	nt	ITD
47	M	54	M5	2	11	5.50	1	95	100	46	45	99	Normal	wt

Abbreviations: female, F; male, M; French-American-British classification system of AML subclasses, FAB; non-erythroid colonies, nERY; intermediate colonies, IM; erythroid colonies, ERY; not tested, nt; wild type, wt; internal tandem duplications, ITD. The colony data are presented as the number of colonies per 50,000 seeded cells. The membrane molecule expression is presented as the percentage of positive cells [12].