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Cellular cytotoxic drug sensitivity in children with acute leukemia and Down's syndrome: an explanation to differences in clinical outcome?

TO THE EDITOR

During the last decade several groups have reported that acute myeloid leukemia (AML) in children with Down's syndrome (DS) has a significantly better outcome than AML in other children.[1,2] In acute lymphoblastic leukemia (ALL), on the other hand, the clinical outcome tends to be worse for DS than for non-DS children.[3,4] The reason for this difference between AML and ALL is not known.

In an ongoing study we prepared leukemic cells at diagnosis from 62 children with AML and 222 children with ALL for test of *in vitro* sensitivity to cytotoxic drugs. Five children with AML (8%) and five children with ALL (2.3%) also had DS, figures similar to those previously reported in population-based studies, and this enabled us to compare drug sensitivity in tumor cells from DS and non-DS children.

Leukemic cells from fresh bone marrow or blood samples were assessed for their *in vitro* drug sensitivity by the fluorometric microculture cytotoxicity assay (FMCA) against a panel of drugs used in the current Nordic protocols for AML[2] and ALL,[5] respectively. The FMCA is a non-clonogenic assay, very similar to the more widely used MTT assay,[6] and is based on the measurement of fluorescence generated from hydrolysis of fluorescein diacetate to fluorescein by cells with intact plasma membranes.[7] A survival index (SI) was calculated as the fluorescence signal of cells after 72 h of drug incubation as a percentage of that of unexposed controls.

Leukemic cells from DS children with AML were significantly more sensitive (P = 0.03–0.002) than blast cells from non-DS children to cytosine arabinoside (Ara-C), doxorubicin, dexamethasone (see Figure 1a) and amsacrine (not shown). A trend in the same direction was found for etoposide (VP16) and 6-thioguanine. DS patients with AML were significantly younger (mean 2.1 years) than non-DS patients (7.9 years), but a statistically significant difference was found also when comparing the DS samples with those of age-matched controls, and there was no clear correlation between age and *in vitro* sensitivity among non-DS patients with AML.

Three Down patients had FAB subtype M1 and two had M7 AML.

In contrast, leukemic cells from DS children with ALL were significantly less sensitive (P = 0.02–0.001) than cells from non-DS patients to dexamethasone and asparaginase, with a similar trend for vincristine, doxorubicin and Ara-C (Figure 1b). The mean age for DS children with ALL was 10.8 years and for non-DS patients 6.5 years (NS). Two DS patients fulfilled standard risk and three intermediate risk criteria according to the Nordic ALL protocol.

Findings similar to ours have previously been described in DS children with AML,[8,9] while in ALL a single study on six DS patients showed no significant differences for most drugs tested (ALL cells of DS patients were slightly more sensitive to anthracyclines).[9]

The inferior clinical outcome in ALL has been attributed to excessive therapy-related toxicity in DS children, but this would not explain the difference between AML and ALL. Our data, which have to be confirmed in larger series of patients, indicate that at least part of the explanation for the differences in clinical outcome between DS and non-DS children should be sought among mechanisms for tumor cell drug resistance. The fact that the difference in drug sensitivity between DS and other children was diametrically opposed for AML and ALL, suggests cell lineage-specific rather than general cellular alterations associated with the presence of three copies of chromosome 21 as suggested by Taub *et al*.[8] Presently, we do not understand the mechanism(s) behind the differences in drug sensitivity between DS and non-DS children, but we think our data point at an area for further fruitful research.

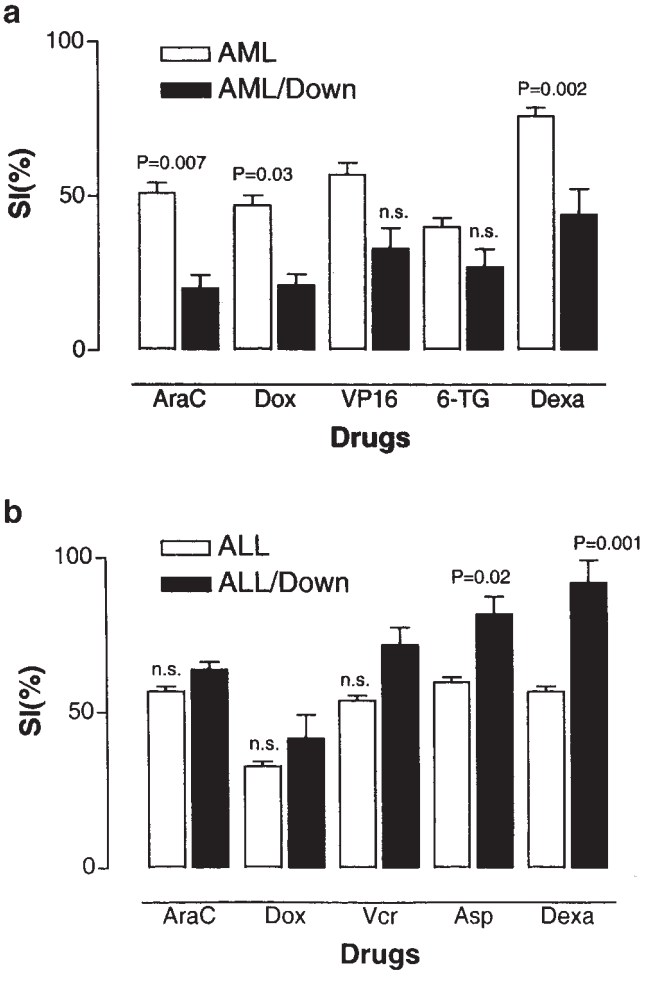

Figure 1 *In vitro* sensitivity of leukemic blast cells from children with Down's syndrome and acute myeloid leukemia (AML; n = 5), upper panel, or acute lymphoblastic leukemia (ALL; n = 5), lower panel, compared to non-Down children with leukemia (n = 57 and 217, respectively). SI denotes surviving cells, in percentage of untreated control, after 72 h incubation with cytosine arabinoside 0.5 μg/ml (Ara-C), doxorubicin 0.5 μg/ml (Dox), etoposide 5 μg/ml (VP16), 6-thioguanine 10 μg/ml (6-TG), dexamethasone 7.1 μg/ml (Dexa), vincristine 0.5 μg/ml (Vcr), asparaginase 10 U/ml (Asp). The results are presented as mean values plus s.e.m. The statistical significance levels (Student's t-test) are indicated; NS, non-significant.

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Received 20 October 1999; accepted 22 December 1999

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Acknowledgements

This work was supported by Lions' Cancer Research Foundation in Uppsala and by the Children's Cancer Foundation in Sweden. The contribution of patient samples from members of the Nordic Society of Pediatric Hematology and Oncology (NOPHO) is gratefully acknowledged.

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Reported cryptic rearrangements of CBFβ-MYH11 in acute myeloid leukaemia are not due to false priming of contaminating DNA

TO THE EDITOR

The presence of inv(16)(p13q22) at diagnosis of acute myeloid leukaemia (AML) is one of several good prognostic, cytogenetic indicators,[1] and such cytogenetic data are now used to stratify patient therapy (eg UK MRC AML 12 trial). The inv(16) chromosomal abnormality results in disruption of the CBFβ gene and creation of the CBFβ-MYH11 fusion gene.[2] Transcripts from this fusion gene can be detected by RT-PCR and several groups, including ourselves, have shown that PCR techniques not only confirm positive cytogenetic findings for inv(16) but also allow detection of additional cases in which this translocation is not apparent.[3–7] In some cases, this is because the inv(16) abnormality is subtle and only detectable in good metaphase preparations, but in most cases these cryptic rearrangements are undetectable by high quality conventional cytogenetics. This finding has led us to suggest that all cases of AML should be screened for the presence of CBFβ-MYH11 fusion transcripts (and AML1-ETO transcripts).[3,8]

Recently Hackwell *et al*[9] reported a very high proportion of 'false positive' results using the primer sets from our study. They demonstrated that this is due to the 5′ nested CBFβ primer cross-hybridising with an intronic sequence of contaminating MYH11 genomic DNA, which results in the amplification of a 210 bp sequence from DNA of similar size to the expected 209 bp CBFβ-MYH11 RT-PCR product. This raised the possibility that the cryptic rearrangements reported by ourselves were spurious, and for this reason we have re-analysed 10 out of the 12 cases of cryptic rearrangement that we previously described. In the other two cases, there was no RNA for further analysis.

In each of these 10 cases, RT-PCR amplified a 209 bp band and no bands were produced from normal control RNA samples. Using genomic DNA from 10 individuals we were, however, able to confirm the production of a similar sized band. The PCR products from the 10 cases with purported cryptic CBFβ-MYH11 rearrangements, five cases of cytogenetically apparent inv(16) and five of the genomic DNA samples were then subjected to digestion with the restriction enzyme *Stu*I. This enzyme will digest the product of a CBFβ-MYH11 fusion into two bands of 109 bp and 100 bp but the genomic DNA-derived product does not contain any cutting sites. No digestion was observed in the genomic DNA-derived products, but complete digestion was observed in the five cases of inv(16) and all 10 cases of cryptic CBFβ-MYH11 rearrangements (Figure 1). In our original study, we confirmed the nature of the fusion product by sequencing across the breakpoint in three out of the 12 cases. We now carried out sequencing a further seven of these cases and identified the presence of a cryptic rearrangement in all of them.

This study thus confirms that cryptic rearrangements of CBFβ-MYH11 occur at a frequency sufficient to justify molecular screening of all cases of AML. The discrepancy between our finding of no 'false positive' results and the false positivity in approximately 80% of samples by Hackwell *et al*[9] is not immediately apparent. The same primer sets were used suggesting that false positives can arise due to

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Received 3 December 1999; accepted 27 December 1999