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REVIEW ARTICLE

Microarray technology in pediatric malignancies: an insight towards individualized therapy

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Abstract:

Accurate and rapid diagnosis, prognostication and monitoring of malignancy are essential for development of curative chemotherapy. Microarrays are such technology which promises to monitor many biological samples at the whole genome or transcriptome scale on a single chip. This technique is becoming increasingly useful in molecular biology and in medicine. Microarrays are the most popular technique for gene expression profiling (GEP). The main purpose of GEP is to finding groups of genes with similar expression patterns and groups of samples with similarly expressed genes. In studies of acute leukemias, it is possible to correlate the expression of over 30,000 genes with a number of specific leukemic features including hematopoietic lineage, immunophenotype and cytogenetics. Microarrays are useful in diagnosis, classify, monitor response to treatment response to chemotherapy, minimal residual disease and clinical outcomes such as metastasis, recurrence, survival or second malignancies. Gene expression profiling can be helpful in addition to conventional techniques. Microarray technology could improve the current procedures for diagnosis and risk assessment in pediatric cancer. A key goal in cancer research is to identify the total complement of genetic and epigenetic alterations that contribute to tumorigenesis. We are currently witnessing the rapid evolution and convergence of multiple genome-wide platforms that are making this goal a reality. These approaches have identified abnormalities in key pathways, including lymphoid differentiation, cell cycle regulation, tumor suppression, and drug responsiveness.

Keywords: microarray, children, diagnosis, individualized therapy

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Profile of gene expression profile in a diagnostic and therapeutic strategy in cancer children

Accurate and rapid diagnosis, prognostication and monitoring of malignancy are essential for development of curative chemotherapy. Currently laboratory methods are based on analysis of level of cell morphology, immunophenotype, cytogenetic, protein and gene expression profiles of cancer cells [1-4]. A major challenge for the treatment of children with cancer is to improve and refine diagnostic and risk classification schemes.

It is not possible to investigate a large number of genes using traditional methods. Microarrays are such technology which promises to monitor many biological samples at the whole genome or transcriptome scale on a single chip [5]. This technique is becoming increasingly useful in molecular biology and in medicine. Microarrays have potential to analyze the expression level of thousands genes in a single reaction in quickly and efficient manner.

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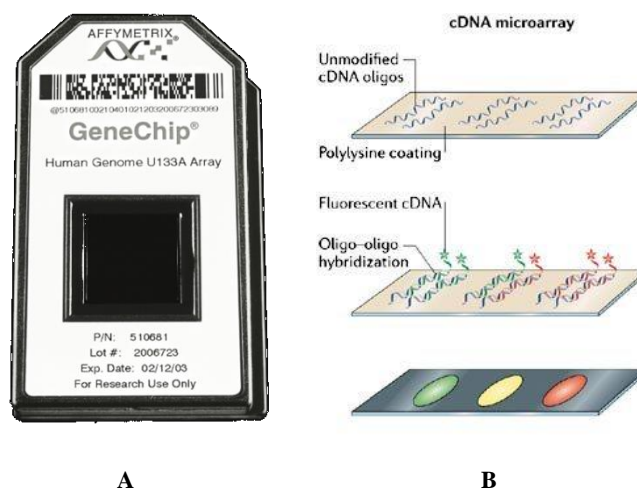


Figure 1. A - One of the most widely used chips in the study of gene expression profiles in acute leukemias GeneChip® Human Genome U133A array (<http://www.affymetrix.com>), B - Scheme of interaction on DNA microarray chip (<http://www.nature.com>)

Arrays technologies are as tools to identify genes whose expression correlates with predict risk of relapse, tailor treatment intensity and monitor response to therapy [3].

Recent studies have demonstrated the ability to stratify acute leukemias on the basis of gene expression patterns. Such analysis can provide new insights into the biologic and molecular mechanisms of drug response and facilitate the development of more effective treatment schemes.

Principles of microarray technology

A typical array platform is a series of many microscopic spots of DNA, cDNA or oligonucleotides that are used as probes attached to a solid support (glass slides, silicon chips or nylon membrane) [5, 6]. Each array contains thousands of different probe sequences arranged in a defined matrix (Figure 1). Most widely used microarrays can be categorized into two groups: (1) robotically spotted cDNA microarrays and (2) microarrays produced by synthesizing the oligonucleotides probe directly on the supporting substrate [6, 7]. A microarray study is multi-step process involves steps like: probe design, array fabrication, RNA extraction and labeling, hybridization, scanning and data analysis (Figure 2) [8].

The RNA samples are obtained from analyzed tissue (interest and reference) and are reverse transcribed into cDNA. In the next step they are assigned to different dye labels [6, 7, 9]. Array spots are hybridized with a cDNA (microarray) or cRNA (GeneChip) sample (called target) under high-stringency conditions. Following hybridization,

the microarray is washed to remove unbound and non-specific material. The presence or absence of a DNA or RNA of interest is visualized using a fluorogenic or chemiluminescent detectors. Detected probe-target hybridization is quantified to determine relative abundance of nucleic acid sequences in the sample. The intensity of the signals from probe-target interaction can be used to estimate whether the expression of a particular genes is up-regulated, downregulated, unchanged or absent [6, 7, 10]. Microarrays are the most popular technique for gene expression profiling (GEP) [8, 11]. The main purpose of GEP is to finding groups of genes with similar expression patterns and groups of samples with similarly expressed genes. Many experiments measure an entire genome simultaneously, so expression of each gene present in a particular cell may be estimated. Analysis of genes expression involves measuring the relative amount of mRNA expressed in two or more experimental conditions. Changes in levels of a particular transcripts suggest a pathological condition.

Application of microarray technology

There are two primary molecular biology applications for an array experiment: identification of sequence (gene/gene mutation) and determination of genes activity (expression profiling) [11]. Microarrays are used especially to measure changes in expression level. With the help of microarray technology, a measurement of the activity of thousands of genes simultaneously can be performed [12]. In this way it is possible to create a better picture of cellular function. Gene expression profiles can be used in: (a) gene

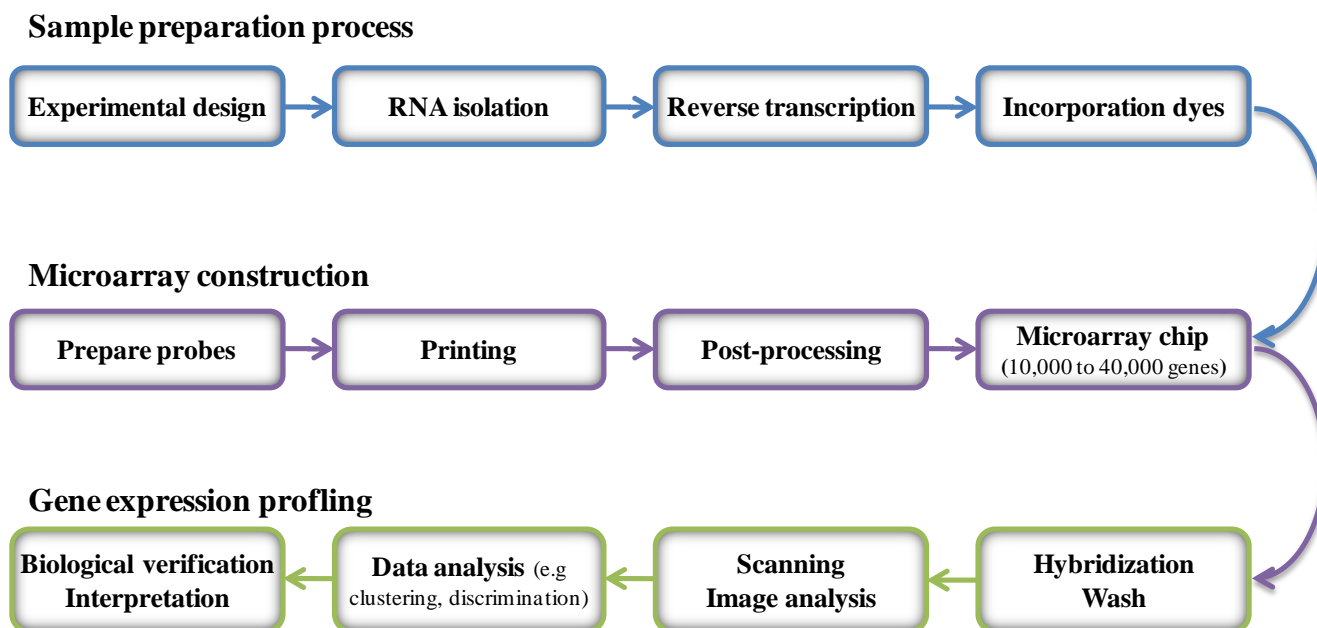


Figure 2. A microarray experiment

discovery, (b) biochemical pathways elucidation, (c) disease diagnosis, (d) drug discovery - pharmacogenomics, and (e) toxicological research - toxicogenomics. This method has been used to examine gene expression profiles of various hematologic and nonhematologic tumors. In studies of acute leukemias, the researchers are able to correlate the expression of over 30,000 genes with a number of specific leukemic features including hematopoietic lineage, immunophenotype and cytogenetics. Microarrays are useful in diagnosis, classify, monitor response to treatment response to chemotherapy, minimal residual disease (MRD) and clinical outcomes such as metastasis, recurrence, survival or second malignancies [2, 3, 13-17]. Gene expression profiling can be helpful in addition to conventional techniques.

Microarray technology could improve the current procedures for diagnosis and risk assessment in acute leukemias [3, 18]. Malignancies are usually classified according to their histology, location, or the occurrence of specific molecular markers. The development of new technologies has led to the necessity of applying more efficient and universal methods of analysis, enabling a better diagnosis of the type of cancer and the determination of risk groups. Microarray technology constitutes the completion of conventional analytical methods. It shortens the time for a complete and precise characterization of cancer cells. Molecular classification based on microarray technology reflects the biological and clinical variety of neoplastic diseases. Gene expression

profiles enable precise diagnosis, scoring and grading, and risk factor stratification. This technique might thus lead totargeted therapy in each type of cancer. This type of strategy has already been used in several types of pediatric cancer, such as leukemia, lymphoma, and sarcoma [19, 20].

Acute lymphoblastic leukemia

Prediction of outcome based on expression signatures

One of the most useful applications of this methodology is to define gene sets that predict outcome. Treatment of childhood ALL is based on the concept of tailoring the intensity of therapy to groups of patient's risk of relapse. Recently, genome-wide studies were started in which patients are stratified according to gene expression profiling. Based on the expression profiles of the leukemic blasts it is possible to predict the relapse for T-ALL and hyperdiploid B-precursor ALL [17], *TEL-AML1*, *MLL*-rearranged and cases that lacked cytogenetic changes. The study performed by Carrol *et al.* [18] suggest that gene expression profiling can be helpful in prediction of slow early response for patients with precursor B-ALL. They were not able to correlate specific genes signature with rapid early response, what may suggest the existence of a variety of molecular mechanisms operate within individual cases to response to chemotherapy. Cario *et al.* [21] identified a characteristic gene expression profile which distinguished patients without minimal residual disease at

end induction and following week 12 from those with detectable MRD at week 12 (group high risk of treatment failure). The signature was characterized by low expression of genes controlling cell cycle progression and apoptosis. Many chemotherapeutic drugs are cell cycle phase-dependent, what may contribute to lower sensitivity of leukemic cells to commonly used chemotherapeutic agents in high-risk patients. Teuffel *et al.* [22] observed low expression of ribosomal proteins in pediatric ALL with an unfavorable outcome. Bullinger *et al.* [13] determined alteration in gene expression levels from 116 adults with AML. They have shown that 133-gene model can accurately predict overall survival including the intermediate risk group. Yagi *et al.* [23] used microarray for identification set of 35 genes related with poor prognosis in childhood ALL.

Drug resistance in leukemia microarray studies

Microarray technology holds great promise in identify indicator genes respond to drug exposure, discover novel gene interactions, complex regulatory networks and cross-communication between different pathways during various anticancer agent [6]. Definition of mRNA signatures can be used to estimate how the cancer cells react to a particular drug and distinguish between drug-resistant and drug-sensitive ALL cells. This method is helpful in defining whether resistance to chemotherapy can be represented by change in transcriptomic profiles and which genes are involved in drug resistance [24]. Expression signatures were able to identify patients that were eventually at risk to fail with conventional therapy. By investigating the differences in gene activity between untreated and treated tumor cells, or between blasts at relapse and at new diagnosis, it will be possible to understand how different therapies affect tumors and ability to develop more effective and less toxic treatment [18].

Several microarray studies of untreated and pre-treated acute leukemias have demonstrated that gene expression patterns can predict antileukemic drug response (Tables 8 and 9). One of the most important array experiment of the leukemic blasts is the study performed by Holleman *et al.* [25]. They identified a characteristic transcriptomic patterns which distinguished ALL samples taken from pediatric patients with leukemic cells either sensitive or resistant to four widely used antileukemic agents: prednisolone (PRD), vincristine (VCR), asparaginase (ASP) or daunorubicin (DNR). The expression of 172 human gene probe sets which correlated with resistant profile was shown to discriminate primary childhood ALL cells. Discriminating genes belonged to numerous functional groups, while no single gene was linked to resistance of four tested agent. Out of the 124 genes correlating with response to treatment, 121 have not

previously been linked with resistance to investigated drugs [25]. The same research group identified also gene expression signatures that distinguish ALL cells with multiple drug resistance profile [26]. Only 15% of identifying genes were common in both experiments. The level of expression for all common genes was concordant between the single and cross-resistance analyses. In the next GEP study, focusing on 70 apoptosis genes Holleman *et al.* [27] identified *BCL2L13*, which was related both to L-asparaginase resistance and treatment outcome, independent from known prognostic factors in 2 independent cohorts of children with B-lineage ALL.

Acute lymphoblastic leukemia

Genetic subtypes of acute lymphoblastic leukemia (ALL) are used to determine risk and treatment in children. 25% of precursor B-ALL cases are genetically unclassified and have intermediate prognosis. Efforts were undertaken to identify children with ALL at initial diagnosis who are at high risk for inferior response to therapy by using molecular signatures. For prediction of early response, genes that correlated to marrow status on day 7 were identified on a training set and were validated on a test set. An additional signature was correlated with long-term outcome, and the predictive models were validated on three large, independent patient cohorts. A 24-probe set signature was identified that was highly predictive of day 7 marrow status on the test set. Pathways were identified that may play a role in early blast regression. Another 47-probe set signature was found that was predictive of long-term outcome in our data set as well as three large independent data sets of patients with childhood ALL who were treated on different protocols. Surprisingly, no sufficient evidence was found for the added significance of these genes and the derived predictive models when other known prognostic features, such as age, white blood cell count, and karyotype, were included in a multivariate analysis. Genes and pathways that play a role in early blast regression may identify patients who may be at risk for inferior responses to treatment. A fully validated predictive gene expression signature was defined for high-risk ALL that provided insight into the biologic mechanisms of treatment failure [28]. New treatment strategies are needed to improve outcome for this newly identified high-risk subtype of ALL [29].

Relapsed acute lymphoblastic leukemia

Almost a quarter of pediatric patients with acute lymphoblastic leukemia (ALL) suffer from relapses [30]. The biological mechanisms underlying therapy response and development of relapses have remained unclear. In an analysis of matched diagnosis-relapse pairs of ALL patients using genome-wide expression arrays on purified leukemic cells, in roughly half of the patients, very few

differences between diagnosis and relapse samples were found, suggesting that mostly extra-leukemic factors (for example, drug distribution, drug metabolism, compliance) contributed to the relapse. Further analysis on 20 sample pairs with clear differences in gene expression, reasoning that these would allow to better study the biological mechanisms underlying relapsed ALL. After finding the differences between diagnosis and relapse pairs in this group, four major gene clusters were identified, corresponding to several pathways associated with changes in cell cycle, DNA replication, recombination and repair, as well as B-cell developmental genes. Another cancer genes commonly associated with colon carcinomas and ubiquitination to be up-regulated in relapsed ALL were also identified. Thus, probably about half of the relapses are due to the selection or emergence of a clone with deregulated expression of genes involved in pathways that regulate B-cell signaling, development, cell cycle, cellular division and replication [31].

Acute myeloid leukemia

Contemporary treatment of pediatric acute myeloid leukemia (AML) requires the assignment of patients to specific risk groups [32]. In microarray analysis of leukemic blasts of AML in pediatric and adult AML samples, class discriminating genes were identified for each of the major prognostic subtypes of pediatric AML, including t(15;17)[PML-RARalpha], t(8;21)[AML1-ETO], inv(16)[CBFbeta-MYH11], MLL chimeric fusion genes, and cases classified as FAB-M7. When subsets of these genes were used in supervised learning algorithms, an overall classification accuracy of more than 93% was achieved. Moreover, it was possible to use the expression signatures generated from the pediatric samples to accurately classify adult de novo AMLs with the same genetic lesions. The class discriminating genes also provided novel insights into the molecular pathobiology of these leukemias. Finally, using a combined pediatric data set of AMLs and ALLs, an expression signature was identified for cases with MLL chimeric fusion genes irrespective of lineage. Surprisingly, AMLs containing partial tandem duplications of MLL failed to cluster with MLL chimeric fusion gene cases, suggesting a significant difference in their underlying mechanism of transformation [15].

Non-Hodgkin lymphoma

T-cell acute lymphoblastic leukemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LL) and are often thought to represent a spectrum of a single disease. The malignant cells in T-ALL and T-LL are morphologically indistinguishable, and they share the expression of common cell surface antigens and cytogenetic characteristics. However, despite these similarities,

differences in the clinical behavior of T-ALL and T-LL are observed. In a microarray analysis of gene expression profiles of pediatric T-ALL and T-LL samples an unsupervised hierarchical clustering of all samples showed complete segregation of T-ALL and T-LL into distinct clusters [33]. The top 201 genes were identified that best differentiated T-ALL from T-LL using significance analysis of microarrays, a supervised statistical approach. Genes representing several functional groups were differentially expressed in T-LL and T-ALL. Prediction analysis of microarrays identified a subset of genes, which accurately classified all T-ALL and T-LL samples with an overall misclassification error rate of 0. Immunohistochemical validation of protein expression of selected genes identified by microarray analysis confirmed overexpression of MLL-1 in T-LL tumor cells compared to T-ALL and CD47 in T-ALL tumors cells when compared to T-LL. Despite significant similarities between the malignant T-cell precursors, clear differences in the gene expression profiles were observed between T-ALL and T-LL implying underlying differences in the biology of the two entities [33].

In a tissue microarray (TMA) analysis of lymphoid neoplasms, it was possible to precisely diagnose Burkitt's lymphoma (BL), diffuse large B-cell lymphoma, mantle cell lymphoma, and B-cell lymphoblastic lymphoma (1 case). In BL, a homogeneous phenotype (CD10(+), Bcl-6(+), Bcl-2(-), MUM1/IRF4-, and Ki-67 approximately 100%) and a stable Epstein-Barr virus integration were found. A distinctive and unusual feature was the frequent plasma cellular differentiation, along with the positivity for CD30 and CD138. The availability of TMAs and immunohistochemistry has enabled us to precisely categorize tumors that might have been diagnosed as "high-grade/aggressive" lymphomas on the basis of cell morphology alone [34].

Central nervous system tumors

Pediatric gliomas comprise a clinically, histologically, and molecularly very heterogeneous group of central nervous system (CNS) tumors. In addition, these tumors are largely different from their counterparts occurring in adults, although they are histologically indistinguishable and uniformly classified by the current WHO classification for CNS tumors. Pilocytic astrocytoma (WHO grade I), mainly arising in the posterior fossa, is the most common representative in children, whereas glioblastoma multiforme (WHO grade IV) predominates in adults [35]. When radical surgical resection is possible in low-grade gliomas, it will likely cure the patient. If complete surgical resection is not possible, however, for example in brainstem gliomas, which are defined by their anatomic localization rather than by their histological or molecular features, therapeutic options are limited and prognosis is

usually poor. Recent genome-wide analyses applying different microarray-based methods to investigate DNA copy number aberrations, mRNA expression signatures, and methylation patterns have shed some light on the pathways involved in the pathogenesis of pediatric gliomas [36]. Mitogen-activated protein kinase (MAPK) and PI3K/AKT signaling were identified as prominent oncogenic pathways in astrocytic tumors in several studies, whereas NOTCH signaling was implicated in the pathogenesis of a subset of intracranial ependymomas. Future therapeutic strategies targeting these (and other) pathways or conferring epigenetic modifications in the tumor might contribute to a better treatment outcome of patients with unresectable or disseminated tumors at diagnosis. Consideration of reliable molecular markers for outcome prediction will most likely result in a better stratification of patients into different risk groups with adjusted treatment intensity in the future [35, 36].

Advances in the field of genomics have recently enabled the unprecedented characterization of the cancer genome, providing novel insight into the molecular mechanisms underlying malignancies in humans. The application of high-resolution microarray platforms to the study of medulloblastoma has revealed new oncogenes and tumor suppressors and has implicated changes in DNA copy number, gene expression, and methylation state in its etiology. Additionally, the integration of medulloblastoma genomics with patient clinical data has confirmed molecular markers of prognostic significance and highlighted the potential utility of molecular disease stratification. The advent of next-generation sequencing technologies promises to greatly transform our understanding of medulloblastoma pathogenesis in the next few years, permitting comprehensive analyses of all aspects of the genome and increasing the likelihood that genomic medicine will become part of the routine diagnosis and treatment of medulloblastoma [37].

Neuroblastoma

The biological heterogeneity of neuroblastoma results in a varied outcome ranging from spontaneous regression to fatal tumor progression. Microarray expression profiling and genetic polymorphism arrays may help identify key genes that differ in aggressive neuroblastomas from those observed in tumors associated with a favorable outcome. Oligomicroarray analysis revealed the overexpression of 283 genes in favorable tumors that were associated with either regressing or maturing tumors. Three candidate genes, including *DHRS3*, *NROB1*, and *CYP26A1*, were selected that were significantly overexpressed in favorable tumors by quantitative real-time RT-PCR. No cases with overexpression of all three genes showed poor outcomes. In post-chemotherapeutic tumors, the expression levels of these genes increased in the cases where patients survived

but decreased in the fatal cases. Thus, using microarray expression profiling, it was possible to identify genes that exhibit altered gene expression in neuroblastoma tumors associated with a favorable outcome. These candidates warrant further study for use in risk assessment and/or as therapeutic targets in neuroblastoma [38]. Using this approach it was also possible to forecast accurately individual survival times for neuroblastoma patients from gene expression data [39].

Osteosarcoma

Osteosarcoma (OS) is an aggressive bone malignancy that primarily affects children and adolescents. Patients with metastatic disease at diagnosis have only a 20% survival rate. The poor survival rate of these patients is largely due to their lack of responsiveness to chemotherapy. The therapy regimen of high-grade osteosarcoma includes chemotherapy followed by surgical resection and postoperative chemotherapy. The degree of necrosis following definitive surgery remains the only reliable prognostic factor and is used to guide the choice of postoperative chemotherapy. The therapy regimen of high-grade osteosarcoma includes chemotherapy followed by surgical resection and postoperative chemotherapy. The degree of necrosis following definitive surgery remains the only reliable prognostic factor and is used to guide the choice of postoperative chemotherapy.

Gene expression screening of nonmetastatic high-grade osteosarcoma patients with cDNA microarray showed that *HSD17B10* gene expression was up-regulated in poor responders and that immunohistochemistry expression of *HSD17B10* on biopsy before treatment was correlated to response to chemotherapy. Other results include correlation of *IFITM2*, *IFITM3*, and *RPL8* gene expression to chemotherapy response. A statistical correlation was found between polysomy 8 or gain of *RPL8* and good response to chemotherapy. These data suggest that *HSD17B10*, *RPL8*, *IFITM2*, and *IFITM3* genes are involved in the response to the chemotherapy and that *HSD17B10* may be a therapeutic target [40].

However, the mechanisms underlying osteosarcoma chemoresistance remain unknown. When the effect of cisplatin, doxorubicin and etoposide was examined on OS cell lines and chip analysis was used to examine differential gene expression, a correlation between increasing metastatic potential and increasing chemoresistance was observed in cell line and sub-line model. Microarray analysis of these cell lines revealed the differential expression of several genes potentially involved in chemoresistance including *ABCG2*, *ADD3*, *NMT2*, *WNT5a* and *PTN*. With the microarray technology, the identification of genes contributing to chemoresistance and determining the role these genes play it is possible to

identify patient responsiveness and overcoming chemoresistance in osteosarcoma patients [41].

Ewing sarcoma

In Ewing's sarcoma family of tumours (ESFT), the clinically most adverse prognostic parameters are the presence of tumour metastasis at time of diagnosis and poor response to neoadjuvant chemotherapy. Genes differentially regulated between metastatic and localised tumours, were identified using microarrays. Functional annotation of differentially regulated genes revealed 29 over-represented pathways including PDGF, TP53, NOTCH, and Wnt1-signalling. Regression of primary tumours induced by polychemotherapy was found to be correlated with the expression of genes involved in angiogenesis, apoptosis, ubiquitin proteasome pathway, and PI3 kinase and p53 pathways. These findings could be confirmed by *in vitro* cytotoxicity assays. A set of 46 marker genes correctly classifies these 20 tumours as responding versus non-responding. Expression signatures of initial tumour biopsies can help to identify ESFT patients at high risk to develop tumour metastasis or to suffer from a therapy refractory cancer [42].

Microarray technology enables to find accurate diagnosis. Small-cell round tumors are often misdiagnosed. In the study aimed to verify the diagnosis of 17 small-cell round tumors of the bone and soft tissue, which were histologically similar to Ewing's sarcoma (EWS), performed on paraffin sections, by using tissue microarray (TMA) technology, immunohistochemistry, cytogenetic (FISH) and molecular biological (QRT-PCR) methods, classical EWS was found to be in 8 patients, large-cell EWS in 1 patient; atypical EWS in 1, and endothelial ES in 1. Two patients were diagnosed as having primitive neuroectodermal tumor (PNET), synovial sarcoma was present in 1 patient, embryonic rhabdomyosarcoma in 1, high-grade undifferentiated sarcoma in 1 and diffuse B-cell large-cell lymphoma in 1. TMA makes it possible to perform a number of diagnostic procedures on the same block containing a copious number of tumor samples and to assess the results of their use. It is emphasized that the diagnosis of small-cell round tumors requires the use of a package of the currently available methods providing the qualitative characteristics of each neoplasm [43].

Wilms tumor

Treatment of Wilms tumor has a high success rate, with some 85% of patients achieving long-term survival. However, late effects of treatment and management of relapse remain significant clinical problems. If accurate prognostic methods were available, effective risk-adapted therapies could be tailored to individual patients at diagnosis. Few molecular prognostic markers for Wilms

tumor are currently defined, though previous studies have linked allele loss on 1p or 16q, genomic gain of 1q, and overexpression from 1q with an increased risk of relapse.

Specific patterns of gene expression in cDNA microarrays were identified in samples from favorable histology Wilms tumors taken from primary nephrectomies at the time of initial diagnosis. Part of these tumors relapsed within 2 years. Genes differentially expressed between the relapsing and nonrelapsing tumor classes were identified: these genes encode proteins with diverse molecular functions, including transcription factors, developmental regulators, apoptotic factors, and signaling molecules. This small subset of genes whose expression potentially can be used to predict tumor outcome in new samples [44].

In another study of children <24 months with stage I favorable histology Wilms tumors <550 g [very low risk Wilms tumors (VLRWT)], two distinctive clusters were identified. One cluster included tumors with epithelial differentiated tubular histology, paucity of nephrogenic rests, lack of LOH for 1p, 16q, and 11p, absence of relapse, and a unique gene expression profile consistent with arrest following mesenchymal-to-epithelial transition. The second cluster included tumors with mixed histology, intralobar nephrogenic rests, and decreased expression of WT1. Two subsets comprising a total of 56% of VLRWT are identified that have pathogenetic and molecular differences and apparent differences in risk for relapse. If these predictors can be prospectively validated, this would enable the refinement of clinical stratification and less arbitrary definition of VLRWT [45].

Rhabdomyosarcoma

The pathologic classification of rhabdomyosarcoma (RMS) into embryonal or alveolar subtype is an important prognostic factor guiding the therapeutic protocol chosen for an individual patient. Unfortunately, this classification is not always straightforward, and the diagnostic criteria are controversial in a subset of cases. Ancillary studies are used to aid in the classification, but their potential use as independent prognostic factors is rarely studied.

In order to identify immunohistochemical markers of potential prognostic significance in pediatric RMS, a single tissue microarray containing paraffin-embedded pediatric RMSs was immunostained with antibodies against p53, bcl-2, Ki-67, CD44, myogenin, and MyoD1. On univariable analysis, immunohistochemical expression of myogenin, bcl-2, and identification of a gene fusion were associated with decreased 5-year RFI (relapse-free interval) and 10-year OS (overall survival). Thus, diffuse immunohistochemical reactivity for myogenin in RMS correlates with decreased RFI and OS, independent of histologic subtype, translocation status, tumor site, or stage [46].

Liver tumors

Microarray technology enables to understand the biology of hepatoblastoma, the most common type of pediatric liver tumor. Although a number of cytogenetic and molecular abnormalities have been described for this type of embryonal tumor, its pathogenesis is still poorly understood. In an attempt to explore the role of different signaling pathways in this disease, the expression patterns of different histologic subtypes of hepatoblastoma using cDNA microarray analysis was analyzed, together with qualitative reverse transcription, polymerase chain reaction, and immunohistochemistry. Wnt signaling pathway, critical both in development and in neoplasia, appears to be particularly relevant in these tumors. Mutations of the beta-catenin gene are present in over 90% of hepatoblastomas, leading to activating transcription of a number of target genes. The pattern of beta-catenin expression and type of mutation in groups of tumors are crucial to understand the corresponding differences in their gene expression profiles. These findings are consistent with a relationship between poor histologic phenotype and beta-catenin activation, indicating the potential utility of targeted gene expression assays to identify molecular events related to the pathogenesis and prognosis of hepatoblastomas [47].

Retinoblastoma

Many retinoblastomas show genomic alterations in addition to mutational loss of both normal RB1 alleles. The hereditary form of retinoblastoma (Rb) is associated with a germ line mutation in one RB allele and is characterized by the occurrence of multiple, bilateral Rb tumors and a predisposition to the development of second cancers. In the study of gene expression profiles in the unaffected parents of patients with hereditary Rb relative to normal individuals, a distinct difference was observed in the patterns of gene expression between unaffected Rb parents and normal controls. By use of the prediction analysis for microarrays and principal component analysis methodologies, significant differences between the two groups were identified when as few as nine genes were analyzed [48].

The most frequent of changes in Rb are gains on chromosomes 1q and 6p and losses on 16q. In gene expression analysis by cDNA microarray hybridization, an increased copy numbers of loci on chromosome 1q were present in 45% primary tumors. Two regions of gain emerged, one in 1q32 and another in 1q21. Tumors with 1q gains showed higher RNA expression of several genes in these 2 regions. The clinical manifestation of tumors with and without gains was similar with regard to many aspects, including size, necrosis and calcification. This association with clinical manifestation indicates that gains

on 1q are significant for the biology of Rb. The genes on 1q with copy number gains and overexpression are candidates that need to be tested for their individual contribution to the progression of Rb [49].

Summary

Integration of clinical, morphologic, phenotypic, cytogenetic, and molecular data has become the basis of novel prognostic prediction and therapeutic strategies in pediatric leukemia. Similarly, integration of new genetic and molecular data with clinical, and other diagnostic information will be crucial for accurate classification of pediatric tumors, risk stratification, and successful development of new therapies for pediatric oncologic patients [47].

Significant progress has been made in understanding the molecular basis of pediatric malignancies. Mechanisms of pediatric acute leukemia induction include hyperdiploidy, aberrant expression of proto-oncogenes, and activation of transcription factors or kinases by aberrant fusion genes. Molecular analysis of these alterations has facilitated the recognition of distinct groups with different sensitivity to therapy, and identified potential targets for antileukemic agents. Similar analysis of pediatric soft tissue and bone tumors also resulted in the identification of specific fusion genes, and their characterization has contributed greatly to understand their biology. Molecular assays for these rearrangements have become important tools in classifying these tumors, providing important prognostic data. However, the understanding of mechanisms involved in the pathogenesis of many other pediatric malignancies, including some embryonal tumors, believed to arise due to perturbation of the normal developmental program, is still vastly incomplete.

A key goal in cancer research is to identify the total complement of genetic and epigenetic alterations that contribute to tumorigenesis. We are currently witnessing the rapid evolution and convergence of multiple genome-wide platforms that are making this goal a reality. These approaches have identified abnormalities in key pathways, including lymphoid differentiation, cell cycle regulation, tumor suppression, and drug responsiveness.

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