MTHFR C677T, MTHFR A1298C and RFC1 G80A. were evaluated. The differences in the distributions of the genotype between all patients and healthy controls were evaluated. For the patients treated with MTX, the association of the genotype with histological response, MTX plasma levels and adverse effects following as NCI Common Toxicity Criteria were examined by chi-square test.

RESULTS: The distributions of MTHFR C677T and A1298C genotypes were not different with those of controls, but the distribution of RFC1 genotype (GG in 22(23%), AG in 37(39%), and AA in 36(38%)) was significantly different with that of controls (GG in 54(29%), AG in 95(51%), and AA in 38(20%)). The ratio of homozygous for the RFC1 A80 allele was significantly higher in patients than in controls. Frequency of grade 3 or higher hepatic disorder after MTX administration was correlated with RFC1 AA allele in position 80 and without MTHFR genotypes in position 677 and 1298. Genotypes of MTHFR and RFC1 were no correlated with MTX serum levels, histological response and other adverse effects.

CONCLUSION: The distribution of RFC1 AA genotype in position 80 was significantly different between patients and controls in Japanese. The polymorphism of RFC1 gene influenced in hepatic disorder after MTX administration for osteosarcoma patients. This finding may be helpful to predict and to prevent severe hepatic disorder.

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Candidate molecular markers associated with endocrine resistance in breast carcinoma

J. Vendrell[1], S. Ghayad[1], I. Bieche[2], F. Spyratos[2], C. Dumontet[3], R. Lidereau[2], P. Cohen[1]
[1]INSERM U590, Faculté de Pharmacie de Lyon, Lyon, France; [2] INSERM U735, Laboratoire d'Oncogénétique, St Cloud, France; [3] INSERM U590, Domaine Rockfeller, Lyon, France

Gene-expression profiles could be a powerful predictor of the response of estrogen receptor (ER)-positive breast cancer patients to endocrine treatment. To this purpose, we used two different cell models of resistance to anti-estrogens: MVLN/CL6.7/CL6.8 cells and VP229/VP267 cells selected after exposure to tamoxifen (Tam), respectively in vitro and in vivo. We newly characterized the cross-resistance developed by the CL6.7 and the VP267 cells, but not by the CL6.8 cells, to fulvestrant (ICI 182,780), a pure ER antagonist used in second-line therapy for patients who have relapsed after Tam treatment. Using a candidate gene approach, we identified by RTQ-PCR 53 genes, the expression of which was deregulated in at least one of the three resistant cell lines (CL6.7, CL6.8 or VP267 cells). We selected 9 candidate genes as putative predictive/prognostic markers to further explore by RTQ-PCR their expression in ER-positive breast tumor samples from patients who relapsed or not under Tam treatment. Deregulation of expression of 7 genes was significantly associated with tamoxifen failure, relapse-free survival and overall survival. In conclusion, this study allowed the identification of new molecular markers associated with tamoxifen failure, and suggested their clinical potential in the management of ER+ breast cancer.

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The role of alterations in BRCA1, BRCA2, TP53 and ATM genes in sporadic breast tumors

I. Ticha[1], J. Soukupova[1], M. Janatova[1], Z. Kleibl[1], P. Pohlreich[1]
[1]Charles University in Prague First Faculty of Medicine, Inst. of Biochemistry and Experimental Oncology, Prague, Czech Republic

Background: The tumor suppressor genes BRCA1, BRCA2, TP53 and ATM are involved in maintenance of the genome integrity. They belong to predisposing genes in hereditary breast cancer, however, their role in sporadic breast tumors remains uncertain. The goal in this study was to analyze the role of these genes in tumorigenesis of sporadic breast cancer. Methods: We evaluated genetic material from 71 tumor and corresponding peripheral blood samples of unselected breast cancer patients for germline and somatic mutations in BRCA1, BRCA2, TP53 and ATM genes. Further, we studied promoter methylation and loss of heterozygosity (LOH) in the corresponding loci. The mutation analyses included entire coding regions of the studied genes and were performed using protein truncation test, MLPA and sequencing. Promoter methylation was determined by methylation specific MLPA and bisulfite sequencing. Results: Allelic losses of BRCA1, BRCA2, TP53 and ATM were found in 14/65 (21.5%), 19/69 (27.5%), 23/62 (37.1%) and 15/70 (21.4%) informative tumor samples, respectively. The sequencing revealed two somatic mutations in BRCA1 (2/71; 2.8%) and one inherited (1/71; 1.4%) and nine somatic (9/71; 12.7%) mutations in TP53. The TP53 frameshift mutation c.340-370del31 (p.L114AfsX46) was novel. We failed to detect any alterations in ATM and BRCA2 coding sequences. Promoter methylation has been found only in BRCA1 (2/59; 3.4%) and TP53 (2/59; 3.4%). No gene alterations were found in 22/62 (35.5%) informative tumor samples.Conclusion: The high occurrence of allelic losses suggests the role of analyzed genes in sporadic breast tumorigenesis. However, acquired mutations were common only in TP53 and promoter methylation was identified only two-times in both BRCA1 and TP53. These results suggest that the role of analyzed genes is limited to the subset of sporadic breast tumors and alternative ways of breast tumorigenesis should be considered.Supported by: IGA MZ 9051-3/2006, MSM 002160808

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Screening for inherited mutations in the Czech high risk breast cancer patients - analysis of 400 families

Z. Kleibl[1], P. Pohlreich[1], J. Stribrna[1], J. Soukupova[1], I. Ticha[1], M. Mateju[2], M. Zikan[3], J. Novotny[2], J. Kotlas[4], J. Sevcik[1]
[1]Charles University in Prague First Faculty of Medicine, Inst. of Biochemistry and Experimental Oncology, Prague, Czech Republic; [2] Charles University in Prague First Faculty of Medicine, Dept. of Oncology, Prague, Czech Republic; [3] Charles University in Prague First Faculty of Medicine, Dept. of Gynaecology and Obstetrics, Prague, Czech Republic; [4] Charles University in Prague First Faculty of Medicine, Inst. of Biology and Dept. of Medical Genetics, Prague, Czech Republic

Background: The role of genetic susceptibility in breast and ovarian cancer has been intensively investigated. In the Czech Republic, 3% to 5% of breast cancer cases are due to inherited mutations in BRCA1 and BRCA2 genes and the risk of breast cancer is also influenced by mutations in other genes. Inherited mutations in low penetrance genes, ATM and CHEK2, are associated with about 2-fold increase in breast cancer risk; germline mutations of TP53 are associated with high risk of early onset breast cancer. The aim of our study was to determine the frequency and types of cancer-predisposing mutations in high-risk breast and ovarian Czech families.

Materials and Methods: We evaluated DNA and RNA samples from 400 breast or ovarian cancer patients. A complete sequence analysis of the BRCA1, BRCA2, ATM and TP53 coding sequences was performed. Large deletions in BRCA1/2 and the most common mutation in CHEK2 (c.1100delC) were detected by multiplex ligation-dependent probe amplification (MLPA); other gene alterations were determined by protein truncation test (PTT) and DNA sequencing.

Results: Of the 400 analyzed families, 117 (29.3%) carried pathogenic mutations, including 86 (21.5%) in BRCA1, 23 (5.8%) in BRCA2, 5 (1.3%) in ATM, 2 (0.5%) in CHEK2 and 1 (0.3%) in TP53. One novel truncating mutation was found in ATM, three in BRCA1 and four in BRCA2 genes. The four most common recurrent mutations in BRCA1 (c.300T>G, c.1806C>T, c.3819_3823del5, and c.5385dupC) explained 70.9% (61/86) of BRCA1 related patients and the c.5385dupC was detected in 47.7% (41/86) of mutation positive women. Five different large deletions in the BRCA1 gene were identified in 9 families (2.3%); at BRCA2 no deletions were detected. Inherited deletions of BRCA1 varied in size (from 2 exons to 17 exons) and appeared among patients diagnosed before 40 years (7/9).

Conclusions: The MLPA ensures a sensitive test for screening of genomic rearrangements. In the Czech Republic, large deletions accounted for 10.5% (9/86) of patients with detected alterations in BRCA1.

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Genomic data integration - application to understanding the biology of glioblastoma

R. Kashivan[1], J.D. Park[1], V. Wasnikar[1], R. Shahinian[1], S. Shams[1]
[1]BioDiscovery, Inc., El Segundo, CA, USA

Technological advances has enabled scientists to collect an astonishing quantity of high-quality measurements from various biological process and events, such as gene expression, DNA copy number, miRNA expression, methlyation and so forth. A great challenge now facing the scientists is now how to analyze and integrate such data into a cohesive picture of the molecular state of the cell. We have developed novel methodologies and implemented these in a system called Nexus Copy Number to enable scientist to make such analyzes and integration. In this paper we will outline the process in analyzing large number of glioblastoma samples processed as part of The Cancer Genome Atlas (TCGA) project. This data set provides an ideal showcase for integration of data as it involves data coming from many different array platforms, including Agilent 244K, Affymetrix SNP 6, and Illumina 550K, as well as different data modality spanning copy number changes, mRNA and miRNA regulation and Methylation data. We will demonstrate how regions of genomic change can be identified and correlated with phenotype data. We will then use a novel "hotspot" detection algorithm to identify the regions where multiple genomic events coincide. The genes in these regions are then selected for enrichment analysis against various GO categories to yield possible biological processes that are involved in glioblastoma.