

to produce prostacyclin in the spinal cord. Our study also supports the hypothesis that the asymptomatic interval after radiation is characterized by sequential physiological changes that are imperfectly reflected in routine histological study and that even in the histologically unaffected spinal cord, severe impairment in substructures and biochemistry of irradiated spinal cord are present.

Key words: Prostacyclin, radiation myelopathy, low dose irradiation, spinal cord.

247

PUBLICATION

Germline mutations in BRCA1 and 2 genes in Polish population, modulation of cancer risk by tri- and dinucleotide repeats in AR (androgen receptor) and ESR1.2 (estrogen receptor) genes

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Introduction: Germline mutations in BRCA1/2 genes predispose to breast and ovary cancers. However, the penetrance of mutations is incomplete (70–90% for breast cancer and 20–50% for ovary cancer). The aim of this work was to identify possible other genetic factors: AR (androgen receptor) gene and ESR1, 2 (estrogen receptor) gene with much lower penetrance which can modulate the penetrance of BRCA mutations.

Methods: Patients with breast and ovary cancer and healthy persons with family history of these cancers were recruited through Chemotherapy Clinics and Genetic Counseling. Controls were identified among the town clerks. Each person filled out detailed questionnaire including family history and signed the consent. Germline mutations in BRCA1 and 2 genes were detected using direct sequencing and when the spectrum of mutations was determined, ASA and RFLP PCR method was used for screening of mutations.

CAG and GGN repeats in AR gene, TA repeats in ESR1 gene and CA repeats lengths in ESR2 gene were measured also in ABI PRISM DNA sequencer. The primers for PCR reactions were labeled with FAM and NED fluorescence dyes and run with the length marker GS-500.

Results: Since 1997, 252 carriers of germline mutations in BRCA1/2 genes belonging to 178 families were identified. Mutation 5382insC in BRCA1 was prevalent in Polish population and it was found in 63% of all carriers. When Amsterdam criteria were applied for pedigree analysis and direct sequencing was used to detect mutations, we found germline mutations in 45% families. For further analysis ASA and RFLP PCR detecting 6 most prevalent mutations in both genes were used. Germline mutations were found in 8.2% of consecutive breast cancer cases below 50 years of age, 13.5% of consecutive ovary cases regardless of age, 12.5% of synchronous bilateral breast cancer and 34.2% of metachronous bilateral breast cancer. CAG repeats length polymorphisms in exon1 of AR gene did not modify significantly the risk of cancer in HBOC families. GGN longer repeats in the same gene were associated with increased risk of developing malignancy in the group of carriers of BRCA1/2 mutations diagnosed with breast and/or ovary cancer (OR = 3.44, $p = 0.00005$). Short TA repeats in ESR1 gene also increased the risk of cancer in the same group under study (OR = 3.14, $p = 0.00007$). By contrast short CA repeats in ESR2 gene showed slight protective effect against malignancy (OR = 0.68, $p = 0.08430$) at the border of significance.

Discussion: In our study we proved that germline mutations in BRCA1 and 2 genes can be also detected in the patients without family history of cancers and strict Amsterdam criteria allow only to identify the part of HBOC families in the population. Analysis of tri- and dinucleotide repeats in AR and ESR1, 2 genes respectively indicates that other genetic factors are able to modify very high penetrance of mutations in BRCA genes.

248

PUBLICATION

Pyruvate kinase activity in colon, stomach and breast tissue as a marker of malignancy

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Pyruvate kinase (PK; E.C. 2.7.1.40) is one of the key enzymes in the synthesis of pyruvate, which is the most important metabolite in aerobic and anaerobic metabolism of carbohydrates. Since the pioneering work of Wartburg (1956), the differences in metabolic profile between normal and malignant tissues are the subject of many studies directed towards advancement in diagnostics and therapy of cancer.

The family of PK enzymes comprises 3 different classes of isoenzymes, which are designated according to the tissue where they are predominant: L isoenzyme – the main isoenzyme of the liver and erythrocytes, M – predominant in muscles and brain, K – in kidneys. These enzymes and their dimeric hybrids are designated as: M₄, KM₃, K₂M₂, K₃M and K₄. Many studies showed the aberrations in isoenzymic profile of PK in malignant hepatomas, gliomas, meningiomas, carcinomas of the lung and in experimental tumors, in comparison with isoenzymatic profiles in corresponding normal tissues.

In our study, we determined the total activity of PK and PK-K₄ isoenzyme activity as well as the effects of the inhibition of PK induced by L-alanine in samples of 20 colon carcinomas and corresponding surrounding tissue (2 and 10 cm distant from tumor), 14 gastric carcinomas and corresponding surrounding tissue (2 and 10 cm distant from tumor) and 15 samples of breast carcinoma and 14 samples of benign dysplasia of the breast.

The results obtained in our study showed significantly increased total activity of PK in malignant tissue samples in comparison with normal tissue of colon (2×) and stomach (3.5×) or with benign breast hyperplasia (10×). We observed significantly higher proportion of PK-K₄ isoenzyme in total PK activity in malignant tissues, showing that the increase in total PK activity is due to PK-K₄ isoenzyme activity increase. The activity of PK-K₄ isoenzyme was 4x higher in colon carcinoma samples, 6x higher in gastric carcinoma samples and 50× higher in breast carcinoma samples in comparison with non-malignant tissue samples. The percentage of the L-alanine induced PK inhibition was significantly increased in malignant tissue samples. Our results showed that the increase in PK-K₄ isoenzyme activity is a significant, highly specific and sensitive parameter, which may enable the differentiation between malignant and normal tissue.

249

PUBLICATION

Interactions of genetic polymorphisms of folate metabolizing enzymes in modifying colorectal cancer risk in a Hungarian population-based case-control study

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Complex interactions of candidate polymorphic genes in CRC risk assessment make individual association estimates difficult. Pathway guided risk evaluation is possibly more adequate for gene-gene interaction studies. TS enhancer region (TSER), TSER G>C SNP, and TS1494del6, MTHFR C677T, cSHMT C1420T genetic variants were analyzed in 325 Hungarian primary CRC patients, and compared with the genotype data of a 200 individual control group of same population origin.

Population analysis showed a significant TSER heterozygote deficiency overall, and also in the rectum cancer (RC) subgroup, and a slight heterozygote excess in RC at cSHMT locus. No disequilibria were found at the TS1494del6 and MTHFR loci, or for any of the polymorphisms in the control population. The OR between TSER homo-/heterozygotes was higher among RC vs. CC (1.63 CI 1.08–2.4, vs. 1.42 CI 0.92–2.18), and rooted mainly in a 3R3R frequency increase for all sites, compared to the control. An important gender difference was apparent: we found 3R3R women most susceptible for RC and men for distal CC (OR 2.06 CI: 1.09–3.8; and 2.7, 1.28 to 5.8, respectively). Variant homozygote cSHMT genotypes (TT) were less affected by CRC (OR 0.50, CI 0.27–0.93), with closely the same effect in both RC and CC, which was significant for the women subgroup alone ($p = 0.05$), as well. No associations with TS SNP and TS1494del6 occurred, nor the reported protective effect of the MTHFR TT variant was seen on our study group, conversely, we found an increased risk for TT genotype, especially for proximal cancers in men. In a multivariate logistic regression analysis TSER 3R3R and cSHMT CC were found independent positive risk factors for CRC, with a cumulative risk of the compound genotype 3R3R&CC OR = 1.78 CI: 1.0 to 3.0; and for the least affected 2R3R&TT OR = 0.42 CI: 0.18 to 0.98, while MTHFR and sex were not influencing alone. Instead, modeling the overall and also the site-specific susceptibilities, important two-way interaction terms (e.g. between MTHFR and cSHMT genotypes) modifying risk emerged, raising the hypothesis that hierarchical models could be useful in folate-related molecular epidemiology studies of CRC.

Our findings support the proposals of pathway-driven multi-locus assessments of genetic susceptibility for CRC, and were able to identify independent risk factors with additive properties at TSER and cSHMT loci, and also MTHFR-cSHMT gene-gene interactions in Hungarian CRC population.