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Review article
Pregledni članak

APPLICATION OF GENOMICS IN CLINICAL ONCOLOGY

PRIMENA GENOMIKE U KLINIČKOJ ONKOLOGIJI

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Summary: Genomics is a comprehensive study of the whole genome, genetic products, and their interactions. Human genome project has identified around 25,000–30,000 genes, and prevailing presence in tumor pathogenesis, high number of mutations, epigenetic changes, and other gene disorders have been identified. Microarrays technology is used for the analysis of these changes. Postgenome age has begun, and the initial results ensure the improvement of molecular tumor diagnostics and the making of a new taxonomic tumor classification, as well as the improvement, optimization and individualization of anti-tumor therapy. First genomic classifications have been made of leukemias, non-Hodgkin lymphoma, and many solid tumors. For example, 4 molecular types of breast carcinoma, three types of diffuse B cell lymphoma, two types of chromophobic renal carcinoma have been identified. Also, gene structures for favorable and unfavorable outcome in leukemia, breast cancer, prostate, bronchi, and other tumors have been identified. It is absolutely possible to diagnose the primary outcome of tumors with which standard tumor position may not be proved using standard diagnostic tools. Pharmacogenomic profiles have ensured better definition of interindividual differences during therapy using antineoplastic drugs and the decrease of their toxicity, as well as individual treatment approach and patient selection with which favorable clinical outcome is expected. Pharmacogenomics has impacted the accelerated development of target drugs, which have showed to be useful in practice. New genomic markers mtDNA, meDNA, and miRNA have been identified, which, with great certainty, help the detection and diagnostics of carcinoma. In the future, functional genomics in clinical oncology provides to gain knowledge about tumor pathogenesis; it will improve diagnostics and prognosis, and open up new therapeutic options.

Keywords: microarray assay, genomics, pharmacogenomics, gene expression, cancer, prognosis, prediction

Kratak sadržaj: Genomika je sveobuhvatna studija celokupnog genoma, genskih produkata i njihovih interakcija. Projekat ljudskog genoma identifikovao je oko 30.000 gena i preovlađujuće prisustvo intergenskih sekvenci. U onkogenima, supresornim genima tumora i dr. genima koji imaju ulogu u patogenezi tumora, identifikovan je veliki broj mutacija, epigenetskih promena i dr. genskih poremećaja. Za analizu ovih promena upotrebljava se mikroarej tehnologija. Postgenomska era je počela, i prvi rezultati omogućavaju da se poboljša molekularna dijagnostika tumora i izvrši nova taksonomska klasifikacija tumora, kao i da se poboljša, optimalizuje i individualizuje antitumorska terapija. Izvršene su prve genomske klasifikacije leukemija, non-Hodgkin limfoma i mnogih solidnih tumora. Na primer, identifikovana su 4 molekularna tipa karcinoma dojke, tri tipa difuznog B ćelijskog limfoma, dva tipa papilarnog karcinoma bubrega. Takođe, identifikovane su genske signature za povoljan i nepovoljan ishod u lečenju leukemije, karcinoma dojke, prostate, bronha i dr. tumora. Apsolutno je moguće dijagnostikovati primarno ishodište u tumora kod kojih se standardnim dijagnostičkim sredstvima ne može dokazati primarno ležište tumora. Farmakogenomski profili omogućili su bolje definisanje interindividualnih razlika u toku terapije antineoplastičnim lekovima i smanjenje njihove toksičnosti, kao i individualni pristup u lečenju i selekciji pacijenata u kojih se očekuje povoljan klinički ishod. Farmakogenomika je uticala na ubrzani razvoj ciljanih lekova, koji su se u praksi pokazali svrsishodnim. Identifikovani su novi genomski markeri mtDNA, meDNA i miRNA, koji sa velikom sigurnošću pomažu u detekciji i dijagnostici karcinoma. U budućnosti, funkcionalna genomika u kliničkoj onkologiji omogućiće upoznavanje patogeneze tumora, poboljšaće molekularnu dijagnostiku, prognozu, i otvoriti nove terapijske opcije.

Ključne reči: mikroarej esej, genomika, genska ekspresija, kancer, prognoza, predviđanje

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Introduction

In the postgenome genomocentric era, accelerated research of the human genome using microarray technology has occurred. It has been assessed that the human genome has got around 25,000–30,000 ge-

nes, but around 75% genomes contain so-called intergenic DNA or non-coding sequences. The International HumMap Project identifies the variations in DNA sequences that are common among humans (1, 2). More than 291 cancer-causing genes have been identified, which is around 1% of the human genome, and around 6% tyrosine and serine kinases have been associated with human cancer (2, 3). Genomics is the comprehensive study of whole sets of genes, gene products, and their interactions (1, 3).

Cancer is a heterogenous group of diseases occurring due to the accumulation of genetic mutations of cancer-related genes, chromosomal instability, and epigenetic changes (4). These abnormalities influence the expression of genes that control tumor growth, apoptosis, invasiveness, metastatic potential and responsiveness or resistance to chemotherapy (5). Identification of these genes that are mutated or the loss of function of antioncogenes in cancer is the central aim of cancer research (6). Microarrays technology has made it possible to identify all of the tumor-specific mutations, to make the molecular profile of individual tumors at the DNA, RNA, and protein levels and to test the genomic response to particular drugs (7). The ability to measure the expression of the thousands of genes in a tumor specimen has revolutionized our ability to describe cancers. Recent studies suggest the usage of DNA microarray technology for class discovery and class prediction, while a major confounder in the generation of gene expression profiles is likely to be the necessity of using small tissue samples (1 μg) (8). Techniques for the »molecular profiling« include: DNA expression profiling, single nucleotide polymorphism (SNP), array-based comparative genomic hybridization (aCGH), genomic resequencing arrays, and serum/tissue proteomics (9, 10).

Nowadays, there is a high interest in transferring microarray technology to clinical oncology because of: the contribution in tumor subtypisation, the identification of persons with increased tumor risk, the identification of the tumor of unknown result, the determination of optimal individual treatment, the identification of predictive and prognostic genomic markers, and the development of new target medicines (2–10). However, routine application of microarray technology in clinical practice requires more significant improvements in the standardisation of microarrays and further improvement of bioinformatics and genome structures in relation to standard methods. The working group on biomedical technology dealing with the creation of a Human Cancer Genome Project (HCGP) aims at forming a large collection of the samples of all tumor types and at making their complete genome specification including multiple technologies. There are now over 4.4 million sequences in the national Center for Biotechnology Information (NCBI) database of Expressed Sequence Tages (ESTs). The Cancer Genome Atlas (CGA) is a pilot project to determine all genomic changes involved in all types of human cancer (11, 12).

The National Cancer Institute (NCI) and National Human Genome Research Institute (NHGRI) today try to accelerate the understanding of the molecular basis of cancer through the application of genome analysis, and the creation of the network of national centers for bioinformatics. Also, in Europe, German Research Center and in Australia MC Callum Center develop a large scale of cancer genomics programs. This paper describes the possible application of DNA microarray in clinical oncology.

Basics of DNA microarrays

DNA microarray is a technique that provides global analysis of gene expression at the level of transcription. Microarrays have been used extensively to simultaneously monitor the expression of thousands of genes from human tumor samples. Because of gown, microarray extends wide bridges between basic science and clinical oncology (13). Microarrays analyses are used in clinical oncology: to identify altered genes or biochemical pathways associated with particular disease, to identify new molecular classes of disease (class discovery), and to predict diagnosis and classification of unknown samples (class prediction) (14).

A DNA microarray, also known as gene or genome chip, DNA chip, biochip, or gene array, is a miniaturized microsystem containing cDNA fragments from thousands of different genes that are immobilized, or attached, at fixed locations (spots) on glass or other matrix. The production of spotted microarrays is a highly automated process to print cDNA or oligonucleotides on the support. The sample spots sizes in microarray are less than 200 microns in diameter, and these arrays usually contain thousands of spots (*Figure 1*) (15).

In general, today, there are two platforms: »cDNA« and »oligonucleotide microarrays« are currently used by a majority of investigators and both are effective. Complementary DNA arrays contain polymerase chain products (PCR) of 500–5000 bp cDNA collections that can be focused on genes expressed in a particular cell type. Oligonucleotide microarrays offer greater specificity than cDNAs, because they can be deposited or synthesized directly on the surface of a support, and because they can be tailored to minimize chances of cross-hybridisation, and sequences up to 25–70 bp have been effective (9, 15). For a microarray experiment, 10–40 mg of high quality RNA is necessary (15).

The whole basic concept of microarray technology is based on hybridization probing. A typical DNA microarray experiment involves the following steps: DNA types, chip fabrication, sample preparation, assay, readout, and bioinformatics. In the first step, total RNA or mRNA is isolated from the source tissue

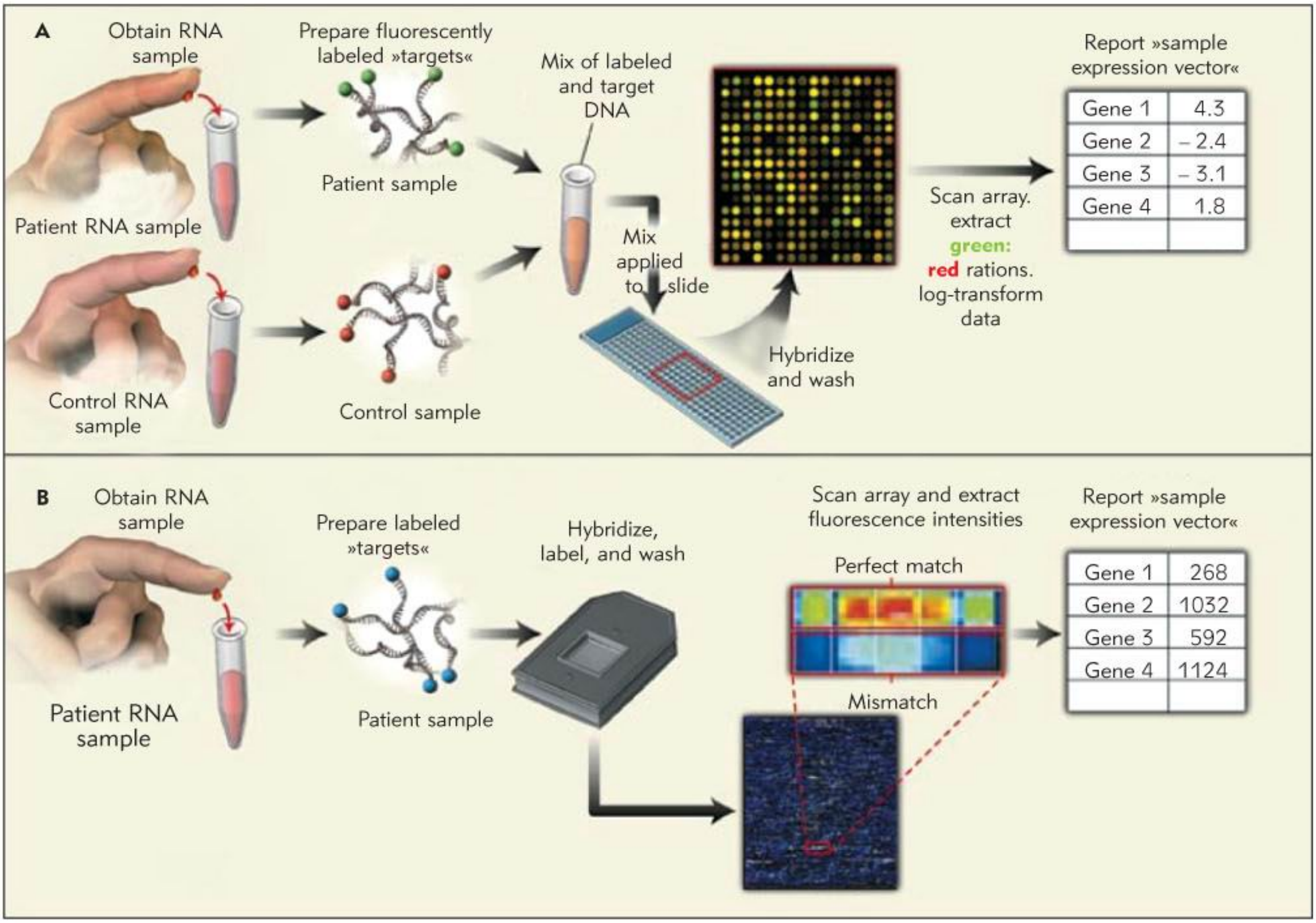

Figure 1 Schematic illustration of DNA microarray analysis.
Panel A: two-color analysis. Panel B: single-color analysis. Taken over from: Quackabush J (N Engl J Med 2006; 354: 2463–721).

or cells and converted to cDNA by reverse transcription labeled with a fluorescent dye, hybridized to the probes on the microarray and detected by phosphor-imaging or fluorescent scanning. After hybridization, the slide is scanned using two different wave lengths, corresponding to the dyes used. The scanning processes generate an image file. The final step in microarray experiments is bioinformatics analysis (15). An image analysis software is used to calculate the intensity of each spot on the array and to store these measurements in a text file. For statistical analysis and visualization of gene expression data development there is a large number of software tools (e.g. Gene Spring, Gene Cluster, dCHIP). For the analysis of the voluminous data there are two methods of pattern recognition: unsupervised and supervised. The expression data can be pictorially summarized, where each row represents a single gene, and each column represents expression levels. The Microarray Gene Expression Data Society (MGEDS) (www.mged.org) recommends the use of a set of Minimum Information About a Microarray Experiment or MIAME (www.mged.org/workgroups/MIAME/miame.checklist.html) (16, 17).

The significant contribution that bioinformatics has made to genomic research is the development and maintenance of nucleotide sequences data bases. There are six major data bases of publicly available information: Gene Bank, EMBL, DNA Data Bank of Japan, Gene Expression Omnibus (GEO) of the National Center for Biotechnology Information at NIH, and ArrayExpress of the European Bioinformatics Network (HuGENet). For example, the current Gene Bank contains more than 17 billion bases from more than 100.000 species. Also, the Cancer Gene Expression Database is an integrated database of gene expression and clinical information (http://cged.hgc.jp) (5, 8, 18). Catalogue of Somatic Mutations in Cancer (COSMIC) is designed to store and display somatic mutation information, and related details contain information relating to human cancer (COSMIC) (http://www.sanger.ac.uk/genetic/CGP/cosmic).

The applicability of DNA microarrays in molecular diagnosis of cancer

Microarrays have been used to study various tumor types, most notably lymphomas and cancers:

breast, colon, lung, prostate, ovary, gastric, melanoma and carcinoma of unknown primary. Gene expression profiling for cancer diagnosis was originally demonstrated in 1999 with microarrays to study the expression of 6,817 human genes in 72 acute lymphatic leukemia cases (ALL) (8, 19). Today, the interpretation of new classes of ALL is not possible without accurate class labels leukemia-specific genetic translocation. Xiong H. and Chen YW identified the seven subtypes of ALL: bcr-abl, E-2A-PBX1, MLL, T-ALL, TEL-AML-1, hyperdiploid > 50 chromosomes, and »others« (20). The subtype TEL-AML-1 in t(12, 21) has good prognosis, and fusion transcript is a prognostic indicator for ALL (19–21). These data indicate the response to chemotherapy is »molecular hard wired« for therapy. MLL leukemias are overexpression of FLT3 which may be a particularly poor response to conventional chemotherapy. Detection of IGkV and TCR genes rearrangements is the most sensitive predictor of relaps in ALL and an excellent marker of minimal residual disease (22).

Acute myeloid leukemia is a heterogeneous molecular disease. Bullinger et al. and Valk et al., 2004, identified five molecular subgroups of AML: PML-RAR, AML1-ETO, CBFB-MYH11, MLL and CD34+ normal – with »poor prognosis« (21, 22). However, Willson et al. (23), 2006, divide AMLs into six cluster groups: A, B, C, D, E, and F. In cluster A they demonstrated mutations of NPM1 genes and overexpression of WTL gene, and genes that promote apoptosis (LTB1, Casp3). In cluster B they identified overexpression of ABCG2, MDR1, BCRP, and MRX genes. Also, in cluster C they identified overexpression of genes for: IRF1, IL-10RA, and MALT-1. In cluster D was defined a »high proliferative signature« and identified down regulation of HOXA9 and HOXA10. The final, cluster F, was defined as having NPM1 mutations and a »monocyte gene signature« that impacts prognosis and therapy of AML (23). However, in AML with normal kariotype they identified two clusters, type I and type II, with different survival rates. CBF is a relatively frequent subtype of AML and carries mutations in the KIT gene (24).

Chronic myelogenous leukemia (CML) is a myeloid stem cell neoplasm with a disease-defining chromosomal translocation, the Philadelphia chromosome (Ph). This translocation fuses two normally separate genes, BCR and ABL, resulting in the BCR-ABL, the oncogene responsible for the development of CML (25). The fused Bcr/abl gene and its gene products provide specific markers for diagnosis and disease monitoring. Screening the Ph chromosome for CML is currently not recommended. However, PCR assay is a powerful tool for the detection of subclinical minimal residual diseases (25, 26). During Bcr/abl translocation, the chromosome 9 breakpoint involves a large 200 kb region within the alteration first exone. The breakpoint on the chromosome 22 is a cluster of three much smaller regions of the Bcr gene (M-Bcr, m-Bcr, and μ-Bcr) (27). Chronic lymphocytic leukemia (CLL) is the leukemia most often found in humans and it is an indolent but inexorable disease with no cure. The presence of somatic mutations in the immunoglobulin genes of CLL cells defined a group that had stable or slowly progressing disease (*Figure 2*) (28). By contrast, patients with unmutated CLL cells had aggressive disease. The 70-KD zeta-associated protein (ZAP-70) is anomalously expressed in CLL cells with unmutated IgV_H genes and may enhance the signaling process in BCR. ZAP-70 expression correctly predicts IgV_H mutations status in 93% of patients. Patients whose leukemic cells express unmutated IgV_H regions often have a progressive disease, whereas patients whose leukemic cells express mutations in IgV_H regions more often have an indolent disease (29).

Multiple myeloma may be, on the basis of expression analysis of B cells, divided into 6 subtypes: MAF, MAFB, CCND1-, CCND3-, MMSET, and others (30).

Diffuse large B cell lymphoma (DLBCL) is the most frequent and aggressive non-Hodgkin lymphoma in adults. The diversity and clinical presentation and outcome, as well as the pathologic and biologic heterogeneity, suggest that DLBCL comprises several disease entities that may require different therapeutic approaches. The gene expression profiling has identified three major molecular subgroups of DLBCL: germinal center B cell like (GCB), activated B cell like (ABC), and primary mediastinal (PM-BCL) or type 3 (*Figure 2*) (31). The GCB group is characterized by frequent REL amplifications, BCL-2 translocations, and ongoing somatic hypermethylation of the immunoglobulin genes. However, ABC and PMBCL have continuing activation of the nuclear factor kB(NF-kB), including IRF-4 and cyclin D2 (31). Achilles heel screen in ABC subgroup is CARD 11/MALT1BCL 10 genes (32). PMBCL is a subtype of DLBCL which has frequent loss of MHC II proteins and better survival than DLBCL. The GCB subgroup expressed high BCL-6 levels, but the ABC subgroup did not express BCL-6. However, ABC subgroup is characterized by high expression of B lymphocyte-induced maturation protein-1(BLIMP-1) which is a transcriptional repressor and required for terminal B cell differentiation (31, 33). The ABC lymphoma have been up-regulated: ACAI, CASP8, FADD-like, PIM2, and PBX2 (34). Mediastinal large B-cell lymphoma (MLBC) is a recently identified subtype of DLBCL that characteristically presents as localized tumors in young female patients. MLBCL had high levels of expression of IL-13R, and downstream effectors of IL-13 signaling (JAK-2, STNT1, TNF, and TNFR-1) (35). Suguro et al. (36) identified »CD5+« and »CD5-« genes signatures. The »CD5+« signature includes downregulation of ECM genes such as POSTN, SPARC, COL1A1, COL3A1, CTSK, MMP9,

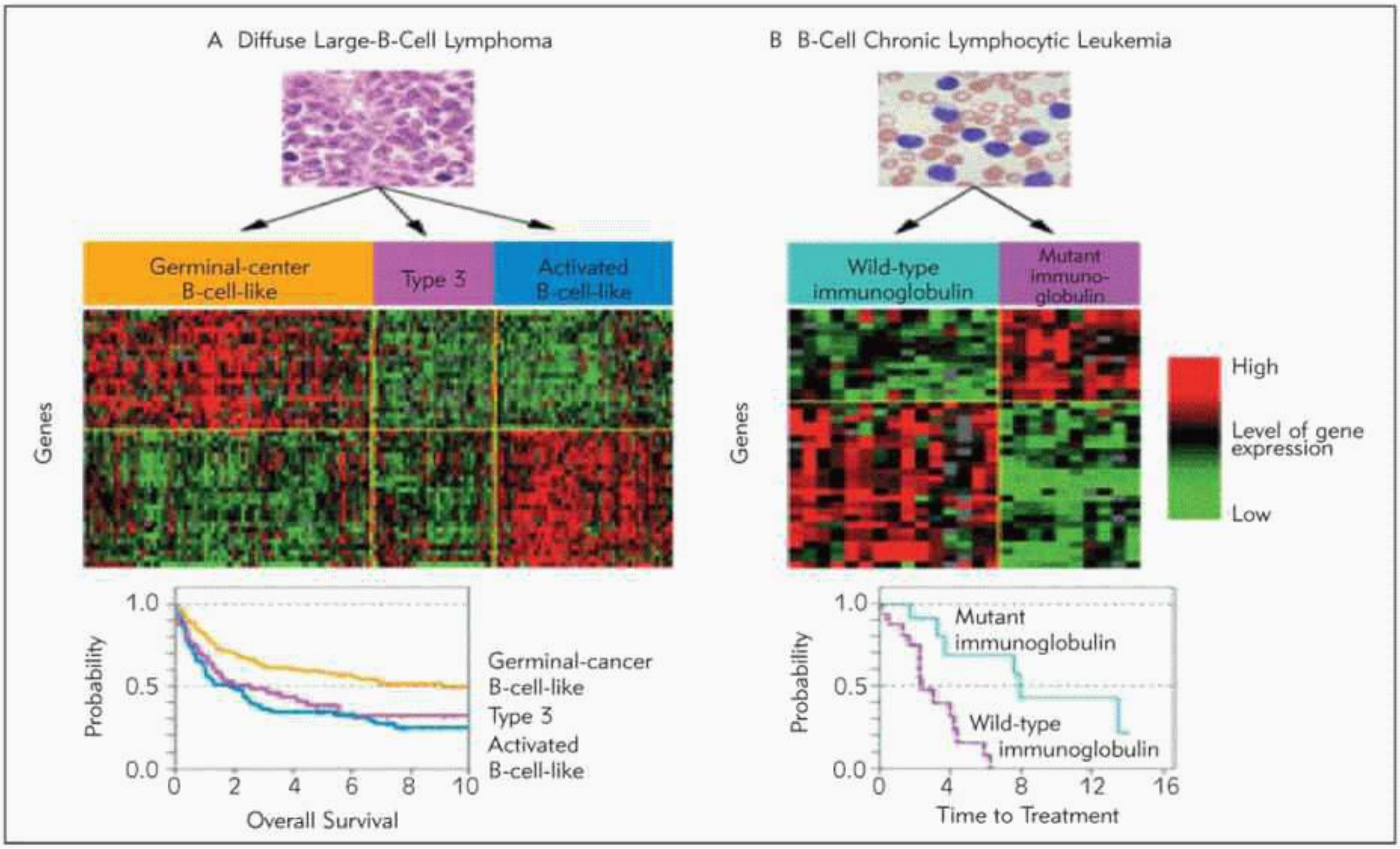

Figure 2 Genomic expression profiles of diffuse large-B-cell lymphoma and chronic lymphocytic leukemia.

and LAMB3. Also, three discrete subsets of DLBCL with genes signatures: oxidation phosphorylation, B cell receptor/proliferation, and host response was identified (36, 37). The distinction between Burkitt's lymphoma and DLBCL is unclear and clinically important. The molecular Burkitt's lymphoma (mBL) signature consisted of »58 genes«, including genes NF-kB (i.e. BCL 2A1, FLIP (CD44, NFkB1A, BCL 3, and STAT 3) that are known to distinguish ABC or GCB subgroups (38).

The analyzed gene expression in follicular lymphomas (FL) has the signature of the immune response-1 »ir-1« which includes selected genes of T-cell activation and function and is highly expressed in macrophages. The immune response-2 »ir-2« signature includes genes which are known to be preferentially expressed in macrophages and dendritic cells. The overexpression of the »ir-1« signature correlates with improved prognosis, but that of the »ir-2» signature correlates with a poor outcome (39, 40). Mantle cell lymphoma MCL) is an aggressive, highly proliferative B-cell non-Hodgkin lymphoma, characterized by the specific translocation t(ll;l4)(q13; q 32) and expression of cycline D1. The characteristic gene expression signature of MCL allowed us to identify a new subgroup that is cyclin D1 negative (41).

Breast cancer is the leading cause of cancer death among women, where the clinicopathological features of tumors are used to prognosticate and guide therapy. The genomic alteration, which frequently occurs in breast cancer and defines key pathogenetic events, is also a potentially useful diagnostic, prognostic, or predictive factor (42). Two major genes, BRCA1 and BRCA2, are associated with susceptibility to breast and ovarian cancer. Mutations in either of these genes confer a lifetime risk of breast cancer of between 60% and 85% and lifetime risk of ovarian cancer of between 15% and 40%. Testing for germline mutations in BRCA1 and BRCA2 is an important tool for predicting the risk of breast cancer and ovarian cancers and developing management strategies. Habel LA, 2006, identified four groups of risk of breast cancer death among lymph node negative patients. Combining recurrence score, tumor grade, and tumor size provides better risk classification than any one of these factors alone (43). Sorlic et al. developed an »intrinsic gene set« and, using hierarchical clustering analysis, classified breast cancer into four main groups:

- luminal cell like with three subgroups: A, B, and C, – tumors that express ER and show profiles similar to normal luminal;
- based cell like: ER-negative that express genes usually expressed by basal/myeloepiteluace;
- HER 2: tumors with consistent overexpression of HER 2;
- normal breast like (*Figure 3*) (44).

The luminal A of the ER positive subgroup has the highest expression of ER, GATA, binding protein

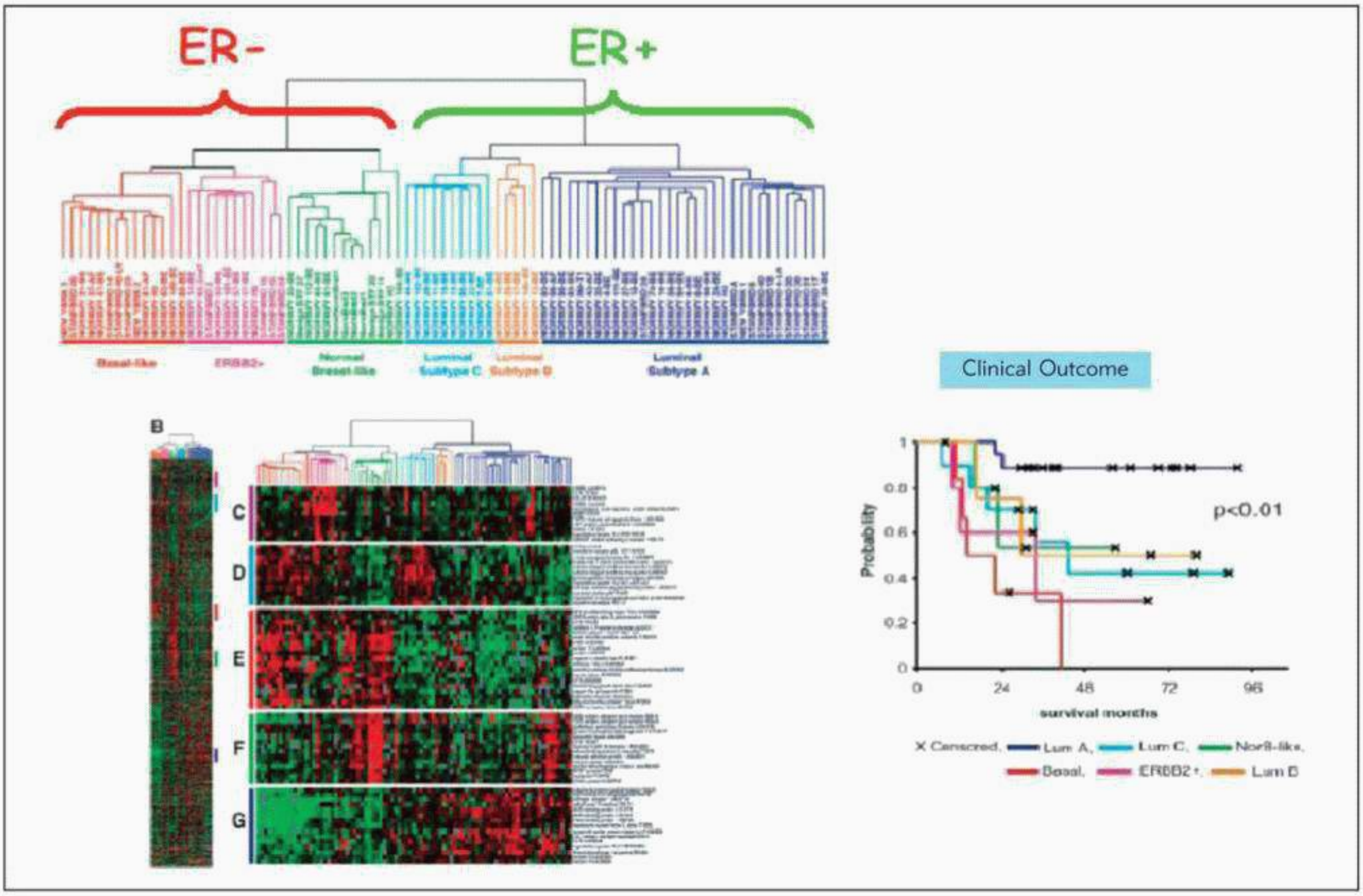

Figure 3 Molecular Classification of Breast Cancer.
To take over from: Sorlic T. et al. PNAS 2001; 98 (19): 10869–74.

3, XBBP-I, HNF 3 alfa and LIV – 1. Luminal B and C tumors showed moderate expression of genes in the ER cluster. The absence of expression of such markers in some ER positive cancers could assist in identifying with BCL-2, and PS-2, HER 2 and EGFR are in inverse correlation with ER expression (44, 45). Cheny et al. identified the expression profile »258 vs. 34« that may identify patients with breast cancer who have a high risk for locoregional relapse after mastectopy (46).

Lung cancer is the leading cause of cancer mortality worldwide, yet there exists a limited view of the genetic lesion driving this disease (47). In non-small cell lung cancer (NSCLC), panels of genes have been found which differ in their expression from normal lung tissue, and thus provide a set of markers that may be useful as diagnostic or therapeutic targets. The adenocarcinoma can be classified into two major types, terminal respiratory unit (TUR) type, and non-TRU type (48). Raponi et al. (49) identified prognostic signatures »50 mRNA transcripts« that may identify patients in an early NSCLC stage who may benefit from adjuvant therapy after surgical treatment. Higher HLJ 1 (DnaJ-like heat shock protein) expression levels are associated with better patient outcome (50). Overexpression of WEGF, PPARγ, cathepsin L, and PAU, ACTN4, and PGK1 is in correlation with poor survival (51). On the basis of ODC gene expression with signature »54«, the groups of adenocarcinoma with »poor prognosis« and »good prognosis« were identified (52). In squamosa forms of lung cancer, a high level of keratin type genes and stratifin was exprimated (53). High serpin B3 is associated with chemoresponsiveness and had a prognostic value in untreated SCC or adenocarcinomas (54). Also, 85% of NSCLCs are associated with promoter hypermethylation ADAMTS8 gene (55). Non-small lung carcinoma is a heterogeneous disease. Chen H. et al. (56) identified the signature of »five genes« (DUSP6, MM9, STAT1, ERBB3, LCK) that can predict the clinical outcome and targets for the development of adjuvant therapy for NSCLC. Squamous cell lung carcinomas and carcinoid tumors show high-level expression of neuroendocrine genes, insulinoma-associated gene GRP and chromoganin A, thymosin-β, $p18^{ink4c}$, p53, p63, claritin, and stratifin, a known regulator of squamous cell differentiation. Bahttachryee et al. (57) defined four subclasses of primary lung adenocarcinoma (C1 to C4). Borczuk AC et al. defined so-called »invasiveness signatures« with TGF-βRII, and E-cadherin overexpression (58). Epidermal growth factor receptor (EGFRR1) mutations analyses might lead to a refinement of the molecular distinctions of

NSCLS subtypes with clinical relevance. The EGFR1 mutations show different frequencies between white and Asiatic patients. Mutations in the EGFR occur in 10–20% of NSCLC, specifically adenocarcinomas, and are associated with the response to EGFR thyrosine kinase inhibitors (Erlotinib and Gefitinib). However, the results of screening of NSCLC for EGFR mutations have been negative. The presence of EGFR mutations was a significant predictor of shorter postoperative survival for TRU type, independent of disease stage (48, 59). Hypermethylation of the TSLC/ IGFSF4 and FIHT genesis was associated with tobacco smoking and a poor prognosis in NSCLC (60). In smokers with and without lung cancers overexpression of about 100 different genes was identified. Many of these genes were drug-metabolizing and antioxidant genes. Expression of a number of genes correlated with cumulative smoking history (61). The Duke University Medical Center developed the Lung Metagene Predictor genomic test to predict patients with early stage lung cancer (62).

Colorectal cancer (CRC), the second most common cancer in the Western world, is best not considered as a homogenous disease, but as covering a spectrum in terms of its molecular properties and its pathological diversity. The molecular differences between adenomas and carcinomas amounted to 1800 and 50 discriminating genes were identified capable of distinguishing the two stages. The »colochip« is a microarray specified to see 460 genes that are expressed in CRC, normal colonic mucosa and liver metastases. Microarray assay gene expression studies in CRC have so far shown the possibility to distinguish between normal and tumor tissue, between different stages of disease, and different tumor locations (left-side vs. right-sided) (63). Patients with advanced CRC consistently contained mutant APC DNA molecules in their plasma in more than 60% cases. The levels ranged from 0.01% to 1.7% of the total APC molecules (64). For early detection of CRC and the FAP syndrome Digital Protein Trunction Assay (DTPA) is used, which detects mutations in the APC gene. Mutations have been detected in 61% CRC in Dukes B 2 stages, in 50% adenomas, which are <1 cm, in 36% CRC on splenic flexure and 60% CRC on proximal flexure (65).

On the basis of the knowledge of molecular pathogenesis of prostate, in non-hereditary forms of the carcinoma, alterations in GSTP1, NKX31, PTEN, P27 genes and androgenous receptor were discovered. Epigenetic hypermethylation was also discovered in: GPX3, SFRP1, COX2, DCK3, GSTM1, and NKX3 genes (66). In the androgen-dependant group of carcinoma, there is increased gene expression of: RNA, metabolism, cell cycle, adhesion, and angiogenesis. However, in the androgen-independent carcinoma, exprimated genes for the synthesis of protein and protein transport are increased, and the expression of the genes responsible for apoptosis is decreased (67). It was determined that there is a correlation between gene expression and clinical parameters and responses to therapy (68). The gene ratio-based diagnosis of prostate cancer using fine needle aspiration could serve as a useful adjunct to standard histopathological techniques (69). Multiclass molecular classification of prostate cancer can help separate poorly differentiated from well-differentiated cancer. The multiclass classifier is highly accurate, but is not perfect. This finding suggests that a successful clinical classification may require the introduction of parallel platforms such as DNA microarray assay into the clinical setting. Petersen LE et al. discovered 16 genes (DGCR5, FLJ0618, RIS1, PRO1895, ABCB9, AKO7203, GLOGA5, HARAS, AKO24152, HEP27, PPIA, SNRPF, SULT1A3, SECTM1, EIF4EBP1, and 571435) which are markers for early detection of prostate cancer and prognostic markers for cancer relapse detection (70). The GSTP 1 is the most frequently methylated gene in prostate cancer. Attempts have been made to detect prostate cancer by identifying plasma and serum, prostate secretion, voide urine and prostate biopsy specimens (specificity 98%, and sensitivity 73%). Hypermethylation of GSTP 1 genes in prostate cancer can correlate with pathological grade or clinical stage and independent cancer (71).

Whang et al. (72) performed specific genes expression profiles of melanoma, which can rapidly aid in tumor classification and identification. EORTIC melanoma group identified a genomic »signature of 254 genes«, which predicts clinical outcome in primary cutaneous melanoma after patients having undergone standard treatment. Also, metastatic profile was identified that was linked to the small GTPase RhoC. Microarray gene expression can be used to define responders and nonresponders. The overall BRAF/NRAS frequency in mutation hotspot is not significantly different among cutaneous melanoma subtypes (73). In metastatic melanoma, in the third and fourth stage of illness, signature »30 survival related prognosis« group with longer survival was identified (74). The prognosis of metastatic melanoma with expression of NEDD9 is also poor. The chronic sun induced damage has substantially more BRAF mutations than melanomas that arise in the area of the trunk, arms and legs that are intermittently exposed to the sun (75). However, CCND1 amplification occurs predominantly in acral regions. Increased susceptibility to melanoma is associated with the loss of germ line CDKN2A gene. In 2006 the new transciption factor MC-1 correlating with melanoma progression was identified and it is used to discover mutations in skin tumors caused by solar UV radiation. The loss of cytoplasmatic P-cadherin is a prognostic marker for melanoma progression (76). Also, ASK/HuDbF4, TRP and Ferritin High Chain (FTL) are independent predictors of malignant melanoma type.

Predicative and prognostic genomic markers

In recent years, in parallel with traditional clinical criteria for the assessment of illness flow, genome markers have been widely used for the prediction and prognosis of clinical and biological tumor flow. In the area of oncohematology, nowadays, several genome markers are used. For example, with the patients with AML disease younger than 55 and who have normal karyotypes, the presence of FLT3 ITD indicates the increase of relapse and poor disease prognosis (22). Also, those with AML disease with mutations in NPM1 and CEBPA genes have got favorable prognosis, but other diseased patients with expression profile: FLT3 ITD, MLL PTD, and ETS have got »poor« prognosis (*Table II*) (23, 24). Methylation types TEL-AML1 and BCR-ABL ALL have got poorer prognosis than non-methylation types (72). Oncohematologists, for the prognosis of those with diffuse large B cell lymphomas (DLBCL) disease, use the International Prognostic Index (IPI) based on clinical data, morphology, and other tools. However, nowadays, they may, on the basis of this genome profile and IPI, with high accuracy and specificity, determine 5-year survival rates: in GCB group it is 59%, in ABC it is 30% and in PMBCL it is 64% (*Figure 2*) (32, 83). This »CD5+« is a clinically distinct subgroup, which is associated with poor prognosis and has more aggressive clinical features (36). With Mantle cell lymphomas, cyclin D1 gene is a prognostic survival factor (41). With this disease, the disregulation of cycline E is a strong predictor of poor prognosis (83).

In clinical oncological practice, there are ongoing studies of various genomic signatures in the patients with cancer of: breast, colorectum, prostate gland, kidney, urinary bladder, lungs, pancreas. For example, approximately 65% of women with breast cancer diagnosis have lymph node negative disease, and 85% of these are expected to be alive and free from distant metastases after 10 years. With such malignant phenotype »70 gene signature« with the women younger than 50, the groups of patients with good and poor prognosis have been identified. In the group with poor prognosis, gene structure »70« indicated that metastatic potential was 6.4 times higher than in the basic group (44). Also, HER-2 gene and decreased expression of β-catetin in breast tumor were related with poor prognosis (45, 84).

The prognosis of colorectal cancer (CRC) is based on TNM or the Dukes staging system in four stages. However, it was clinically observed that B and C stages include groups with good and poor prognosis. This clinical heterogeny has confirmed genome researches into »signature 43« which include osteopontin and neuregulin (63). Sporadic CRC with microsatellite instability have a better prognosis than microsatellite stable tumors. The carriers mutations in mismatch repair genes (MLH1, MSH2 and MSH6) have the same total survival as the non-carriers of these mutations (85). Barier (81) defined the genetic signature for II and III CRC stage in colorectum mucosa, that may be used for the evaluation of surgical resection. The prognosis of CRC among patients with hereditary nonpolyposis colorectal cancer (HNPCC or the Lyinch syndrome) is better than among those with sporadic CRC (85).

The estimate of prognosis of the patients with non-small cell lung cancer is substantially improved due to genome discoveries. For example, higher levels of HLJ 1 (DnaJ-like heat shock protein) indicate

Table I Alterations of miRNA expression in human cancers.

Altered miRNA	Locus	Cancer type	Directly regulated targets
Down regulated			
Let-7 family	multiple	Lung associated of a poor prognosis	with RAS family
miR15a/16	13q14.2	B-CLL	Bcl-2
miR-143/145	5q32	Colorectal (miR 145 breast)	ERK5, MAPK7
miR-125b(lin4)	11q24.1	breast	ND
Upregulated			
miR-17-92	13q31.3	Lung, breast, colon, pancreas, prostate, hepatocellular cancer	E2F1, TGFbeta, RII
miR -106a	Xq26.2	Colon, pancreas, prostate	RB1
miR -155	21q21.3	M. Hodgkin, Non-Hodgkin lymphoma, breast, lung	ND
miR -221/222	Xp11.3	Papillary thyreoid cancer, glioblastoma	KIT
miR -21	17q23.2	Breast, colon, lung, pancreas, prostate, stomach, glioblastoma	ND
miR -372/373	19q13.42	Testicular germ cells tumors	LATS 2
miR -191	3p21.31	Colon, lung, pancreas, pro tae, stomach	

ND: not determined
To take over from: Volina S. et al (PNAS 2006; 103 (7): 2257.

better NSCLC prognosis than the expression of hypermethylation of the TSLC/IGFSF4 and FIHT genes (48, 59, 52, 56).

Predicting of treatment results in anticancer therapy

Cancer drug therapy is undergoing a transition from the previous pregenomic cytotoxic era to the new postgenomic era. Future mechanism based on therapeutic agents would be designed to act on molecular targets that are involved in the malignant progression of human cancer. Such agents would show greater therapeutic selectivity for cancer versus normal cells. Heterogeneity in patients' response to chemotherapy is consistently observed in populations. Pharmacogenomics is the study of inherited differences in interindividual drug disposition and effects, with the goal of selecting the optimal drug therapy and dosage for each patient. Pharmacogenomics is especially important for clinical oncology, as severe systematic toxicity and unpredictable efficacy are hallmarks of cancer therapies. Current therapies of cancer have exhibited limited success with efficacy in only 20–40% of cases. Moreover, penalty of administering optimal therapy that employs high doses of extremely toxic drugs is severe due to the associated side effects. Pharmacogenomics can lead to optimized therapy regimens, resulting in improved quality of life and life expectancy in cancer patients. The first step in developing molecular diagnosis is to identify genetic markers predicative of toxicity or efficacy. The following 3 analytic tools are used to identify these markers: genotyping/haplotyping, LOH, and mRNA expression analyses. The genetic polymorphisms in drug metabolizing enzymes and other molecules are responsible for much of the interindividuals differences in the efficacy and toxicity of many chemotherapy agents. The prediction of cancer treatment outcome based on gene polymorphisms is becoming possible for many classes of chemotherapy agents (84). Cancer research has provided insight into the processes responsible for cancer growth and identified numerous molecular targets for cancer therapy (e.g. apoptosis, angiogenesis, cell-cycle regulation, signal transduction, and invasion). A novel therapeutic target in angiogenesis is Avastin, and in cell-cycle it is Flavopiridol. For example, Imanitib (Gleevec) in CML induces dramatic and often durable clinical responses in most patients. The therapeutic index is high. The specific methylation changes in MGMT and MLH1 genes can alter the response to different therapeutic agents in cancer. Other methylation-regulated genes that could serve as biomarkers in cancer therapy include drug transporters, genes involved in microtubule formation and stability, and genes related to hormonal therapy response.

Combination chemotherapies in cancer are usually selected by a trial-and-error approach. It should now be possible to use gene expression profiling to assess the interaction of anticancer agents in order to optimize combination chemotherapy. In ALL, a comparison of samples before and after treatment with typical agents (mercaptopurine and methotrexate) yields a set of genes with distinct regulation patterns linked to treatment. Thiopurines are a family of drugs that include mercaptopurine and azathioprine. Thiopurine methyltransferase (TPMT) polymorphisms have been associated with therapeutic efficacy and toxicity of mercaptopurine. TPMT activity is highly variable and polymorphic in all large populations; approximately 90% of individuals have high activity. By using microarray assay it is possible to detect the three signature mutations in TPMT2, TPMT3A, and TPM3C. These results can then be prospectively used to determine safe starting doses for thiopurine therapy (86).

A paradigm for understanding the pathogenesis of AML has been proposed in which co-operation was required between class I mutations, which have antiapoptotic properties, and class II mutations, which lead to inhibition of cellular differentiation, for the expression of the leukemic phenotype (*Table II*).

In addition, chip analysis has shed light on antiandrogen resistance in the treatment of prostate cancer. In androgen-dependent prostate carcinoma high expression genes are discovered (RNA metabolizing, cell-cycle, adhesion, and angiogenesis). However, in androgen-independent prostate cancer high expression genes for synthesis and transport of protein, and low expression genes for apoptosis are discovered (67). The presence or absence of HER-2 amplification can be used to differentiate patients who may

Table II Mutations classes and leukemias and target therapies.

Examples	Potential therapies
Class I	
FLT3	FLT3 inhibitors (PRE 412, CEP 701, MLN 518, Thalidomide, Lendidomide)
KIT	c-kit or Bcr-abl inhibitors (Imatinib, Desatinib, AMN107)
RAS	Farnestyl transferase inhibitors (Tipifarnib, High dose cytoarabine)
BCL-2 high expression	Bcl-2 antisense (Oblimeksen)
PI3K kinase activation	mTOR inhibitors (e.g. Rapamycin)
Class II	
PML-RAR	All-trans-retinoid acid
RUNX1-MTG	Epigenetics therapies (5-aza-C)
CBFB MYH 11	Epigenetics therapies (5-aza-C, ATRA, SAH1)
MLL PTD	Epigenetics therapies (5-aza-C)

have a response to antibody HER-2 (Trastuzumab, Herceptin) therapy from those who will not have a response. The likelihood of tumor regression with Herceptin therapy may be as high as 35% among patients with tumors that strongly overexpress HER2 (67). The interleukin-17BR is a predictor for the risk of recurrence in women with node-negative, ER-positive breast cancers who had received adjuvant treatment with tamoxifen (85).

The EGFR, also known as ErbB1 or HER 1, has important roles in the proliferation and metastasis of tumor cells. It is frequently overexpressed in common solid tumors and has become a favored target for orally administrated EGFR inhibitor Gefitinib for non-small cell lung cancer. In phase I/II studies, Gefitinib was active against NSLC (9% to 12%) across a broad range of doses, and 30% or more of patients had stable disease (32). The finding of heterozygous mutations suggested that the mutation caused a gain of function of EGFR. Mutant receptors were more sensitive to inhibition by Gefitinib (67). In 2006, at Duke University, researchers determined the panel of genomic tests for the detection of toxic effects to chemotherapy (86). Lam S, Ling R. defined predicative genomic signatures for chemotherapy response in non-small cell lung cancers patients with genome BAC CGH microarrays (87). Citin KV at al. defined the signature on the cervical tumors resistent and sensitive to radiotherapy or chemotherapy (Carboplatin/Taxol or Cisplatin/Taxo) (88). The combination of genomic profiling using microarray analysis and the development of targeted therapy hold promise of individualizing prognostics and therapy (89).

The combined genotyping of dihydropirimide dehydrogenase (DPD)and TSER functional variants might be useful in selecting patients who are likely to tolerate and respond to 5-FU therapy (84). The determination of the UGT1A1 genotypes may be clinically useful for predicting severe toxicity to Irinotecan (84). Mayo Clinic developed the UGT1A1 test for patients with advanced colorectal carcinoma which have a serious adverse reaction to Compostar (FDI, August, 2006).

The polymorphisms in the XPD gene were significantly associated with treatment outcome. Twenty four percent of patients with the lys/lys genotype achieved an objective response to therapy with DDP. Polymorphisms in the XRCC1 and GSTs genes also have to be associated with DDP agent response (84). Silencing of PEBP4 expression may be a promising approach for breast cancer (90).

The subcutaneous metastases of melanoma are more responsive to immunotherapy with IL-2 than nodal and visceral metastases. The subcutaneous metastases highly expressed PRAME and TRP-1, IL-I6,IL-21,Lck,IFI I6 (71). In non-small cell lung cancer, expression profiles can be defined to predict the chances of successful treatment with commonly used anticancer drugs such as DDP, taxanes or gemcitabine (91).

Paik et al. (85) validated the use of an RT-PCR assay of 21 genes to predict the likelihood of distant recurrence in node negative, estrogen receptor positive patients treated with tamoxifen. Paik's assay calculates a distinct recurrence score (RS) on the basis of tumor expression of 16 cancer related genes and five reference genes. The RS categorizes the patients as being at low risk (score <17), intermediate risk (score 12 to 30), and high risk (score >30). The RS is a tool that can stratify patients to identify low risk patients who would not need chemotherapy (85).

Novel genomic markers

In the postgenome era, the research of novel genome cancer markers, micro RNAs, methylate DNA, and mitochondriae DNA began.

MicroRNA (miRNA)

MicroRNAa (miRNA) are endogenous 22-nt small and non-coding class small RNA genes that function as negative gene regulators with mRNAs, and inhibit their expression (92). In the human genome, more than 300 or 400 miRNAs have been discovered, and the estimated number of mirNA genes is as high as around 1000 (93, 94). Micro RNAs regulate basic cellular functions including proliferation, differentiation and apoptosis. As a group, miRNA are estimated to regulate 30% of genes in the human genome. The expression of miRNAs is highly specific for tissue development in stages of tumors. The expression of miRNAs genes is deregulated in cancer (95). More than half of miRNAs are located at sites in the humane genome that are frequently amplified, deleted, or rearranged in cancer. Mutations in miRNAs or polymorphisms in the miRNAs may contribute to cancer predisposition and progression (96). The overexpression or underexpression has been shown to correlate with tumor types. The overexpression of miRNA could result in down regulation of suppressors genes, until their underexpression leads to oncogene up-regulation. The expression profiles can be used for the classification, diagnosis and prognosis of human cancers (95, 96). Specific miRNA signature has been identified by now in lung, breast, stomach, colon, prostate and pancreatic cancers, and other hematological diseases (*Table II*).

The identified frequence copy number of abnormalities of Dicer, Argonate 2, and other miRNA is in breast cancer 72.8%, ovarian cancer 37.1%, and melanoma 85.5% (96). The elevated expression of miR-21, miR-15, and down regulation of miR-125b and miR-145 are useful for distinction between normal and cancer breast tissues. In hepatocellular carcinoma a higher expression of miR-18, pre-miR-18, and miR-224 was identified and also lower expression of miR-199a, miR-195, miR-200a, and miR-125a (97). The overexpression of has-let-7g, miR-181b, and has

miR-200c is associated with colon cancer, and has-let-7g and has-miR181b are indicators for chemoresponse to 5-Fluorouracile (98). High levels of expression of has-miR-155 and low levels of has-let-7a-2 correlated with poor prognosis of survival in patients with lung adenocarcinoma. The overexpression of miR-15a/miR-16 and miR-146 was found in chronical lymphocytic leukaemiae (CLL) and multiple myeloma (99).

About 10% of patients with CLL have mutations in genes for miRNAs. Thirteen miRNAs genes were identified that represent a unique genetic signature and could potentially be useful to distinguish between the two types of CLL (100).

Methylated DNA (meDNA)

Epigenomics has discovered highly specific DNA methylation markers for predicting the aggressiveness of breast and prostate cancer, lung, colorectal cancer, AML and other cancers (101). The BRCA 1 methylation is frequent (9.1%) in primary sporadic breast cancer. The BRCA1-methylated tumor is significantly associated with ER negativity. In medullary histology BRCA 1 methylated gene types are not found (101, 102). The methylated septin 9 DNA (me DNA) in blood is found in up to 52% of patients with all stages of colorectal cancers at high levels of specificity – 95% in asymptomatic individuals over 50 years of age (103). The circulating methylated tumor related DNA in serum of melanoma patients could be used to monitor patients during treatment and to determine the disease status (102, 103). The identification is hypermethylation in promoter genes CCCNA1G, CDKN2A, CRABP1, MLH1, NEUROG 1 and MGMT, into colorectal cancer (103). The methylation profile of a group of four genes (DKK3, SFRP2, PTEN, and p73) may be a potential new biomarker of risk prediction in ETR6/RUNX1-positive acute leukemia (104). DNA hypermethylation has been associated with drug resistance acquired during cancer chemotherapy, and therefore the re-expression of methylation silenced genes resulted in increased sensitivity to existing chemotherapy. The treatment of cancer with demethylating agent (Decitabine) could lead to re-expression of caspase-8 and the restoration of sensitivity to chemotherapy.

Mitochondrial DNA (mtDNA)

Mitochondrial DNA is a 16 569 bp double-stranded, circular DNA encoding 13 respiratory chain protein subunits, 22 tRNAs and two rNAs. It is also composed of a 1.2 kb noncoding region, the Displacement-loop (D-Loop), which contains essential transcription and replication elements.

The mutation rate is 10–17-fold higher in mtDNA than in the nuclear DNA. mtDNA mutations may lead to a dysregulation of oxidative phosphorylation that can enhance production of the carcinogenic ROS. Somatic mutations have been reported in many human tumors. In head and neck squamosis cell carcinomas mutations in the noncoding region of the D-Loop, D310 were described. The D-loop mutations should be considered as a cancer biomarker that can be useful for the early detection of head and neck carcinoma (105). Mitochondrial DNA mutation may by involved in medular thyreoid cancer tumorogenesis and progression (106). Similar results were obtained in prostate and oesophagus adenocarcinoma. The D-loop mutations were associated with poor prognosis and absence of benefit from adjuvant 5-FU chemotherapy in colon cancer. Also, mtDNA depletion increased sensitivity to cisplatin.

Mitochondrial DNA has a high mutation rate due to the damage produced by free radicals, the lack of protective action by histones and the limited capacity of repair of the mtDNA. A high incidence of specific mtDNA alterations has been reported for gastric, prostate, pancreatic, skin, colorectal, urinary bladder, thyreoid, oesophageal, breast, uterine cancers and chromophobe renal cell cancer (107).

Mitochondrial DNA plays a role in respiration and the cells energy conversion mechanism. Since the late 1990s, researches at the John Hopkins University School of Medicine have observed changes in mtDNA sequences in solid cancers. The National Institute of Standards and Technology (NIST) has developed a relatively simple diagnostic test »Temperature Gradient Capillary Electrophoresis (TGCE)« which is a sensitive and high-throughput screening tool for identifying mtDNA variation (108). For example, in primary lung tumor eight sequence variants of mtDNA were identified. Two of the sequence variants identified (22%) were found in the D-loop region, which accounts for 6.8% of the mitochondrial genome. The other sequence variants were distributed through the coding region. In lung tumors, the majority of sequence variants occurred in the coding region (109). The mtDNA mutations are an early indicator of malignant transformation in prostatic cancer tissue (110). Serial genetic analysis of the mtDNA methylation profile can be used for predicting the aggressiveness of breast cancer and monitoring cancer patients during treatment (110). The high frequencies of D310 alteration in primary breast cancer combined with the high sensitivity of the PCR-based assays provide a new molecular tool for cancer detection (111).

The future of clinical cancer management will be based on the development of functional genomics and related disciplines, with specific emphasis on the chip-based analysis of tumors. Information rich gene expression dataset will be applied clinically to predict accurate diagnosis, prognosis and therapeutic options.

References

1. Quackenbush J. Microarray analysis and tumor classification. N Engl J Med 2006; 354: 2463–72.
2. Abdullah-Sayani A, De Mesquita JMB, Van de Vijver MJ. Technology insight: tuning into the genetic orchestra using microarrays-limitations of DNA microarrays in clinical practice. Nature Clinical Medicine 2006; 3 (9): 501–16.
3. Hunter T. Protein kinases and disease in the postgenomic era. http://pasteur.fr/applications/eurocont/proteinkinase/14hunterabstract.pdf (I2/I2/2006).
4. Bernner Ch, Duggan D. Oncogenomics. Molecular approach to cancers. USA, Arizona, Totoma, New Jersey: Humana Press; 2004.
5. Liu ET, Krishna R, Karturi R. Microarrays and clinical investigations. N Engl J Med 2004; 350: 1505–97.
6. Landanyi M, Gerald WL. Expression profiling of human tumors. Diagnostic and research applications. Human Press, USA; 2003.
7. Ramaswamy S. Translating cancer genomics into clinical oncology. N Engl J Med 2004; 350: 1814–7.
8. Fey MF. Genomics and proteomics: expression arrays in clinical oncology. Ann Oncol 2004;Suppl 4:iv163 –5.
9. Wadlow R, Ramaswamy. DNA microarrays in clinical cancer research. Current Molecular Medicine 2005; 5: 111–20.
10. Gabriele I, Moreti F, Pierotti MA, Marncola FM, Foa R, Belardelli FM. The use of microarray technologies in clinical oncology. J Translation Medicine 2006; 4: 1–5.
11. COSMIC. htpp:// www.sanger.ac.uk/genetics/CGP/cosmic (2/14/2006).
12. Report of working group on biomedical technology, February 2005. Recommendation for Human Cancer Genome Project. http://www.genome.gov/ pages/about/NACHGR/may 2005 NACHGR Agenda. Report of Working Group on Biomedical. Technology.pdf. (11/14/2006).
13. Goldsmith ZG, Danasekeran N. The microevolution: applications and impacts of microarray technology on molecular biology and medicine (review). Int J Molec Med 2004;13: 483–95.
14. Weaver ChH, Deutrer D. Medical genomics: implications for clinical oncology. Current Topics in Oncology 2005.
15. Nambiar PR, Boutin SR, Raja R, Rosenberg DW. Global gene expression profiling: a complement to conventional histopathologic analysis of neoplasia. Veterinary Pathology 2005; 42: 735–52.
16. Stoeckert CJ Jr, Causton HC, Ball CA. Microarray databases: standards and ontologies. Nat Genet 2002; 32: 469–73.
17. Brazma A, Hingamp P, Quackenbush J, Sherlock G, Spelman P, Stoeckert CJ Jr, et al. Minimum information about a microarray experiment (MIAME) – toward standards for microarray data. Nat Genet 2001; 29: 365 –71.
18. Ioanidis JPA. A rapid map for efficient and reliable human genome epidemiology. Nat Genet 2006; 38: 3–5.
19. Grimwade D, Haferlach T. Gene expression profiling in acute myeloid leukemia. N Engl J Med 2004; 350; 16: 1676–8.
20. Xiong H, Chen YW. Kernel-based distance metric learning for microarray data classification. BMC Bioinformatics 2006; 7: 299.
21. Yeoh EJ, Ross ME, Shirtleff SA, Williams WK, Patelo D, Mahariz R, et al. Classification subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene profiling. Cancer Cell 2002; 1 (2): 133–43.
22. Bench AJ, Erber WN, Scott MA. Molecular genetic analysis of haematological malignancies: Acute leukemias and myeloproliferative disorders. Clin Lab Haematol 2005; 27 (3): 148.
23. Willson C, Davidson GS, Martin SB, Andries E, Potter J, Harvary R, et al. Gene expression profiling of adult acute myeloid leukemia identifies novel biologic clusters for risk classification and outcome prediction. Blood 2006; 108 (2): 685–96.
24. Mrozek K, Bloomfied CD. Chromosome aberrations, gene mutations and expression changes, and progression in adult AML. Hematology 2006; 1: 169–77.
25. Houghton SG, Cockerill FR. Real-time PCR: overview and applications. Surgery 2006; 191 (1): 1–5.
26. Lee WI, Kantarijan H, Glasman A, Talpaz M, Lee MS. Quantitative measurement of BCR/abl transcripts using real-time polymerase chain reaction. Ann Oncol 2002; 13: 781–8.
27. Tefferi A, Dewald GW, Litzow ML, et al. Chronic myeloid leukemia: current application of cytogenetics and molecular testing for diagnosis and therapeutic. Mayo Clin Proc 2005; 80 (30): 390–402.
28. Colin GA, Ferracin A, Cimmino A, et al. A micro RNA signature associated with prognosis and progression in chroniclymphocytic leukemia. N Engl J Med 2005; 353: 1793–801.
29. Mirshahid HR, Abraham J. Genomic profiling in clinical oncology. Postgraduate Medicine 2006; 119 (2): July-August.
30. Zhan F, Huang Y, Colla S, Stewart JP, Hanamura I, Gupta S, et al. The molecular classification of multiple myeloma. Blood 2006; 108: 2020–8.
31. Bea S, Zefl X, Wright G, et al. Diffuse large B cell lymphoma subgroups have distinct genetic profiles that influence tumor biology and improve gene expression based survival prediction. Blood 2005; 106 (9): 3183–90.
32. Studt LM. Molecular diagnosis of the hematological cancers. N Engl J Med 2003; 348 (18): 1777–85.

33. Pasgualucci L, Compagno M, Holdsworth J, et al. Inactivation of the PRDM1/BLIMP1 gene in diffuse large B cell lymphoma. N Engl J Med 2006; 354 (2): 311–17.

34. Polsen CB, Broup R, Nielsen FC, Borregoard N, Hansen M, Gronbaek K, et al. Microarray-based classification of diffuse large B-cell lymphoma. Eur J Hematol 2005; 74 (6): 453.

35. Savage KJ, Monti S, Kutok JL, Cattoretti G, Neubarger D, De Leval L, et al. The molecular signature of mediastinal large B cell lymphoma diffuse from that of other diffuse large B-cell lymphomas and their features with classical Hodgkin lymphoma. Blood 2003; 102 (13): 3871–9.

36. Suguro M, Tagava H, Kagoma Y, et al. Expression profiling analysis of the CD5+ diffuse large B cell lymphoma subgroup: development of a CD5 signature. Cancer Sci 2006; 97: 868–74.

37. Monti S, Savage KJ, Kutok JK, et al. Molecular profiling of diffuse large B cell lymphoma reveals a novel disease subtype with brisk host inflammatory response and distinct genetic features. Blood 2005; 105 (5): 1851–61.

38. Humnel M, Beutnik S, Berger H. A biological definition of Burkitt's lymphoma from transcriptional A genome profiling. N Engl J Med 2006; 354 (23): 2419 –30.

39. Connors JM. Improving diagnosis and treatment of lymphomas with gene expression profiling. (Hematologist/jfm 05/review. 8/30/2006).

40. Roberts RA, Wright G, Rosenwold AR, et al. Loss of major histocompatibility class II gene and protein expression in primary mediastinal large B cell lymphoma is highly coordinated and related to poor patient survival. Blood 2006; 88 (1): 311–8.

41. Schraders M, Pfundt R, Straatman HM, et al. Novel chromosome imbalances in mantle cell lymphoma detected by genome wide array based comparative genomic hybridization. Blood 2005; 105 (4): 1686–93.

42. Bergamashi A, Kym YH, Wang P, Sorile T, Hernandez-Bousard T, Longing PE, et al. Distinct patterns of DNA copy number alteration are associated with different clinicopathological features and gene expression subtypes of breast cancer. Genes Chromosomes Cancer 2006; 45 (11): 1033–40.

43. Habel LA, Shak S, Jacobs MK, et al. A population based study: tumor gene expression and risk of breast cancer death among lymph node negative patients. Breast Cancer Res 2006; 8 (3): R 25.

44. Sorlic T, Perou CM, Tibshirani R, Aas T, Geisler S, Johansen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. PNAS 2001; 98 (19): 10869 –74.

45. Reis-Filho JS, Westbury C, Pierga JM. The impact of expression profiling on prognostic and predicative testing in breast cancer. J Clin Pathol 2006; 59: 225–31.

46. Pritchard KI, Shepherd LE, O'Malley FP, et al. HER 2 and responsiveness of breast cancer to adjuvant chemotherapy. N Engl J Med 2006; 354: 20: 2103–11.

47. Cheny SH, Horng CF, West M, Huang E, Pittmans J, Tsou MH, et al. Genomic prediction of locoregional recurrence after mastectomia in breast cancer. J Clin Oncol 2006; Oct 01: 4594–4602.

48. Tonon G, Kin-Wong K, Maulik G, et al. High-resolution genomic profiles of human lung cancer. PNAS2005; 102: 9625–30.

49. Raponi M, Zhang Y, Yu J, Chen G, Lee G, Taylor JMG, et al. Gene expression signatures for prediction prognosis of squamous cell and adenocarcinoma of the lung. Cancer Res 2006; 66: 7466–72.

50. Takeuchi T, Tomida S, Yatabe Y, Kosaka T, Osada H, Zanagisawa K, Mitsudomit T, Takahashi T. Expression profile-defined classification of lung adenocarcinoma shows close relationship with underlying major genetic changes and clinicopathologic behaviors. J Clin Oncol 2006; 24 (11): 1679–88.

51. Albin A, Pfeffer U. A new tumor suppressor gene: invasion, metastasis, and angiogenesis as potential key targets. J Natl Cancer Inst 2006; 98 (12): 800–1.

52. Granville CA, Dennis PA. An overview of lung cancer genomics and proteomics. Am J Resp Cell Mol Biol 2005; 32: 169–76.

53. Carbone DP. Lung cancer biology and transcription research. Program and abstracts of ASCOI 38th Annual Meeting, May 18–21, 2002, Orlando, Florida, Abstract -2.

54. Rom WN, Tchon-Wong KM. Functional genomics in lung cancer and biomarker detection. Am J Resp Cell Molecular Biology 2003; 24: 153–6.

55. Petty RD, Kerr KM, Murray GI, Nielson MC, Rooney PH, Bisset D, Collie-Duguid ESR. Tumor transcriptome reveals the predicative and prognostic impact of lysosome protease inhibitors in non-small cell lung cancers. J Clin Oncol 2006; 24 (11): 1729–44.

56. Chen HY, Yu SL, Chen CH, Chang GC, Chen CY, Yuan A, et al. A five-gene signature and clinical outcome in non small-cell lung cancer. N Engl J Med 2007; 356: 11–20.

57. Battacharyee A, Richards WG, Staunton J, Li C, Monti S, Vasa P, et al. Classification of human lung cancer by mRNA expression profiling reveals distinct adenocarcinoma subclasses. Golub@genome.wi.mit.edu (10/12/2006).

58. Liloglou T. DNA methylation profiling in lung cancer. 19th, European Association for Cancer Research Congress: Chip-based technologies – Agilent satellite symposium – abstract. July 3rd, 2006, Budapest.

59. Zakovski MF, Landanzi M, Kris MG. EGFR mutations in small-cell lung cancers in patients who have never smoked. N Engl J Med 2006; 355 (2): 213–5.

60. Spira A, Schmgr F, Beane J, Shah V, Liu G, Brody J. Impact of cigarette smoke on the normal airway transcriptome. Chest 2004; 125: 113–S.

61. Brody J, Spira A. State of the art. Chronic obstructive pulmonary disease, inflammation, and lung cancer. Proc Am Thoracic Society 2006; 3: 535–7.

62. Potti A, Mukherjee S, Petersen R, Dresman HK, Bild A, Koontz J, et al. A genome strategy of refine prog-

nostic in early stage non-small cell lung cancer. N Engl J Med 2006; avg. 570–80.

63. Eschrich S, Yang I, Bloom G, et al. Molecular staging for survival prediction of colorectal cancer patients. J Clin Oncol 2005; 23: 3526–35.
64. Dehl F, Dressman D, He Y, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumours. PNAS 2005102: 45: 16368–73.
65. Traverso G, Shabar A, Levin B, et al. Detection of APC mutation in fecal DNA from patients with colorectal tumors. N Engl J Med 2002; 346: 311–20.
66. Nelson W, De Marzo A, Isaacs WB. Prostate cancer. N Engl J Med 2003; 349: 366–81.
67. Wu J, et al. Processing oligonucleotide array data. Nature Biotechnology 2004; 22 (6): 656–8.
69. Bueno R, Loughlin KR, Powell MH, Gordon GJ. A diagnostic test for prostate cancer from gene expression profiling data. J Urol 2004;172 (2, part1): 903– 6.
70. Petersen LE, Ozen M, Erdem H, et al. Artificial neural network analysis of DNA microarray-based prostate cancer recurrence. CIBCB 2005: 1–8.
71. Hanson JA, Gillespie JW, Grower A, et al. Gene promotion methylation in prostate tumor associated stromal cells. J Natl Cancer Inst 2006; 98 (4): 255–61.
72. Wang E, Panelli MC, Zavaglia K, et al. Melanoma-restricted genes. J Translation Med 2004; 2: 34.
73. Saldanha G, Potter L, Da Forno P, Pringle JH. Cutaneous melanoma subtypes show different BRAI and NRAS mutations frequencies. Clin Cancer Res 2006; 12: 4499–505.
74. Mandruzzato S, Callegaro A, Turcatel G, Francescato S, Montesco MC, Chirian-Silenti V, et al. A gene expression signature associated with survival in metastatic melanoma. J Translation Med 2006; 4: 50.
75. Meltzer PS. Genetic diversity in melanoma. N Engl J Med 2005; 353: 2104–7.
76. Bauer R, Wild PJ, Bataille F, Pauer A, Klinkhammer-Schalke M, et al. Prognostic relevance of P-cadherin expression in melanocyte skin tumors analysed by high-throughput tissue microarrays. J Clin Pathol 2006; 59: 699–705.
77. Nambiar S, Hengge UR. Gene expression patterns in melanoma reveal two independent predictors. Workshop: Microarray technologies in clinical oncology; potential and perspectives. June 30, 2005. Rome, 2005.
78. Tzankov A, Eschwendtner A, Augusti F, Figl M, Oberman EC, Dimhofer S, Went P. Diffuse large B-cell lymphoma with overexpression of cycline E substantives, poor standard treatment response and interior outcome. Clin Cancer Res 2006; 12: 2125–32.
79. Dollad-Filhart M. Quantitative in situ analysis of β-catetin expression in breast cancer shows decreased expression is associated with poor prognosis. Cancer Res 2006; 66 (10): 5487–94.
80. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Unteh M, Smith I, et al. Trastuzumab after adjuvant chemotherapy in her-2 positive breast cancer. New J Eng Med 2005; 353 (16): 1659–72.
81. Barrier A, Van der Laan MJ. Colon cancer prognosis prediction by gene expression profiling. University of California, Berkeley, 2005 (http://www.bepress.com/ucbbiostat/paper178, 8/8/2006).
82. Barenston RA, Tenesa A, Farrington SM, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. N Engl J Med 2006; 354: 2751–63.
83. Baltić V, Bogdanović G, Baltić M, Jurišić V, Stojiljković B. Apoptoza i maligna transformacija ćelija – klinička iskustva. U: Apoptoza. Eksperimentalna i klinička iskustva. Novi Sad: SANU, Ogranak u Novom Sadu; 2004. p. 81–97.
84. Waters JW, Mc Leod HL. Cancer pharmacogenomics: current and future applications. BBA 2003; 1603 (2): 99–111.
85. Paik S, Shak S, Tang E, et al. A mutagene assay to predict recurrence of tamoxifen treated, node-negative breast cancer. N Engl J Med 2004; 351: 2817– 26.
86. Nevins J. New genomic tests guide choice of chemotherapy in cancer patients. October 23, 2006 (http://www.Gammae.duke.edu/press/news 10230.6, 11/1/2006).
87. Lan S, Ling V. Application of pharmacogenoma for rational chemotherapy of lung cancers. http://www.genomicbe.ca/research-tech/research-projects-health-lung-cancer.htm (12/9/2006).
88. Citin KV, Albanasa L, Fuji K, et al. Application of expression genomic for predicting treatment response in cancer. Ann NY Acad Sci 2005; 1058: 186–95.
89. Ross MI. Early-stage melanoma: staging criteria and prognostic modeling. Clin Cancer Res 2006; 12: 2312s–2319s.
90. Wang X, Li N, Li H, Liu B, Qiu J, Chen T, Cao X. Silencing of human phosphatidylethanolamine-binding protein 4 sensitizes breast cancer cells to tumor necrosis factor alfa-induced apoptosis and cell growth arrest. Clin Cancer Res 2005; 11: 7545–53.
91. Kikucho T, Diago Y, Katagiri T, Tsunoda T, Okada K, Kakiuchi S, et al. Expression profiles of non-small cell lung cancers on cDNA microarrays: identification of genes for prediction of lymph node metastasis and sensitivity to anti-cancer drugs. Oncogene 2003; 22 (14): 2192–205.
92. Lu J, Getz G, Misaka GG, Alvarez-Savedra E, Lamb J, Peck D, et al. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834–8.
93. Meltzer PS. Cancer genomic: small RNAs with big impacts. Nature 2005; 435: 745–6.
94. Osada H, Takashi T. MicroRNAs in biological processes and carcinogenesis. Cancerogenesis 2007; 28 (1): 2–12.
95. Zhang L, Huang J, Yang N, Grwshock J, Megraw MS, Giannakakis A, et al. MicroRNAs exhibit high frequency genomic alterations in human cancer. PNAS 2006; 103 (24); 9136–41.

96. Calin GA, Croce CM. Micro RNA signatures in human cancers. Nature Rev Cancer 2006; 6: 857–66.

97. Volina S, Calina GA, Liu CG, Ambs S, Cimmno A, Petrarcca P, et al. A microRNA expression signature of human solid tumors defines cancer genes targets. PNAS 2006; 103 (7): 2257–61.

98. Nakajaima GO, Hayashi K, Xi Y, Kudo K, Uchiada K, Takasaki K, et al. Non-coding microRNAs has-let-7g and has-miR181b are associated with chemoresponse to S-1 in colon cancer. Cancer Genomics Proteomics 2006; 3: 317–24.

99. Rossi A, Bonatti S, Mallardo M, Martinelli V, Ciancia R, Gravetti A, et al. MicroRNAs: possible role in the molecular etiology of chronic myeloproliferative diseases. Haematology 2006; 91 (S3).

100. Tamaru Y, Hyashizava Y. Cancer Research with noncoding RNA. Cancer Sci 2006; 97 (12): 1285–90.

101. Model F, Ebert M, De Vos T, Tetzner R, Schuster M, Lesche R, Sledziewski A, Day RW. Detection of methylated DNA in plasma from colorectal cancer and controls by real-time PCR analysis. http://www. epigenomics.com (9/12/2006).

102. Koyanagi K, Mori T, O'Day SJ, Martinez SR, Wang HJ, Hoon DSB. Association of circulating tumor cells with serum tumor related methylated DNA in peripheral blood of melanoma patients. Cancer Res 2006; 66 (12): 6111–7.

103. Ogino S, Brahmandaw M, Kawasaki T, Kirkner GJ, Loda M, Fuchs CS. Epigenetic profiling of synchronous colorectal neoplasias by quantitative DNA methylation analysis. Modern Pathol 2006; 19 (8): 1083–90.

104. Roman-Gomez J, Jimenez-Velasco A, Agirre X, Castillego JA, Navaro G, Calasanz MJ, et al. CpG island methylator phenotype redefines the prognostic effect of t(12;21) in childhood acute lymphoblastic leukemia. Clin Cancer Res 2006; 12: 4845–50.

105. Livre A, Blons H, Houller AM. Clinicopathologic significance of mitochondrial D-loop mutation in head and neck carcinoma. Br J Cancer 2006; 94: 692–7.

106. Abu-Amero KK, Alzahran AS, Zou M, Shi Y. Association of mitochondrial DNA transfersion mutations with medullary thyreoid carcinoma/multiple endocrine neoplasias type 2 syndrome. Oncogene 2006; 25: 677–84.

107. Meierhofer D, Mayer JA, Fink K, Schmeller N, Kofler B, Sperl W. Mytochondrial DNA mutations in renal cell carcinomas reveal no general impact on energy metabolism. Br J Cancer 2006; 94: 268–74.

108. Giralda-Rosa W, Vleuges RA, Musiek A, Sligh JE. High-throughput mitochondrial genome screening method for nonmelanoma skin cancer using multiplexed temperature gradient capillary electrophoresis. Clinical Chemistry 2005; 51: 305–11.

109. Jakupciak JP, Wang WW, Markowitz MF, et al. Mitochondrial DNA as a cancer biomarker. JMD 2005; 7 (2): 97.

110. Parr RL, Dakubo GD, Crandall KA, et al. Somatic mitochondrial DNA mutations in prostatic cancer and normal applying adjunct glands in comparison to agematched prostate samples without malignant histology. JMD2006; 8 (30): 312–19.

111. Parrallei P, Xiao Y, Fliss M, et al. Detection of mtDNA mutations primary breast cancer and fine-needle aspirates. Cancer Res 2001; 61: 7623–6.

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