LABORATORY OF TUMOR BIOLOGY
Medical School-University of Crete
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BACKGROUND

Cancer is a devastating disease caused by derangement of the function of many important genes ultimately leading to malignant cellular transformation, tissue invasion and generation of metastases. Although significant progress has occurred in the treatment of cancer over the last several years, primarily due to our improved understanding of the underlying biological mechanisms, survival of patients with metastatic disease remains poor. Recent developments in the field of molecular biology offer new hope for improving the outcome of cancer patients. Biological pathways critical for the proliferation and survival of cancer cells have been discovered and are now targeted by novel molecular therapeutic agents. The biologic behaviour of tumors can be precisely predicted by analyzing patterns of gene expression. Thus the prognosis for a certain patient can be better determined and the treatment can be tailored accordingly. Moreover, the optimal treatment can now be selected based on the molecular characteristics of the tumor or the host. In this context, the Laboratory of Tumor Biology performs primarily translational research and operates in close collaboration with the Department of Medical Oncology of the University Hospital of Heraklion.

RESEARCH ACTIVITIES

1. Detection and characterization of micrometastatic cells

Occult tumor cells, which are undetectable by classical imaging and laboratory studies, can contribute to disease relapse and generate overt metastases; therefore, their identification in patients with early stage cancer may have substantial impact on determining prognosis and individualizing treatment strategies. For this purpose, epithelial cells have been identified in the bone marrow (disseminated tumor cells; DTCs) or the peripheral blood (circulating tumor cells; CTCs) of patients with stage I-III breast cancer, using immunocytochemistry or molecular techniques. Starting more than 10 years ago, our laboratory focused on the detection of CTCs in the blood of women with early breast cancer. We initially tested different markers such as cytokeratin 19 (CK-19), maspin and carcinoembryonic antigen (CEA), and among them CK-19 proved to be the most sensitive and specific. CK-19 is a cytoskeletal protein expressed on epithelial but not on mesenchymal cells, therefore its detection in the blood is a surrogate marker for the presence of CTCs. Using the LightCycler system, we developed an optimized real-time RT-PCR assay which is highly sensitive and specific and therefore suitable for high-throughput continuous monitoring and quantification of CTCs in the blood of breast cancer patients. By applying this assay we demonstrated that the detection of CK-19 mRNA+ CTCs is an independent prognostic indicator for disease recurrence and decreased survival of women with early breast cancer.

The observation that not every patient with breast cancer and CTCs in the blood develops disease relapse, supports the concept that the biological behavior of CTCs may be different depending on activated pathways and expressed receptors on their surface. To investigate the role of HER2 expression on CTCs, we developed a nested RT-PCR to detect HER2 mRNA+ cells in the blood. We demonstrated that the presence of HER2 mRNA+ cells has independent prognostic and predictive value and that the anti-HER2 monoclonal antibody trastuzumab can effectively target and eliminate chemotherapy- and hormonotherapy-resistant CTCs and DTCs. These observations underscore the importance of HER2 expression on CTCs and open the way to individualized targeted therapies.

Besides HER2, other receptors and pathways may be important for the proliferation and survival of micrometastatic cells. Such new targets may be discovered by the phenotypic analysis of CTCs. We recently isolated and analyzed CTCs for the expression of phosphorylated FAK (p-FAK), phosphorylated PI-3 and Akt kinases (p-PI-3K, p-Akt), and studied the actin organization using confocal laser scanning microscopy. Our findings provide strong evidence that micrometastatic cells express activated signaling kinases which may regulate migration mechanisms, supporting the presumption of their malignant and metastatic nature. We are currently investigating the expression on CTCs of other receptors such as EGFR, VEGFR-2 as well as pro-apoptotic molecules (caspases). In future studies, these survival pathways may be targeted by novel biological agents in an attempt to improve survival of women with breast cancer.

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Recent publications:

1. Stathopoulou A, Gizi A, Perraki M, Apostolaki S, Malamos N, Mavroudis D, Georgoulias V, Lianidou ES. Real-time quantification of CK-19 mRNA positive cells in peripheral blood of breast cancer patients using the LightCycler system. Clin Cancer Res, 2003; 9:5145-51

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3. Stathopoulou A, Ntoulia M, Perraki M, Apostolaki S, Mavroudis D, Malamos N, Georgoulias V, Lianidou ES. A highly specific real-time RT-PCR method for the quantitative determination of CK-19 mRNA positive cells in peripheral blood of patients with operable breast cancer. Int J Cancer. 2006;119:1654-9.

4. Xenidis N, Perraki M, Kafousi M, Apostolaki S, Bolonaki I, Stathopoulou A, Kalbakis K, Androulakis N, Kouroussis C, Pallis T, Christophylakis C, Argyraki K, Lianidou ES, Stathopoulos S, Georgoulias V, Mavroudis D. Predictive and prognostic value of peripheral blood cytokeratin-19 mRNA-positive cells detected by real-time polymerase chain reaction in node-negative breast cancer patients. J Clin Oncol. 2006;24:3756-62.

5. Apostolaki S, Perraki M, Pallis A, Bozionellou V, Agelaki S, Kanellou P, Kotsakis A, Politaki E, Kalbakis K, Kalykaki A, Vamvakas L, Georgoulias V, Mavroudis D. Circulating HER2 mRNA-positive cells in the peripheral blood of patients with stage I and II breast cancer after the administration of adjuvant chemotherapy: evaluation of their clinical relevance. Ann Oncol. 2007;18:851-8.

6. Kallergi G, Mavroudis D, Georgoulias V, Stournaras C. Phosphorylation of FAK, PI-3K, and impaired actin organization in CK-positive micrometastatic breast cancer cells. Mol Med. 2007 ;13:79-88.

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E, Stathopoulou A, Lianidou E, Chouverakis G, Sotiriou C, Georgoulias V, Mavroudis D. Different prognostic value of Cytokeratin-19 mRNA-positive Circulating Tumor Cells according to estrogen receptor and HER2 status in early breast cancer . J Clin Oncol 2007;in press.

2. Tumor immunology and immunotherapy

Our laboratory focuses on the induction of antigen-specific T lymphocyte responses to tumors and the development of immunotherapy approaches for cancer treatment. The immune system is a complex network that protects the body from pathogenic organisms. In addition, the immune system can play a crucial role in the protection against cancer. The T cells are essential regulators and effectors of immune responses. CD8+ T cells, after activation by antigen-presenting cells (APC), differentiate into cytotoxic T cells (CTLs) which can destroy cells presenting tumor-specific or -associated antigens, thus controlling tumor growth and formation of metastasis.

A major problem of tumor immunotherapy is that most human tumor-associated antigens are also present in normal tissues, and therefore T cells are often affected by tolerance directed against their "dominant" (high affinity for HLA) peptides. To overcome the tolerance-related blunting of T cell responses, "cryptic" (low affinity for HLA) peptides have been used for the activation of an anti-tumor immune response. Since, the human telomerase reverse transcriptase (hTERT) is highly expressed in most human malignancies and correlates with poor prognosis, we used a modified (tyrosine substitution in P1 to increase affinity to HLA), HLA-A*0201-restricted "cryptic" peptide derived from hTERT (TERT572Y) to vaccinate patients with advanced cancer in order to induce immune response.

In our lab, we monitor the immune responses to the therapeutic vaccine Vx001 (TERT572Y) in HLA- A*0201 patients with cancer. The following approaches are used: a) screening for reactivity to the TERT572Y and TERT572 peptides using an IFN-γ ELISpot assay b) identification of peptide-specific CD8+ T cells by IFN-γ intracellular staining using flow cytometry c) assessment of cytotoxic T lymphocyte activity after peptide-specific stimulations by analysis of perforin by ELISpot assay d) phenotypic and functional characterization of peptide-specific CD8+ T cells and e) development of clones of tumor-reactive T cell lines. We expect that this research will improve our understanding of the mechanisms underlying immune responses against tumor cells and might lead to innovative cancer therapies.

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REPRESENTATIVE PUBLICATIONS

1. Mavroudis D, Bolonakis I, Cornet S, Myllaki G, Kanellou P, Kotsakis A, Galanis A, Nikoloudi I, Spyropoulou M, Menez J, Miconnet I, Niniraki M, Cordopatis P, Kosmatopoulos K, Georgoulias V. A phase I study of the optimized cryptic peptide TERT(572y) in patients with advanced malignancies. Oncology. 2006;70:306-14.

2. Bolonaki I, Kotsakis A, Papadimitraki E, Aggouraki D, Konsolakis G, Vagia A, Christophylakis C, Nikoloudi I, Magganas E, Galanis A, Cordopatis P, Kosmatopoulos K,

Georgoulias V, Mavroudis D. Vaccination of patients with advanced non-small-cell lung cancer with an optimized cryptic human telomerase reverse transcriptase peptide. J Clin Oncol. 2007;25:2727-34.

3. Molecular pathways operating in cancer cells

Epidermal growth factor receptor (EGFR) and insulin-like growth factor-1 receptor (IGF-1R) have been linked to the pathogenesis of many human tumor types including breast and lung cancer. Activation of these receptors and the respective downstream signaling cascade is involved in cancer progression through the enhancement of cancer cell proliferation, evasion of apoptosis and the induction of angiogenesis and cell migration. EGFR, and IGF-1R targeting with small molecule tyrosine kinase inhibitors and specific antibodies are novel promising strategies for the inhibition of cancer progression.

Caveolae are vesicular organelles of the plasma membrane that serve as platforms for the congregation of various signaling molecules such as the growth factor receptors. Caveolae are involved in the regulation of cancer cell signaling and facilitate the cross-talk between distinct signaling cascades. Caveolin-1, a principal component of caveolae membranes is overexpressed in solid tumors and has been shown to regulate EGFR signaling. We are currently investigating whether the overexpression of caveolin-1 modulates EGFR and IGF-1R signaling in breast cancer cells. We additionally test the effect of caveolin-1 overexpression on the growth inhibitory effects of specific kinase inhibitors or monoclonal antibodies against EGFR and IGF-1R.

Furthermore we study the inhibitory effects of a monoclonal antibody against IGF-1R either alone or in combination with chemotherapeutic agents in lung cancer cell lines. The underline mechanisms are being explored. In our studies we use cultures of cancer cell lines, MTS proliferation assay, cell cycle and apoptosis analysis, Western blot analysis, and immunofluorescence techniques.

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4. Pharmacogenetics

Pharmacogenetics, the study of the role of inheritance in the individual variation in drug response, could be potentially used for the identification of the optimal drug and dose for each patient. Even though individual differences in drug response can result as an effect of age, sex, disease, or drug interactions, genetic factors also influence both the efficacy of a drug and the likelihood of an adverse reaction. Several lines of evidence indicate that genetic alterations such as polymorphisms, gene transcripts and somatic mutations can serve as predictive markers and probably they can be used to tailor chemotherapy in specific subgroups of patients.

In our laboratory, we develop translational research programs in order to evaluate the prognostic and predictive role of specific genetic markers. Using different genetic approaches, such as the identification of mutations of specific genes using direct sequencing technique (EGFR, K-ras), genetic polymorphism analysis with allelic discrimination (ERCC1, GST), gene expression profiling with the use of qRT-PCR (ERCC1, BRCA1, RRM1 and 2, XPD, TXR1, TSP1), gene amplification and expression by FISH or IHC (c-met), we try to develop algorithms that could be used in the near future for tailoring treatment in cancer patients. The final goal of the

program is the evaluation of these markers in prospective randomized trials ultimately leading to their application in the daily practice.

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