From gene expression profiles to new targeted therapies in acute lymphoblastic leukemia

Monique L. den Boer

Abstract
Gene expression profiles can be used to identify genetic subclasses in childhood ALL. We demonstrated that in vitro drug resistance to different classes of drugs is related to specific expression patterns of genes. The profile of these resistance-associated genes predicts clinical outcome independent from all other known risk factors. Moreover, the prognostic value has been independently confirmed in a second group of patients treated on different protocols. Gene expression profiling has the advantage that it is a screening technique that may lead to new (unexpected) ideas about genes important for therapy response and, moreover, may point to new therapeutic targets. Examples are overexpression of genes involved in glycolysis and MCL-1. Targeting these genes sensitized prednisolone resistant ALL cells to the cytotoxic effect of glucocorticoids.

Introduction
Over the past four to five decades improvements in chemotherapeutic regimens for children with acute lymphoblastic leukemia (ALL) have resulted in cure rates of ~80%. In vivo and in vitro resistance to antileukemic agents are associated with a relatively poor prognosis [1]. However, still very little is known about the underlying genetic defects of drug resistance. Classic resistance mechanisms such as overexpression of multidrug resistance proteins MDR-1, MRP-1, MVP/LRP and BCRP do not seem to play a major role in ALL [2]. Two genetic subclasses of ALL, i.e. those involving rearrangement of BCR-ABL and MLL are associated with a poor outcome but account only for a low number of therapy failures in absolute sense. The majority of therapy failures still occurs in the large so-called favorable genetic subgroups such as hyperdiploid ALL and TEL-AML1 rearranged ALL. This illustrates that the current genetic classification of ALL is not sufficient and that the genes that contribute to therapy responses are unknown. New insights in mechanisms of clinical drug resistance are difficult to obtain because we do not know where to look for. Moreover, many yet known drug resistance-associated genes have been identified in highly manipulated cell line models and hence, many of these “candidate” genes have been shown to be less relevant in patients’ cancer cells. Although gene expression profiling techniques have the disadvantage that they are just “fishing expeditions” the advantage is that the whole genome or at least a very large part of the genome is screened which may give us new ideas on genes that might be important for therapy failure and new drug targeting in ALL.

Gene expression profiling, drug resistance and outcome
We have determined the in vitro cytotoxicity to 4 important drugs used in ALL treatment, i.e. prednisolone, vincristine, L-asparaginase and daunorubicin in 173 children with newly diagnosed ALL and compared the gene expression profile of in vitro sensitive- and resistant cases [3]. Out of 14,500 probe sets we identified genes differentially expressed in B-lineage ALL cases that were either sensitive or resistant to prednisolone (33 genes), vincristine (40 genes), L-asparaginase (35 genes) and daunorubicin (20 genes). Out of the total of 124 genes, 121 had never been linked to drug resistance before. A gene expression score was made that combined the expression data of genes linked to resistance for our 4 drugs. A high gene expression score appeared to be significantly associated with a poor outcome: the hazard ratio for relapse was 3.0 for patients with a high gene expression resistance score compared to patients with a low score. The prognostic relevance of this drug resistance-
associated gene expression score was independent of all other known risk factors and was confirmed in an independent population of patients treated with an other protocol that included the above-mentioned 4 drugs. The hazard ratio for relapse for patients with a high score in this population was even 11.9.\[3\]

Using partly the same set of data we also investigated the differential expression of 70 key apoptosis genes between drug sensitive and - resistant ALL cases \[4\]. No single apoptosis gene was related to resistance to all 4 unrelated drugs. Expression of MCL-1 and DAPK1 were associated with prednisolone resistance whereas BCL2L13, HRK and TNF were associated with resistance to L-asparaginase. Of these 5 genes only BCL2L13 overexpression predicted outcome significantly. This prognostic relevance appeared to be independent from all other known risk factors and was confirmed in an independent second group of patients treated with another protocol.

The above-mentioned studies do not identify the genes that have the strongest predictive value for outcome because the first selection was made on the basis of in vitro drug resistance phenotype. This leads to a selection bias and an underestimation of the prognostic power of gene expression profiling. It has been demonstrated that the different lineages of ALL (B- versus T-lineage) and the genetic subclasses of B-lineage ALL (TEL-AML1, BCR-ABL, hyperdiploid, E2A-PBX1, MLL) have specific gene expression profiles that can be recognize with an accuracy of more than 95%. This suggests that the underlying biology of these subtypes of ALL differs and in conjunction with this also their underlying resistance mechanisms and the gene expression profiles with predictive value for outcome. Indeed we showed that for instance the mechanism of L-Asparaginase differs between TEL-AML1 rearranged ALL and other genetic subtypes of ALL. In the latter the expression of asparagine synthetase was related to L-asparaginase resistance while in TEL-AML1 rearranged ALL this was not the case \[5\]. This also implies that it might be difficult to identify one single gene that predicts outcome in all subtypes of ALL.

The group of Willman discovered a new gene that was named OPAL1 (outcome predictor in acute leukemia) of which a high expression was predictive of a favorable outcome in childhood ALL \[6\]. In two independent cohorts of patients including >400 cases we observed that OPAL1 was highly expressed in TEL-AML1 positive ALL, as was also found by Willman and co-workers. However, we could not confirm the independent prognostic relevance of OPAL1 expression in the total group of ALL patients nor in the genetic subgroups such as T-ALL and TEL/AML1 positive or –negative B-lineage ALL \[7\]. The prognostic relevance of OPAL1 expression seems therefore therapy dependent or it might be difficult to reproduce gene expression findings from one laboratory to another. The fact however that the gene expression profiles of genetic subclasses of ALL are reproducible between different laboratories suggest that the first explanation may be more likely to be true.

**Gene expression profiling may lead to new targeted therapy strategies**

An advantage of micro-array technologies is that they may lead to new and unexpected insights into the background of drug resistance and may lead to new genes or pathways that may serve as therapeutic targets. An important example is that our array analyses revealed that prednisolone resistance in B-lineage ALL is associated with an overexpression of genes involved in glycolysis, i.e. the glucose transporter 3 (GLUT3) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). In addition, prednisolone resistant cell lines were shown to have an increased glycolytic rate. The glycolysis inhibitor 2-deoxy-D-glucose (2-DG) sensitized prednisolone resistant but not-sensitive cell lines to prednisolone induced cell kill. This effect appeared to be specific for prednisolone and not for other drugs. Targeting the enhanced glycolysis in ALL may therefore be a suitable approach to modulate glucocorticoid resistance in ALL \[8\].

A second example is the overexpression of MCL-1 in prednisolone resistant ALL cells. This was found not only in common/pre B-ALL but also in MLL gene rearranged infant ALL. Down-regulation of MCL-1 by RNAi sensitized ALL cells to glucocorticoid induced cell kill. Hence, inhibition of MCL-1 also offers an interesting therapeutic strategy in ALL.
Conclusion

Specific gene expression profiles are associated with resistance to different classes of antileukemic drugs. Profiles of genes associated with in vitro drug resistance have independent prognostic value. Analysis of pathways aberrantly expressed in resistant cells may lead to new therapeutic strategies in ALL.

References