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Acute Myeloid Leukemia

Differences in the expression pattern of apoptosis-related molecules between childhood and adult *de novo* acute myeloid leukemia

Distinct expression patterns of pro- and anti-apoptotic proteins may contribute to different prognoses and therapy outcomes in adult versus childhood acute myeloid leukemia (AML). Therefore, we investigated whether expression levels of apoptosis-related proteins CD95, Bcl-2, Bax, Bcl-xL, procaspase-3, XIAP, cIAP-1, and survivin differ between children and adults with *de novo* AML.

haematologica 2004; 89:363-364

(<http://www.haematologica.org/journal/2004/3/363>)

Prognosis in acute myeloid leukemia (AML) is age-related, with childhood AML having a better treatment outcome than adult AML. Within adults, patients < 60 years old have a better prognosis than patients ≥60 years old. For the current chemotherapy regimens, these prognostic differences are valid for response to induction chemotherapy, event-free survival (EFS) as well as overall survival (OS).¹⁻⁴

It has been speculated that differences in the expression patterns of anti- and pro-apoptotic molecules between childhood and adult acute leukemia might contribute to the different treatment outcomes of age-stratified leukemia groups.⁵ However, systematic investigations on possible expression differences of apoptosis-related molecules in children and adults with acute leukemia are rare. To evaluate this hypothesis, we examined consecutively collected leukemic cell samples from children (n=45) and adults (n=92; < 60 years: n=44, ≥ 60 years: n=48) with *de novo* AML for the expression levels of several apoptosis-related molecules (CD95, Bcl-2, Bax, Bcl-xL, caspase-3, XIAP, cIAP-1, survivin). All samples contained more than 80% leukemic cells based on morphologic criteria.

Surface CD95 expression and intracellular expression of Bcl-2 and Bax were determined by flow cytometry, as described previously, using the PE-conjugated anti-CD95 monoclonal antibody DX2, the FITC-conjugated anti-Bcl-2 monoclonal antibody 124, and the polyclonal rabbit-anti-human antibody I-19 raised against Bax-specific peptide sequences.⁶ Antigen expression distribution in individual cell samples was quantified as relative fluorescence intensity (RFI), determined by the ratio of mean fluorescence intensity of cells stained for the respective antigen to mean fluorescence intensity of the corresponding neg-

ative control. Expression levels of Bcl-xL, caspase-3, XIAP, cIAP-1 and survivin were determined by Western blotting using monoclonal antibodies specific for XIAP (Transduction Laboratories, Lexington, KY, USA), cIAP-1, survivin (R&D Systems, Minneapolis, MN, USA), procaspase-3 (Pharmingen, San Diego, CA, USA), and Bcl-xL (Santa Cruz Biotechnology, Santa Cruz, CA, USA). Data on X-ray films were quantified by scanning-densitometry using the NIH Image analysis system. To normalize for variation in antibody concentration or time of exposure, the protein signal from the respective patient was normalized against the protein signal of the control cell line BJAB (human Burkitt-like lymphoma cell line). Western blot results are expressed in terms of this ratio (relative optical density, ROD).⁷ Differences in the expression levels of apoptosis-related molecules between childhood and adult AML were evaluated using the Mann Whitney-test.

As outlined in Table 1, expression of Bax, procaspase-3, XIAP and c-IAP1 differed between the age groups. The most striking finding in our study was the much higher expression of cIAP-1 in childhood AML than in adult AML. In contrast, all other observed statistically significant age-related expression differences (Bax, procaspase-3, XIAP) were for higher protein levels among the adults than among the children. However, neither the higher expression of cIAP-1 nor the lower expression levels of Bax and procaspase-3 in childhood AML observed in this series fits with the general expectation that expression levels of anti-apoptotic proteins (e.g. cIAP-1) would be lower and expression levels of pro-apoptotic proteins (e.g. Bax, Procaspase-3) would be higher in the prognostically more favorable pediatric AML group.⁵

Only the higher expression of the anti-apoptotic molecule XIAP in the prognostically more unfavorable adult AML matches this expectation.⁷ Possible explanations for these rather unexpected findings may include: (i) the intracellular location of these molecules influences their apoptotic activity. For example, to be pro-apoptotic, Bax must translocate from the cytoplasm to mitochondria, where it triggers cytochrome c release; (ii) post-translational modifications (e.g. phosphorylation) of Bcl-2 family members might be of importance for their functional activity; (iii) measurement of the active form of caspase-3 rather than the inactive proform might be more informative as a potential prognostic marker; (iv) expression analysis of recently characterized molecules (e.g. Diabolo/Smac) counteracting the anti-apoptotic activity of IAP molecules might be helpful to understand the observed expression pattern of apoptosis-related molecules within this study; (v) protein families other than apoptosis-regu-

Table 1. Mean expression levels of apoptosis-related molecules in children and adults with *de novo* AML.

AML groups	Expression levels	CD95	Bax	Bcl-2	Bax/Bcl-2 ratio	Pro-caspase-3	XIAP	Bcl-xL	Survivin	clAP-1
Children < 18 yrs	mean	3.7	5,1	2.6	2.2	3837	2455	2006	378	7827
	n	39	38	38	38	44	44	44	44	23
	SEM	0.3	0.4	0.2	0.2	415	457	339	114	1153
Adults < 60 yrs	mean	4.0	6.9	2.2	3.5	3714	2647	2499	641	2739
	n	43	43	43	43	44	44	44	44	17
	SEM	0.3	0.8	0.2	0.5	540	455	376	176	833
Adults ≥ 60 yrs	mean	3.5	8.4	2.3	4,6	5488	4064	3082	616	2035
	n	44	47	47	47	46	46	46	46	25
	SEM	0.3	0.8	0.1	0.6	656	502	407	151	399
p value		ns	<0.001	ns	<0.001	<0.05	<0.05	ns	ns	<0.001

n: number of examined samples; SEM: standard error of the mean; ns: not significant. CD95, Bax, Bcl-2, P-gp, LRP, and MRP mean expression levels are given as RFI values and procaspase-3, XIAP, Bcl-xL, survivin and clAP-1 expression levels are given as standardized mean values as described in the text.

lating molecules might be more important for therapy response and prognosis in AML.^{8,9} In contrast to the expression patterns of apoptosis-related proteins, neither FAB subtype nor cytogenetics differed significantly between childhood and adult *de novo* AML in these series (data not shown).

To our knowledge, this is the first systematic study investigating possible expression differences of apoptosis-related molecules between childhood and adult AML.

The results obtained indicate that the assumption that anti-apoptotic proteins will be preferentially expressed in adult leukemia whereas pro-apoptotic molecules will be more prominent in childhood leukemia might not be sufficient to explain different therapy outcomes and prognoses in different age groups of patients with AML. As this was a retrospective study with cryopreserved cell samples, further prospective studies should confirm our findings. Additionally, new approaches (e.g. microarray technique) might identify apoptosis-related genes with different expression patterns between childhood and adult acute leukemia.

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Funding: this study was supported in part by grants from the Carreras-Foundation, Mildred-Scheel-Stiftung für Krebsforschung and Deutsche Forschungsgemeinschaft. We would like to thank G. Czerwony, M. Martin and K. Liebezeit for their excellent technical assistance. The cell samples included in this study were sent from various hospitals in Germany participating in the AML-BFM (co-ordinators: U. Creutzig and J. Ritter, Münster) and AML-CG (co-ordinators: T. Büchner and W. Berdel, Münster; W. Hiddemann, München; B. Wörmann, Braunschweig) trials. We would like to thank the co-ordinators of the above studies for their continuous support as well as all clinicians who provided cell samples for our investigations.

Key words: childhood AML, adult AML, apoptosis

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