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Clinical significance of genetic alteration in plasma of colorectal cancer patients

T. Lecomte[1], A. Berger[2], F. Zinzindohoue[2], S. Micard[1], B. Landi[2], P. Beaune[1], P. Cugnenc[2], P. Laurent-Puig[1]. [1] *INSERM, U490, Paris, France;* [2] *Assistance Publique Hôpitaux de Paris, Hopital Europ*en Georges Pompidou, Paris, France*

Background: The objective of this study was to assess the prognostic value of detecting tumor-derived DNA in plasma of patients with colorectal cancer.

Methods: The presence of genetic alterations in plasma DNA from 58 patients with colorectal cancer (stages I-IV) was searched. Tumors were screened for mutations of the gene KRAS2 (codons 12 and 13) and promoter hypermethylation of the tumor suppressor gene p16. KRAS2 mutations were detected using a mutant allele-specific amplification assay (MASA). Promoter hypermethylation of p16 was identified by methylation specific PCR (MSP). The same assays to detect tumor specific gene alterations in the plasma were used. Survival and recurrence rates were assessed in the patients with and the patients without derived-tumor DNA in their plasma.

Results: Out of 58 tumors, 22 contained a KRAS2 mutations, and the identical mutation was detected in the plasma of 10 corresponding patients. Thirty-one out of the 58 tumors contained a promoter hypermethylation of p16. The interpretation of the assay which could identified promoter hypermethylation of p16 was possible in plasma for 24 of these 31 patients. Among these 24 patients, promoter hypermethylation of p16 was detected in the plasma of 21 patients. Finally, KRAS2 mutations or promoter hypermethylation of p16 was detected in the paired plasma of 26 out of the 37 patients in which the two assays (MASA and MSP) were used and conclusive. The two-year overall survival rate was 48% in the 26 patients with derived-tumor DNA detected in plasma DNA, whereas in the 11 patients without derived-tumor DNA detected in plasma DNA, the survival rate was 100% ($p < 0.03$ by the log-rank test). Among these 37 patients, 25 patients had stage I, II or III disease. In this sub-group of patients, the two-year recurrence-free survival rate for the 17 patients with derived-tumor DNA detected in plasma DNA was 66%, as compared with 100% for the 8 patients without derived-tumor DNA detected in plasma DNA ($p=0.04$ by the log-rank test).

Conclusions: The presence of tumor derived DNA in plasma seem to be a determinant prognostic factor for patients with colorectal cancer and may be used to identify patients with a high risk of recurrence who would need an adjuvant therapy.

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Gene expression profiling of malignant mesothelioma cell lines treated with IFN-gamma

E. Kettunen[1], C. Vivo[2], Y. Pilatte[2], S. Knuutila[1], M.-C. Jaurand[2]. [1] *Haartman Institute and Helsinki University Central Hospital, Department of Medical Genetics, Helsinki, Finland;* [2] *University Paris XII, EMI 9909 INSERM, Cr*teil, France*

Purpose: Malignant mesothelioma (MM), a rare tumor type of the pleura is very resistant to presently available therapies. Among the immunotherapies, however, the antiproliferative effect of gamma interferon (IFN-g) in MM has been demonstrated. In order to further characterize the effects of IFN-g in the gene expression level, four MM cell lines showing different sensitivities to the antiproliferative effect of IFN-g and displaying different morphological and molecular features were studied.

Methods: cDNA array technique was used to study the gene expression patterns of 588 cancer-related genes. The MM cell lines were treated with IFN-g for 6 and 72 hours and the gene expression levels between IFN-g-treated and untreated cells were compared. The differences in gene expression of several genes were confirmed by reverse transcription combined with real-time PCR.

Results: The gene expression patterns of several sets of genes in resistant and sensitive, growth-arrested cell lines were shown to differ after IFN-g treatment. In one cluster of genes (COL3A1 and CDC6 as examples) the expression in resistant cells was increased at 6 hours but decreased at 72 hours after IFN treatment, in comparison with untreated cells. In the same set of genes, in sensitive cells the gene expression level remained unchanged or was upregulated at a later stage. In another set of genes (ITGAE as an example), IFNg produced an early downregulation in the resistant cells followed by an increased gene expression at 72 hours. In this set of genes, no changes were shown in sensitive MM cell lines.

Conclusion: By using the cDNA array technique, we have revealed marked differences in expression pathways especially those involved in cell adhesion and cell proliferation, between IFN-g-resistant- and sensitive MM cell lines.

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Gene expression profiling of mantle cell lymphoma and its blastoid variant

Y. Zhu[1], J. Hollmen[2], R. Oinonen[3], K. Franssila[4], Y. Aalto[1], E. Elonen[3], H. Manninen[2], J. Kere[1], S. Knuutila[1]. [1] *Haartman Institute and Helsinki University Central Hospital, Medical Genetics, Helsinki, Finland;* [2] *Helsinki University of Technology, Laboratory of Computer and Information Science, Helsinki, Finland;* [3] *Helsinki University Central Hospital, Internal Medicine, Helsinki, Finland;* [4] *Helsinki University Central Hospital, Pathology, Helsinki, Finland*

Purpose: The genetics of mantle cell lymphoma (MCL) and its blastoid variant are not very well studied. In the present study, we use the cDNA micro-array technology to examine the gene expression profiles of both the normal and the blastoid variant forms of MCL. The aim is to identify genes that may play pathogenic roles in MCL, and in particular genes that may be related to the blastoid variant transformation.

Method: cDNA micro-array analysis was done on the lymph node specimens from 18 MCL patients. The lymphomas were histologically classified according to the revised European-American classification of lymphoid neoplasms (REAL). Eight patients had morphologically normal MCL; samples from 9 patients were classified as the lymphoblastoid variant, and sample from 1 patient was classified as the pleomorphic variant. Atlas Human Haematology array filters were used (Clontech Laboratories, Inc., Palo Alto, CA). The array results were analyzed by using AtlasImage software (Clontech). To define genes as down-regulated and up-regulated, we used regression analysis and principal component analysis

Results: The analysis revealed 16 differentially expressed genes in both normal and blastoid variant MCL groups (10 up-regulated and 6 down-regulated). The up-regulation of Annexin II, CD44 and granulin precursor, as well as the down-regulation of Neprilysin, regulators of G protein signaling (RGS1 and RGS2) and CD66d contribute to the tumorigenesis and metastasis of MCL. The roles of the up-regulation of AF-17 gene and GPR13 genes, as well as the down-regulation of the ADA gene and the EBI2 gene, need to be further studied. The analysis also revealed 12 genes whose expression levels differ between the normal and the blastoid variant MCL groups. The simultaneous up-regulation of the c-myc oncogene, apoptosis regulator bcl2, and pim-1 proto-oncogene in the blastoid variant group but not in the morphologically normal group suggests that the gp130-mediated STAT3 signaling pathway is involved in the transformation of the normal MCL to the more aggressive blastoid variant.

Conclusion: The combination of two statistical methods to analyze the cDNA micro-array experiment results could be a feasible strategy in data analysis. We are able to identify genes that may play important roles in MCL origination, growth, and transformation to the more aggressive blastoid variant. These findings could be used in developing novel diagnostic tools and therapies for MCL.