ISTITUTO SUPERIORE DI SANITÀ

Workshop

Microarray technologies in clinical oncology: potential and perspectives

Istituto Superiore di Sanità
Rome, June 30, 2005

ABSTRACT BOOK

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The workshop gives special attention to the recent applications and challenges of DNA microarray technology in clinical oncology research. Microarray technology represents a powerful tool which has potentially an enormous impact on biomedical sciences and has significantly changed the way questions about diseases are addressed. DNA microarrays have yielded new insights into basic mechanisms of cancer. The current challenge to the scientific community is to carry these new insights further and to translate these into new diagnostic, prognostic and therapeutic applications in the field of clinical oncology. The major aims of the workshop are: i) to illustrate the diagnostic and prognostic value of microarray technology in clinical oncology; ii) to evaluate the application of microarrays for monitoring and predicting responses of cancer patients in clinical trials. Furthermore, a special attention will be dedicated to discuss technological critical issues and prospects of implementation.
Keywords: Microarrays, Oncology

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Il workshop pone particolare attenzione alle recenti applicazioni della tecnologia dei DNA *microarray* in oncologia clinica. Le tecnologie basate sull'utilizzo dei *microarray* rappresentano uno strumento potente che ha un enorme impatto potenziale nell'ambito delle scienze biomediche e ha cambiato in modo significativo l'approccio alla comprensione delle malattie. I DNA *microarray* hanno permesso di ottenere nuove informazioni sui meccanismi di base del cancro. La sfida corrente della comunità scientifica è di implementare queste nuove conoscenze e tradurle in nuove applicazioni diagnostiche, prognostiche e terapeutiche nel campo dell'oncologia clinica. I principali obiettivi del workshop sono: i) illustrare il valore diagnostico e prognostico della tecnologia dei *microarray*; ii) valutare l'applicazione dei *microarray* come strumento di monitoraggio e mezzo predittivo delle risposte dei pazienti oncologici ai trial clinici. Inoltre, una particolare attenzione verrà dedicata a discutere gli aspetti tecnologici critici e le prospettive di implementazione.
Parole chiave: *Microarray*, Oncologia

Scientific Committee: F. Belardelli, R. Foà, L. Gabriele, F. M. Marincola, M. Pierotti

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PROGRAM

Thursday, 30th June 2005

9.00 Registration

9.20 Welcome address
Enrico Garaci

First session
DIAGNOSTIC AND PROGNOSTIC VALUE OF MICROARRAY TECHNOLOGY

Part 1

Chairpersons: **G. D'Agnolo, F. Cognetti**

9.30 *Microarray technologies for the clinical management of solid tumors*
Marco Pierotti

9.55 *Gene expression profiling in myeloid leukemias*
Torsten Haferlach

10.20 *Gene expression profiles in lymphoid malignancies*
Robin Foà

10.45 *Lymphoma profiling, clues for diagnosis and therapy*
Miguel A. Piris

11.10 Break

Part 2

Chairpersons: **P.G. Natali, G. Ciliberto**

11.45 *Gene expression patterns in melanoma reveal two independent predictors*
Ulrich Hengge

12.10 *Messenger RNA portrait of mesothelioma as assessed by microarray* in vitro*,* ex vivo*, and* in situ *analysis*
Bertrand H. Rihn

12.35 *Identification of diagnostic/therapeutic targets in melanoma by array technology*
Marco G. Paggi

13.00 Break

Second session
MICROARRAYS FOR MONITORING AND PREDICTING RESPONSES IN CLINICAL TRIALS
Chairpersons: **E. Garaci, F. Belardelli**

14.00 *Microarrays for the evaluation of gene expression profiles and gene polymorphism in clinical trials of cancer immunotherapy*
Francesco M. Marincola

14.25 *Protein microarrays and perspectives of application in clinical trials*
Monica Panelli

14.50 *Microarray analysis for monitoring the response to interferon*
Eleonora Aricò

15.15 Break

15.35 *Gene expression technologies in the development of new therapeutics in colorectal cancer*
Paul Clarke

16.00 *Impact of DNA microarrays in breast cancer management*
Christos Sotiriou

Round Table
MICROARRAY TECHNOLOGIES IN CLINICAL ONCOLOGY: TECHNOLOGICAL ISSUES AND PROSPECTS OF IMPLEMENTATION
Chairpersons: **R. Foà, F. Belardelli**

16.25 *Round table discussion*
Convenors: **L. Gabriele, T. Haferlach, M.C. Panelli, M. Pierotti, M. Petilli, M.A. Piris, J. Reid, B. Rihn**

17.30 Closure of the workshop

First session

Diagnostic and prognostic value of microarray technology

Part 1

Chairpersons
G. D'Agnolo, F. Cognetti

MICROARRAY TECHNOLOGIES FOR THE CLINICAL MANAGEMENT OF SOLID TUMORS

Marco A. Pierotti, Manuela Gariboldi, Loris De Cecco, James Reid, Lara Lusa, Paolo Radice, Maria G. Daidone
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Several studies have demonstrated that breast cancers with distinct pathological features can be recognized by their gene expression profiles. The accomplishment of a breast cancer molecular taxonomy is therefore expected to allow an improvement of knowledge of its underlining molecular biology and a development of effective tools to predict its clinical behaviour and response to therapy. To contribute both to a molecular classification of breast cancer and to a definition of predictive models for treatment outcome, by using a cDNA microarray platform we have first characterized the gene expression profile of a group of familial breast cancer patients by carrying germ-line mutations of BRCA1 and BRCA2 genes and samples from patients classified as BRCAX. Class comparison analysis show that BRCA1 samples exhibited a significant number of differentially expressed genes compared to any of the other hereditary or non-hereditary groups of samples, whereas the other classes did not show such striking differences, although BRCAX samples seem to segregate in two distinct groups.

As for the use of gene expression profiling to identify clinically relevant classifiers for breast cancer, initial promising results have prompted researchers to push the technology further to develop predictive models for treatment outcome, for a personalized therapy. However, to provide data ready for "prime time" clinical use, investigators need to validate the predictive ability of their findings on independent and suitably large enough cohort of patients. Here we present such an attempt on a recently proposed predictive model for anti-estrogen response following Tamoxifen treatment based on the expression ratio of HOXB13 to IL17RB genes on an independent series of 58 patients with estrogen-receptor-positive breast cancers. Unfortunately, we failed to validate the performance of this predictive model. In an attempt to explore the possibility of developing a gene expression-based predictor of Tamoxifen response, we have also analyzed a group of 40 patients that have responded to Tamoxifen and compared their expression profiles with those derived from 18 patients that, conversely, have not responded to the same treatment. We are currently extending the analysis to 75 more samples. The obtained results are currently under evaluation and will be presented and discussed.

This work is supported by AIRC and CNR-MIUR grants.

GENE EXPRESSION PROFILING IN MYELOID LEUKEMIAS

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So far, comprehensive diagnosis of leukemia requires a combination of cytomorphology, immunophenotyping, and genetic methods. We aimed at developing a new diagnostic tool based solely on gene expression profiling to accurately predict all clinically relevant subtypes of leukemia in adults and to distinguish these from normal bone marrow. Therefore, we analyzed samples from 1337 untreated patients at diagnosis and healthy donors using oligonucleotide microarrays. The first series of 937 cases was hybridized to HG-U133A+B microarrays (Affymetrix). The following 13 subgroups were included: 620 AML (42 t(15;17); 38 t(8;21); 49 inv(16); 47 t(11q23); 75 complex aberrant karyotype (CA); 193 normal karyotype (NK); 176 other cytogenetic abn.); 152 ALL (26 Pro-B-ALL/t(11q23); 12 ALL-t(8;14); 32 T-ALL; 82 c-ALL/Pre-B-ALL); for further comparisons 75 CML, 45 CLL, and 45 bone marrows from healthy volunteers or non-leukemia pts. (nBM). For each disease entity the top 100 differentially expressed genes were calculated in a one-versus-all (OVA) approach. Class prediction was performed using support vector machines (SVM). Prediction accuracy was estimated by 10-fold cross validation (CV) and assessed for robustness in a resampling approach. 891 of the 937 samples (95.1%) were correctly classified (10-fold CV). A resampling approach with 2/3 training and 1/3 test cohort (100 runs of SVM) confirmed this high accuracy (median, 93.8%). In particular, a median of 100% sensitivity and specificity was achieved for AML with t(15;17), t(8;21), and inv(16), as well as Pro-B-ALL/t(11q23), and CLL. The median specificity was at least 99.7% in all subgroups except for AML normal/other (median specificity, 93.7%). The second prospective series comprized 400 unselected cases which were hybridized to the new generation HG-U133 Plus 2.0 microarrays (Affymetrix). To validate the diagnostic accuracy of our approach these cases were processed blinded in parallel to routine diagnostic work-up and classified based on the gene expression signatures discovered in the first series described above. Applying a first classification step as described above the 13 different diagnoses were predicted with an accuracy of 94.5%. Highly predictive genes were further validated using real time PCR and were reproduced with very high correlations. In conclusion, we were able to identify within a routine diagnostic workflow distinct expression profiles for all clinically and prognostically relevant adult leukemia subtypes and their discrimination from nBM based only on gene expression data. Accuracy, sensitivity, and specificity were higher than achieved with each of the gold standard techniques alone used today. Thus, gene expression patterns analyzed by microarrays qualify as a diagnostic tool in a routine setting for leukemia diagnosis and classification and may guide relevant therapeutic decisions in the near future.

GENE EXPRESSION PROFILES IN LYMPHOID MALIGNANCIES

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The introduction of microarray analysis represents a revolution in the scientific field, allowing to investigate thousand of genes in a single experiment. Important biological insights have been revealed by this technique in several hematological neoplasm.

In acute lymphoblastic leukemia (ALL), these studies have enabled to identify specific patterns associated with: 1) known molecular abnormalities, such as ALL1/AF4, E2A/PBX1, TEL/AML1 and to a lesser extent BCR/ABL; 2) immunophenotypic characteristics, namely lineage derivation and the degree of leukemic cell differentiation. In addition, a great effort is at present set to identify genes and/or gene patterns that correlate with different risk stratification groups and different prognostic features.

In chronic lymphocytic leukemia (CLL), this approach has helped to dissect that this disease is a single entity with a unique profile that differs from other lymphoid malignancies. Furthermore, it has permitted to better define the two distinct variants that are characterized by a different IgVH mutational status: these two variants can be discriminated by a small set of genes. Finally, it has allowed to define a similarity between this disease and memory B cells, and has also led to hypothesize that CLL cells from IgVH unmutated may be continuously stimulated *in vivo*, thus showing a gene profile that is reminiscent of the B-cell receptor signalling pathway.

In multiple myeloma (MM), gene expression profiles have provided insights into the disease and its resemblance with the different stages of differentiation of the plasma cell and has offered the opportunity of stratifying patients according to the degree of aggressiveness of their disease. In lymphomas, gene expression profiling have proved to be useful in identifying specific patterns that characterize the various subsets within this vast nosological entity and in certain subtypes –in particular DLBCL and mantle cell lymphoma – has provided useful information, allowing a better risk stratification of patients.

Current efforts are directed to the identification of new therapeutic targets, as well as to the design of pharmacogenomic experiments aimed at predicting response to any given drug, or contrariwise, at identifying genes that are associated with the refractoriness to therapy.

LYMPHOMA PROFILING, CLUES FOR DIAGNOSIS AND THERAPY

Miguel A. Piris
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High-throughput molecular studies have demonstrated that the common forms of cancer carry on a large variety of changes in the expression of relevant genes codifying for the most essential cell functions.

Our effort, working in lymphomas, is currently focused into the recognition of molecular signatures of key genes, which allow redrawing the sequence of events that induce the neoplastic transformation and progression. Genes which are being recognised as master genes in Non-Hodgkin's Lymphoma are bcl6, c-myc, bcl2, MUM1 and NF-kB subunits. Each one of them is identified through a characteristic signature, which allows recognizing its functional and biological relevance.

At the same time, the accumulation of information on multiple markers is allowing to propose predictive systems, mainly based in biological data, which may complement the current standard clinical predictive systems. These biological-based predictor models identify the specific weight of each marker, and associate the most relevant into a single formula. Our group is now proposing predictive systems for Hodgkin's Lymphoma, B-CLL, CTCL, MCL and Large B-cell lymphoma. These predictive systems will need to be validated in prospective studies, before being used for clinical decision. This validation is being performed through the analysis disease-specific genes printed on low-density arrays, in a design focused to specific clinical decisions.

These studies are also making possible the development of Pharmacogenomics projects, aimed to identify expression profiles associated with sensitivity of resistance to specific drugs. Finally they are pinpointing to the presence of potential therapeutic targets.

Massive application of high-throughput molecular analysis is expected to contribute critically to cancer diagnosis and proper treatment, facilitating individualized treatments and making possible the development of new rationally designed drugs.

Part 2

Chairpersons

P.G. Natali, G. Ciliberto

GENE EXPRESSION PATTERNS IN MELANOMA REVEAL TWO INDEPENDENT PREDICTORS

Sandeep Nambiar, Ulrich R. Hengge
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Melanoma is a complex multigenic disease, susceptibility to which is determined by several parallel and stepwise interaction of regulatory molecules affecting growth control, differentiation, cell adhesion and survival. High-throughput oligonucleotide microarray (Human U133A, Affymetrix Inc) based profiling (n=27) with the objective of identifying key molecular regulators in this process revealed several significantly regulated candidate genes, which were subsequently confirmed by Real-time RT-PCR.

Two potential independent predictors, Activator of S phase Kinase (ASK/HuDbf4) and Tumor potentiating region (Tpr) were significantly oveexpressed in primary melanomas, cutaneous melanoma metastases, and metastatic melanoma cell lines (BLM, MV3, M13) as opposed to congenital nevi.

We have shown that approximately 86% melanoma metastases overexpressed ASK/HuDbf4 Tpr as compared to other potential markers for detection of melanoma progression/metastasis namely CD146/MUC18 (13%) and c-Met (53%). Tpr-met hybrid RNA and protein resulting from a chromosomal translocation event have been reported earlier to increase the metastatic potential of melanoma.

We therefore investigated variation in c-Met transcript levels subsequent to si-RNA mediated knock down of Tpr in the highly metastatic BLM cell line and found no metastasis. Additionally, the Spearman correlation of expression of Tpr & c-Met was weak with only 13.69% of the variation in c-Met explained by the variation in Tpr amongst melanoma patients suggesting that Tpr-met hybrids in metastatic melanoma patients is not a high probability event.

We further investigated the effect of conventional cisplatin therapy and novel statins (atorvastatin, fluvastatin and pravastatin)-based therapy on cell viability in BLM cells and found the effect of atorvastatin and fluvastatin to be comparable to that of cisplatin. In addition, atorvastatin decreased the transcription of both ASK/HuDbf4 and Tpr.

These findings support the prospective analysis of statin drugs in the therapy of melanoma.

MESSENGER RNA PORTRAIT OF MESOTHELIOMA AS ASSESSED BY MICROARRAY *IN VITRO*, *EX VIVO*, AND *IN SITU* ANALYSIS

Bertrand H Rihn
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Asbestos fibers are known as mutagenic and carcinogenic for human and are responsible for occupational pulmonary diseases including asbestosis, bronchogenic carcinoma and malignant pleural mesothelioma, the cancer of the external lining of the lung.

Transcriptomes of pleural cells and malignant pleura human samples (mesotheliomas) were compared by the use of microarrays. Gene expression profiling was obtained from i) cultured pleural cells (7,000 genes, *in vitro* study), ii) tumor specimens (10,000 genes, *ex vivo* study), and iii) microdissected pleural cells (10,000 genes, *in situ* study). Results showed dozen of overexpressed genes in mesothelioma that i) promote local invasion, ii) protect cells against oxidative stress and iii) counteract anti-cancer therapies.

In the 3 studies, both FTL (ferritin light chain) and TXN (thioredoxin) overexpressions were consistently associated with the acquisition of the malignant phenotype. In microdissected cells, the expression of other redox-modulated genes, including FTH1 (ferritin heavy chain), GSTP1, MGST3 (microsomal glutathione S-transferase3), PRDX1 (peroxiredoxin1), PRDX4, SOD1, TXNL2 was increased in mesothelioma cells. Since MPM are tumors that are highly resistant to anti-cancer therapies, this cluster of genes may function in order to reduce their sensitivity to anticancer therapies which act mainly through Reactive Oxygen Species production. Implication of some of those genes was also confirmed by retrospective *in silicio* analysis.

The portrait of normal and cancerous pleura achieved at the mRNA level seems meaningful for the understanding of asbestos-mediated carcinogenesis, as for as mesothelioma stratification and management. Indeed the mesothelioma markers described in this study should improve the accuracy of mesothelioma diagnosis and therapy.

IDENTIFICATION OF DIAGNOSTIC/THERAPEUTIC TARGETS IN MELANOMA BY ARRAY TECHNOLOGY

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In order to identify genes relevant for human melanoma progression, we carried out cDNA array experiments employing an *in vitro* model consisting of two cell lines: one, LP, derived from a primary melanoma and the other, LM, from a metastasis found in a supraclavicular lymph node of the same patient. Basic cDNA array data identified about 30 genes differently expressed in the two cell lines. Northern blot analysis confirmed an effective transcriptional modulation for 6 out of 14 genes analyzed. Among these, we reported ferritin light chain (L-ferritin) gene overexpression in the LM metastatic cell line. Artificial L-ferritin down-modulation in LM cells strongly inhibits proliferation and chemoinvasion *in vitro*, and cell growth *in vivo*. In addition, L-ferritin down-modulated LM cells display enhanced sensitivity to oxidative stress, epitomized as increased superoxide dismutase and decreased catalase activities, and to apoptosis. Immunohistochemical analysis of a human melanoma tissue array reveals that ferritin expression level in metastatic lesions results significantly higher ($P<0.0001$) than in primary melanomas. Furthermore, ferritin expression is constantly up-regulated in autologous lymph node melanoma metastases, when compared to the respective primary tumors, in a cohort of 11 patients. Our data suggest that high ferritin expression can enhance cell growth and improve resistance to oxidative stress in metastatic melanoma cells, by interfering with their cellular antioxidant system. The potential significance of these findings deserves to be validated in a clinical setting.

Second session

Microarrays for monitoring and predicting responses in clinical trials

Chairpersons
E. Garaci, F. Belardelli

MICROARRAYS FOR THE EVALUATION OF GENE EXPRESSION PROFILES AND GENE POLYMORPHISM IN CLINICAL TRIALS OF CANCER IMMUNOTHERAPY

Ena Wang, Monica C. Panelli, Ping Jin, Katia Zavaglia, Sara Deola, Eleonora Aricò, Li Xin, Kina Smith, Sonia Voiculescu, Francesco M. Marincola
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Immune responsiveness of solid tumors may be dependent upon factors related to the genetic background of patients or to distinct characteristics of individual tumors that may alter their microenvironment to facilitate or inhibit immune responses occurring naturally in the tumor bearing host. Whether either or both of these categories of factors play a prominent role in determining tumor rejection in natural conditions (spontaneous regression) or during immune therapy remains to be confirmed. Several lines of evidence derived from experimental animal or *in vitro* models suggest that immune responsiveness results from a combination of genetic and epigenetic factors that modulate the activation of effector immune responses at the tumor site. However, direct *ex vivo* observations in humans are scant particularly when tumor/host interactions have been analyzed in the target tissue: the tumor site. Available technology allows nowadays direct and kinetic analyses of such interactions in real time by serial sampling of lesions using minimally invasive techniques such as fine needle aspirates. Serial sampling permits the study of the biology of cancer while leaving the studied lesion in place. This permits prospective evaluation of the natural history of the lesion left in place to identified biomarkers predictor of immune responsiveness to a given treatment. In addition, serial sampling during and after treatment may inform about the actual mechanisms of action of the treatment and its biological effects including the induction of tumor escape mechanism.

In this presentation, we will systematically review the tool and strategies available for this direct *ex vivo* analyses with particular attention to high throughput hypothesis-generating methods. In addition, insights on the postulated mechanism of immune responsiveness in humans will be discussed based on pilot studies performed in this vein.

PROTEIN MICROARRAYS AND PERSPECTIVES OF APPLICATION IN CLINICAL TRIALS

Monica C. Panelli[1], Leonardo Rossi[2], Brian M. Martin[3], Richard L.White[4], Mareva Foster[4] Glen L. Hortin[5], Ramy Moharram[3], David Stroncek[1], Ena Wang[1], Francesco M. Marincola[1]
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Systemic interleukin-2 (IL-2) administration induces an assortment of downstream effects whose biological and therapeutic significance remains unexplored mostly because of the methodological inability to globally address their complexity. We have profiled the transcriptional response of peripheral blood mononuclear cells (PBMC) and tumor microenvironment in patients with metastatic melanoma following 1 and 4 doses of intra-venous IL-2 (720,000IU/kg) administration (Panelli et al., Genome Biology 2002). Genes encoding for chemokines and cytokines related to inflammatory processes (IP-10, MIP-1β, MCP1, MCP-3, GM-CSF/IL-15/IL-3Rβ, IL-1) were found to be over-expressed in the peripheral blood. A general inflammatory profile was also observed within the tumor microenvironment by the up-regulation of genes involved in the innate immune response leading to activation of monocytes/APC and NK cells (GRO-1, MIG, MCP-1, MCP-3, MIP1-α , MIP-β , PARC, IL-8). To better characterize the cytokine outburst that follows systemic IL-2 administration we followed the serum levels of 68 soluble factors in ten patients with RCC undergoing high dose (720,000 IU/kg intravenously every 8 hours) IL-2 therapy (*Panelli et al. J Transl Med. 2004; 2(1):17*). Serum was collected before therapy, 3 hours after the 1st and 4th dose and assayed on a multiplexed protein array platform. This study demonstrated that 1) the serum concentration of more than half the soluble factors studied changed significantly during therapy; 2) changes became more dramatic with increasing doses; 3) subclasses of soluble factors displayed different kinetics and 4) cytokine patterns varied quantitatively among patients. Protein array analysis of these sera was limited by the number of capture antibodies selected for protein detection. We recently expanded the analysis to Surface-Enhanced-Laser-Desorption-Ionization-Time-Of-Flight Mass-Spectrometry (SELDI-TOF-MS) and quantitative protein analysis (nephelometry). All cytokines/chemokines detected by protein arrays were below the SELDI detection limit while novel IL-2 specific changes in expression of acute phase reactants and HDL metabolites could be identified. Serum amyloid protein A (SAA) and C-reactive protein (CRP) expression were consistently up-regulated after 4 doses of IL-2 while other proteins were downregulated. These findings were confirmed by SELDI immunoaffinity capture and nephelometry. Immunoaffinity capture revealed different, otherwise undetectable, isoforms of SAA. A linear correlation between peak area by SELDI and protein concentration by nephelometry was observed. Overall distinct yet complementary information was obtained using different platforms which may better illustrate complex phenomena such as the systemic response to biologic response modifiers.

MICROARRAY ANALYSIS FOR MONITORING THE RESPONSE TO INTERFERON

Eleonora Aricò[1], Stefania Parlato[1], Imerio Capone[1], Tiziana Di Pucchio[1], Lucia Gabriele[1], Licia Rivoltini[2], Ena Wang[3], Monica C. Panelli[3], Francesco M. Marincola[3], Filippo Belardelli[1]
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IFN-α is a pleiotropic cytokine widely used in the treatment of certain types of cancer, including melanoma. However, the mechanisms leading to anti-tumor response remain unclear. Recent studies suggest that IFN-α can enhance the activities of dendritic cells (DCs), supporting the rationale for its use as an adjuvant of cancer vaccines. In this study, we used microarrays to profile gene expression in peripheral blood mononuclear cells (PBMC) from stage IV melanoma patients treated with IFN-α in combination with epitope-specific immunization. PBMC were isolated before and 24 hours following IFN-α administration. Collections coincided with the first cycles of vaccination and another cycle scheduled 42 days later. Total RNA isolated from PBMC underwent two rounds of amplifications to obtain antisense RNA. Test samples and reference sample were labeled with Cy5-dUTP and Cy3-dUTP respectively. Test-reference sample pairs were mixed and co-hybridized to custom made17K cDNA microarrays. Data were analyzed via mAdb Gateway Analysis tool, and further analyzed using Cluster and TreeView software. Unsupervised hierarchical clustering (class discovery) of the complete data set did not segregate PBMC collected before from those collected after IFN-α treatment. However, an IFN-α signature was clearly detectable when supervised clustering (class comparison) was performed. In particular, 35 genes were specifically induced by the IFN-α treatment, during the first and the second round of treatment. Comparison of gene-expression patterns between the samples collected before and after the first IFN-α treatment identified 134 genes differentially expressed at a <0.005 significance level. Interestingly, unsupervised hierarchical clustering based on these genes distinguished the samples collected before and after the second IFN-α administration (class prediction) confirming that this signature is consistently induced by IFN-α treatment. In a separate study, we have also used microarrays to characterize the molecular gene expression profiles DCs generated *in vitro* from monocytes after IFN-α treatment as compared to reference cells. Of note, there was a common signature of IFN-induced genes, which included genes involved in immunological pathways, such as antigen processing and presentation, cytokine and chemokine activity. In conclusion, our study provides a global description of the *in vivo* effects of IFN-α and its molecular signature, thus opening new perspectives into the comprehension of the mechanisms of action and, possibly, into the identification of molecular markers of the clinical response to IFN.

GENE EXPRESSION TECHNOLOGIES IN THE DEVELOPMENT OF NEW THERAPIES IN COLORECTAL CANCER

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Great progress has been made in the last two decades in the identification and characterization of the genetic changes involved in the malignant colorectal transformation process. This is a multistep process that requires a number of key events including the acquisition of self-sufficiency in growth signals that can result from autocrine growth factor stimulation, overexpression of growth factor receptors, or overexpression or activation of the cytoplasmic components of these signaling pathways. One promising therapeutic strategy is to target these defects. Small molecule inhibitors of some of the key signaling molecules, such as Mek, receptor tyrosine kinases, phosphatidylinositol-3-kinase (PI3K), and the Ras family of guanine-nucleotide-binding proteins are currently under development. The Cancer Research UK Centre for Cancer Therapeutics is particularly interested in the development of novel agents that target these pathways, including the molecular chaperone HSP90 that is required for folding, stability and function of key oncogenic protein kinases and PI3K p110α that has recently been reported to be mutated in a number of cancers including colorectal cancer. Our group is particularly interested in using expression profiling to enhance the discovery and development of these novel anti-cancer agents. Our primary interest is in investigating gene expression patterns induced by cancer therapeutics, both in experimental models and as part of their clinical evaluation. We have used expression profiling by microarray to identify gene expression signatures for a number of different drug types. These signatures can be used to confirm of identify the mechanism(s) of drug action, discover genes involved in drug sensitivity or resistance, and develop molecular biomarkers for use as pharmacodynamic and prognostic end-points. The development of novel therapeutics and the role of gene expression profiling will be exemplified by the following recent studies and work in progress:

- The preclinical development of inhibitors of PI3K and HSP90, their potential use in colorectal cancer and the role of gene expression profile in this process.
- Identification of genes altered in the tumour tissue of rectal cancer patients and colorectal cancer cell lines during treatment with current chemotherapeutic agents, including a cluster of genes regulated by c-Myc.

IMPACT OF DNA MICROARRAYS IN BREAST CANCER MANAGEMENT

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In a short space of time, high throughput global gene expression profiling has given us new insights into breast cancer biology. Novel classifications of breast cancer have been proposed based on their molecular differences. The estrogen receptor status has been found to be the most important discriminator of expression subtypes, suggesting that its biology plays a central role in breast cancer carcinogenesis. Furthermore, the molecular profiles suggest an epithelial cell of origin – with overexpression of luminal and basal cell genes. Within the ER negative tumors a new subtype of breast cancer has been discovered – the basal-like group – that has an extremely poor prognosis. Within the ER positive tumors, up to three distinct groups may exist. The implementation of molecular stratification in future breast cancer clinical trials is vital, and may lead to significant therapeutic and management implications for patients. Recent microarray reports have challenged long held views of metastatic progression. Breast cancers may possess a “metastatic signature”, and the ability to metastasize may be an inherent feature of breast cancer tumorgenesis. A 70-gene poor prognostic signature developed by researchers in Amsterdam in the Netherlands may predict prognosis of early breast cancers significantly better than the widely used St Gallen and National Institutes of Health (NIH) consensus criteria, thus potentially sparing many women from unnecessary adjuvant chemotherapy treatment.

The use of microarray technology may facilitate the development of predictors for drug sensitivity. The study of gene expression profiles before and after neoadjuvant chemotherapy may be particularly informative. Gene expression profiles obtained from fine needle aspiration and core biopsies has been found to be feasible, and may be helpful in identifying targets for the treatment of drug resistant carcinomas.

Whilst these discoveries may revolutionize our understanding of breast cancer biology, the challenge for future years is implementation of genomic knowledge into breast cancer clinical trials. This will be the only way to assess the true value of the knowledge and predictors developed by microarray technology. This will require considerable international collaboration and novel clinical trial designs and statistical analyses. Further refinement in the use of the technology and the development of better statistical algorithms are continually evolving in an effort to reduce the errors currently inherent in its use. This relates particularly to the analysis of data involving a low number of samples to a large variables per sample ratio with no predefined hypothesis.

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