Ann Hematol (2001) 80:B35–B37

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Potential of chromosomal and matrix-based comparative genomic hybridization for molecular diagnostics in lymphomas

Introduction

Genome research lead to one of the largest scientific achievements of the last decade. Apart from sequencing the human genome, there was also a huge increase of knowledge regarding genomic aberrations in cancer. In Non-Hodgkin's lymphomas (NHL), some of these findings already are of clinical relevance; specific genomic aberrations are characteristic of distinct subtypes of NHL and correlate with certain morphological, immunological and clinical findings [5]. In addition, some aberrations are useful as clonal markers for the detection of (minimal) residual disease. However, due to limited availability of fresh lymphoma tumor tissues, there are only scarce data regarding the prognostic significance of specific genomic aberrations in lymphomas (see e.g. [11, 13]). In all these studies, chromosomal banding analyses were performed retrospectively in heterogeneous groups of patients. In contrast to such banding analyses, molecular cytogenetic techniques, such as comparative genomic hybridization (CGH) do not depend on the availability of fresh tumor samples. Therefore, these methods allow a retrospective genomic screening of archival tissue samples derived from homogeneous groups of patients treated within the same clinical trial.

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Comparative genomic hybridization within a clinical trial in aggressive lymphomas

We used CGH for a prospective analysis of paraffin-embedded tumor samples obtained from patients, who were treated within the trial NHL-B of the German High-Grade Non-Hodgkin's Lymphoma Study Group. In this trial, all patients received similar therapy regimens (CHOP or CHOEP administered every 14 or 21 days). Two histopathological reference centers of the trial (University of Würzburg; Prof. H.K. Müller-Hermelink, Dr. G. Ott; University of Lübeck: Prof. A.C. Feller, Dr. H. Merz) provided the material for molecular cytogenetic studies. Here, data of the CGH analyses for the first 220 patients are outlined. CGH was successful in 117 cases (53%). In most cases, where CGH was not successful, this was explained by excessive degradation of the DNA due to prolonged fixation using unbuffered formalin.

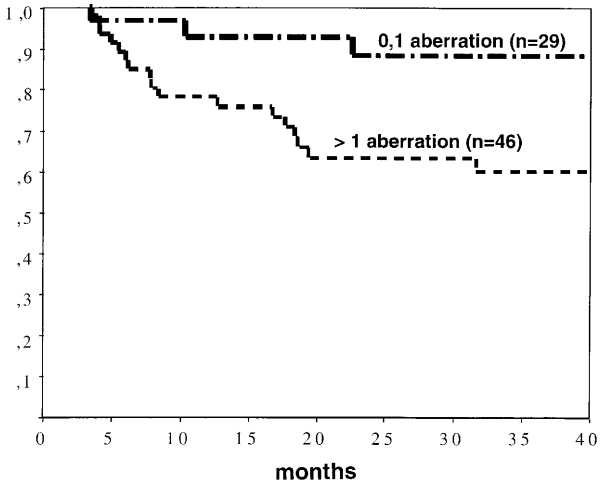


Fig. 1 Overall survival depending on the complexity of the karyotype in 75 patients treated within the trial NHL-B of the German High-Grade Non-Hodgkin's Lymphoma Study Group ($p<0.02$, log rank test)

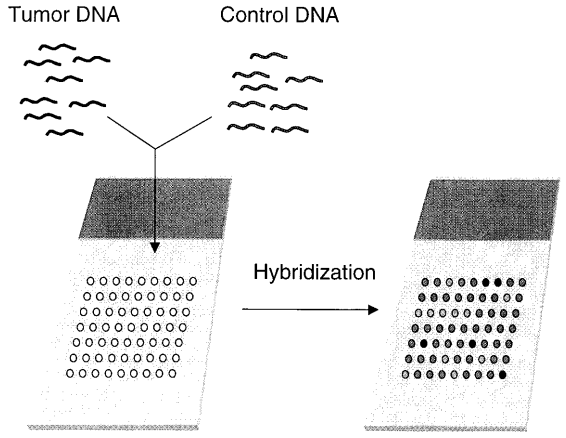


Fig. 2 Schematic illustration of matrix-CGH. Selected DNA fragments are immobilized on a glass slide. Simultaneous hybridization of differently labeled tumor- and control DNA results in characteristic fluorescence signal ratios. Based on these ratios, gains and losses of the respective genomic material within the test genome can be determined

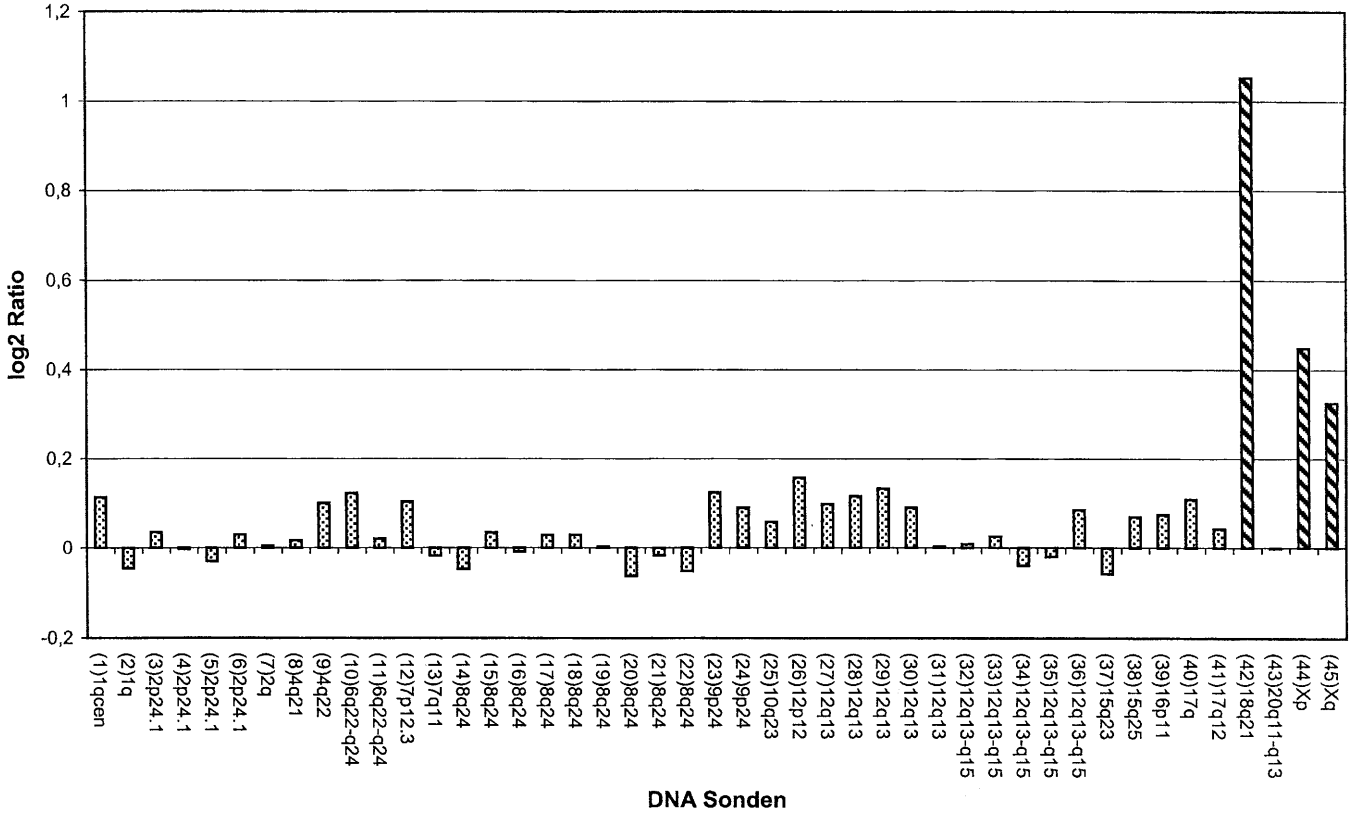
Fig. 3 Example of a matrix-CGH result obtained in a Non-Hodgkin's lymphoma. An array containing 45 different clones was used. The target DNAs are ordered according to ascending chromosomal map locations (horizontal). Logarithmic signal ratio values (log_2) for each clone are shown as vertical columns. In this case, a high copy number amplification on chromosome band 18q21 (clone 42) was diagnosed. In addition, the gender specific copy number difference for chromosome X was detected by two clones (tumor in a female patient versus control DNA obtained from a healthy male volunteer). Signal ratio thresholds for low copy number changes (log_2 <–0.41 and >+0.32) and amplifications ($1og_2$ >1) are equal to those widely used for chromosomal CGH (<0.75 and >1.25 and >2, respectively)

The most frequent genomic aberrations were gains on chromosome arms 7q, 12q and 18q as well as deletions mapping to chromosome arms 4q, 6q and 13q. All these aberrations were present in >15% of the cases. A preliminary analysis of the clinical relevance of genomic aberrations was performed. This analysis suggested a negative prognostic impact for the complexity of genomic aberrations as well as for gains on the long arm of chromosome 18 (see also Fig. 1). With higher case numbers and longer follow-up durations, a more detailed analysis of the prognostic impact of the most frequent genomic aberrations will become possible.

The data obtained within this trial can also be used for the identification of novel correlations between specific genomic aberrations and distinct subtypes of lymphoma. Recently, we demonstrated close genetic relationships between specific lymphoma entities: 11q- and 13q-deletions are significantly more frequent in chronic lymphocytic leukemias and mantle cell lymphomas than in other lymphoma types [3]. For primary mediastinal B-cell lymphomas and Hodgkin´s disease 9p-gain is a characteristic finding [4, 6].

Genomic DNA-Chip analysis: a tool for automated genomic screening in lymphomas

Although a large number of cases were analysed by CGH, this technique has methodological limitations restricting its wide application in clinical diagnostics. These are mainly due to the use of metaphase chromosome preparations as hybridization targets. In each experiment,



chromosomes have to be identified. This step is time consuming and requires well trained personnel. Currently, reliable automation of chromosome identification is not possible. Moreover, only large genomic losses and gains (>3–10 Mbp) are detectable by chromosomal CGH [2, 7]. A novel microarray based approach termed matrix-CGH now seems to overcome most of these limitations [8, 12]. For matrix-CGH, metaphase chromosome preparations are substituted by sets of well defined genomic DNA fragments immobilized on glass slides (see Fig. 2) using automatic roboting devices. Similar to chromosomal CGH, equal amounts of control DNA and differently labeled tumor DNA are hybridized simultaneously onto the array. Results are obtained by automated image acquisition using a laser scanner. Quality control, normalization and statistic array evaluation are then performed with dedicated software tools. Results are summed up as separate logarithmic signal ratio values for each of the tested clones on a given array. By matrix-CGH, smaller aberrations (down to approximately 100 kbp) can be identified and a fully automated evaluation procedure will become possible. However, in contrast to the widespread application of array technology for expression analysis (see e.g. 1; Chan et al. in this issue), so far only few data on genomic aberrations in malignant tumors using matrix-CGH have been published [9, 10, 12].

An example of matrix-CGH for the analysis of a Non-Hodgkin lymphoma is shown in Fig. 3. For this experiment, an array containing 45 different genomic target DNA fragments was used. These DNA fragments contained known oncogenes, tumor suppressor genes and sequences derived from frequently altered chromosomal regions in lymphomas. The tumor DNA was obtained from a Non-Hodgkin´s lymphoma in a female patient. In this experiment, control DNA from lymphocytes of a male donor was co-hybridized. The gender specific copy number difference for chromosome X was sensitively detected by DNA targets no. 44 and no. 45. A high level gain was detected by DNA target no. 42 mapping to chromosomal band 18q21. This clone contains the *BCL2* gene sequence, which is known to be frequently altered in Non-Hodgkin´s lymphomas.

Currently, arrays containing more than 300 different targets are used in our laboratory for molecular diagnostics in lymphomas. Envisioning even larger chips for the comprehensive detection of genomic imbalances relevant to lymphomas, matrix-CGH might become a powerful tool for standardized, high throughput analysis of genomic aberrations in the context of clinical trials.

Acknowledgments This work was supported by the Deutsche Krebshilfe (Project 47/95 Be I) and the IZKF Ulm (Project C12).

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