11   INVITED
Is mTOR a good target for chemoprevention and what patients could benefit from mTOR inhibitors?

J. Soria. *Institut Gustave Roussy, Medical Oncology/Lung Unit, Villejuif, France*

Molecular targeted therapy refers to treatment strategies directed against molecular targets considered to be involved in neoplastic transformation. Such molecularly-targeted agents (MTA) are currently under study in all treatment settings including that of chemoprevention, defined as the use of natural or synthetic agents to interrupt the carcinogenic process, thereby nipping tumours in the bud.
Numerous studies have demonstrated that the Akt pathway is critical for cell survival via phosphorylation of a number of downstream proteins. It plays a very important role in promoting growth and blocking apoptosis in cancer cells such as SCLC. There is increasing evidence that PI3-K/Akt plays an important role in breast cancer tumourigenesis. Akt activity was found to be constitutive in breast cancer cell lines with either HER2 overexpression or a PTEN mutation. Active Akt can be detected in head and neck squamous cell carcinoma (HNSCC) whose pattern of expression and localization correlate with disease progression. Many other transforming events which do not directly modify PI3-K, Akt, or PTEN genetically still cause activation of the PI3-K/Akt/PTEN pathway. Three examples of such transforming events are the BCR/ABL translocation, which is the causative event in chronic myelogenous leukemia, amplification of HER2, and amplification of the EGFR.
Several preclinical studies have indicated that rapamycin or its derivatives specifically inhibit the transforming effect of the PI3-K/Akt pathway. For example, rapamycin inhibits the transforming activity of the oncogenic variants of PI3-K and Akt. There are several mTOR inhibitors under clinical development for cancer therapy. CCI-779 is a rapamycin derivative developed by Wyeth-Ayerst which has completed phase I studies as a single agent administered intravenously or orally. It is currently being investigated in combination studies with other anticancer agents and in a broad spectrum of phase II single agent studies. RAD001 (Novartis) and AP23573 (Ariad Pharmaceuticals) are other mTOR inhibitors undergoing clinical development which are being tested in phase II trials. There is no ongoing chemoprevention trial with these agents. However the PI3K/Akt pathway appears to be a very appealing target for chemoprevention. Indeed, the target is present and abnormal as compared to normal epithelium. The target should influences tumor biology, and the preclinical data obtained with the drug aiming at the target appears favourable (efficacy, toxicity). The key results of the drug in human studies are currently being gathered in terms of efficacy and toxicity from phase I, and phase II trials. While efficacy appears very promising, toxic issues (mucositis, hypertriglyceridimia, hyperglycemia, anorexia) warrant further analysis before embarking on chemoprevention trials with the current agents.

Scientific Symposium
Molecular profiling for early detection and prognosis

12   INVITED
Micrometastases: detection, characterization and clinical relevance

K. Pantel. *Universitätsklinikum Hamburg-Eppendorf, Institut für Tumorbiologie, Hamburg, Germany*

Bone marrow (BM), which can be easily collected from the iliac crest, is the most important site for detecting micrometastatic epithelial tumor cells, which are present in BM samples of 20–40% of patients with carcinomas at various sites; even in the absence of lymph node metastases (stage N0) or clinical signs of overt distant metastases (stage M0). BM samples can also be monitored for the presence of micrometastatic tumor cells after primary surgical treatment, to detect tumor recurrence. Gene expression profiling studies of breast cancer cells indicate that specific molecular pathways are associated with haematogeneous dissemination of tumour cells to BM, whereas these pathways were not involved with lymphatic dissemination. Disseminated tumour cells found in the BM of patients with various types of solid tumours (e.g. breast, colon and lung) can be detected by sensitive immunocytochemical and molecular assays. For epithelial tumors, cytokeratins have become the best marker for the immunocytochemical detection of micrometastatic tumor cells. The presence of disseminated tumour cells in bone marrow predicts the development of overt metastases – both in the bone and other organs. Phenotyping of disseminated tumor cells in BM of early stage patients has also yielded additional prognostic information. In patients with gastric

cancer, the presence of micrometastatic cells that express the urokinase-type plasminogen activator receptor (uPAR) correlates with an unfavourable prognosis. Similar observations have been made in patients with breast cancer; Her2/neu overexpression by tumor cells that have disseminated to the BM predicts poor clinical outcome. So uPAR and Her2/neu might be important for the survival and growth of disseminated tumor cells. Recently, Jonathan Uhr's group presented evidence that the genotype of persistent circulating tumor cells may change towards a Her2-amplified genotype, which might be in line with our previous findings on the selection of a Her2-positive phenotype in BM micrometastases.
The genetic characterization of single disseminated tumour cells isolated from the BM, along with gene expression profiling studies of primary tumour cells, indicate that haematogeneous dissemination is often a very early event in tumour progression. The cells appear to first disseminate from the early primary lesions and then acquire additional genetic defects.
Peripheral blood would be an ideal source for the detection of disseminated tumor cells because of an easy sampling procedure. However, the prognostic significance of circulating tumor cells (CTC) is much less clear than for DTC in BM. An important progress seems to be the development new enrichment systems for CTC. Using new detection systems, it was shown that the presence of CTC in breast cancer patients detected by immunocytochemical or molecular methods correlated with stage and course of the disease. We and others have recently shown a correlation between the detection of CTC in the blood and DTC in BM of patients with primary breast cancer. These findings indicate that CTC detection could have clinical relevance.
Functional analysis of micrometastatic cells remains a challenge, because even after short-term culture their numbers are still small (on average between 1,000 and 10,000 cells). Permanent cell lines have therefore been established, and show gene expression and genomic characteristics that are typical of epithelial tumor cells *in situ*. These could therefore serve as models for functional studies on dormancy of micrometastatic cells.
Single disseminated tumour cells in the blood and bone marrow are targets for adjuvant therapy. These cells show often different properties than cells of the primary tumour, so further molecular analysis will provide additional information and will help to develop antimetastatic therapies.

13   Abstract not received

14   INVITED
Expression profiling of peripheral blood cells (PBC) for early detection

A.L. Børresen-Dale[1], P. Sharma[2]. [1]*Department of Genetics, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway;* [2]*DiaGenic ASA, Oslo, Norway*

Existing methods to detect breast cancer in asymptomatic patients have limitations, and there is a need to develop more accurate and convenient methods. Especially, an accurate method for breast cancer detection based on peripheral blood as a clinical sample will be highly desirable because of the easy accessibility and less-invasive nature by which samples can be obtained.
Results demonstrating that peripheral blood can be used to develop a gene expression based test for early detection of breast cancer will be presented. The rationale for using blood cells as monitors for a malignant disease elsewhere in the body is based on the hypothesis that a malignant growth will cause characteristic changes in the biochemical environment of blood. These changes will affect the expression pattern of certain genes in blood cells.
We initially conducted a pilot study where the expression pattern of 1368 genes in peripheral blood cells of 24 females with breast cancer and 32 females with no signs of this disease were analyzed using macroarrays and the expression data analyzed by PAM. The results were validated using a standard leave-one-out cross-validation approach. We were able to identify a set of genes that correctly predicted the diagnostic class in at least 82% of the samples. The majority of the identified genes had a decreased expression in samples from breast cancer patients, and predominantly encoded proteins implicated in ribosome production and translation control. In contrast, the expression of some defense-related genes was increased in samples from breast cancer patients.
In order to revalidate these findings and to increase the repertoire of informative genes, we have now extended the study with a larger number of breast cancer and non-breast cancer samples and used Agilent WG oligo arrays for large-scale gene expression analysis. The preliminary analysis of the data supports our previous finding that a blood-based gene expression test can potentially be developed to detect breast cancer in asymptomatic patients.