Folate profiling in a methionine dependent mouse colon tumour model

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Cobalamin-dependent methionine synthase catalyses the transfer of a methyl group from methlytetrahydrofolate (MTHF) to homocysteine, forming tetrahydrofolate (THF) and methionine. The reaction recycles intracellular folates, and produces methionine, precursor of S-adenosylmethionine. Methionine synthase is the only known enzyme capable of recycling folates at this point in the one carbon cycle. Since impaired methionine synthase activity may lead to accumulation of MTHF, levels of circulating folates give an insight into methionine synthase activity. Most cancer cells show an inability to grow in a methionine depleted environment even when supplemented with homocysteine (methionine-dependence). This phenomenon provides selective targeting for cancer treatment. A mouse colon adenocarcinoma cell line, MAC15A, has been characterised as methionine dependent. MAC15A tumours were implanted subcutaneously in mice. Erythrocyte folates from tumour-bearing and control mice were analysed by HPLC with fluorescence detection. The predominant folate species were MTHF (65% of total measured folate), formyltetrahydrofolate (33%) and THF (2%). Distribution of folates altered with tumour growth. Initial accumulation of MTHF (day 1-5) was reversed by day 10, suggesting increased methionine synthase activity. This increase may not be sufficient to sustain tumour growth, explaining thus the need for externally provided methionine.

DELINEATION OF A MINIMAL LOSS INTERVAL AND EXCLUSION OF 3 GENES AS CANDIDATES FOR TUMOR SUPPRESSOR GENES ON CHROMOSOME 12 IN MOUSE MALIGNANT LYMPHOMAS

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Our previous studies and many other genetic reports have strongly suggested an existence of a tumor suppressor gene(s) on murine chromosome 12 and syntenic human chromosome 14q32. Putative tumor suppressor gene(s) might reside between D12Mit53 and D12Mit233 in radiation-induced lymphomas. Here we analyzed three genes, Tc11, Ys1 genes and D12Mit233 were observed in 94(45%), 143(68%) and 147(70%) of Y184pR2, from YACs which located in the hot region between D12Mit53 and D12Mit233, and the highest frequency (71%) of allelic loss was observed at the Y184pR2 locus. The LOK patterns of individual lymphomas suggest that putative tumor suppressor gene(s) lies between Y152pR1 and Y184pR2.

Mutations of BRCA1 gene in Czech breast and ovarian cancer families

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Inherited predisposition accounts for 5-10% of all breast and ovarian cancers. The recent characterisation of predisposing genes, BRCA1 and BRCA2, allows the identification of mutation carriers. We examined patients from 81 risk families in the Prague region for germline mutations in BRCA1. Genetic material was extracted from peripheral blood lymphocytes and total RNA was reverse transcribed into cDNA. Three overlapping fragments of exon 11 were amplified on DNA; remaining coding segments of BRCA1 were amplified by nested PCR on cDNA. A combination of protein truncation test (PTT) and sequencing was used to examine the 22 coding exons of BRCA1. Different deleterious mutations were found in 6 women (7.4%). A PTT-analysis revealed four truncating mutations in exon 11. A deletion of an A residue between bases 1129 and 1133 (1129delA) resulted in a termination of translation at codon 340, a deletion of AG at nucleotide 2530 (2530delAG) led to premature translation termination at codon 838; two nonsense mutations (Q563X and Q1111X) changed a C to a T in positions 1806 and 3450. Sequencing of RT-PCR products revealed the complete loss of exon 5 (Exon5del), a mutation that removes a section of the critical RING finger domain of the BRCA1 protein, and the novel in-frame 3-bp deletion at the very beginning of exon 8 (561delCAG) lead to the loss of glutamine 148 from BRCA1. Supported by grants: GAUK 25/2000, LF1-206019-2

Detection of circulating tumour cells in miniature pigs with hereditary melanoma

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Tyrosinase, a key enzyme of melanine biosynthesis, is most widely used as a specific marker for the detection of disseminated melanoma cells. The amplification of tyrosinase mRNA by means of reverse transcription and polymerase chain reaction is a sensitive technique capable of detecting a single tumour cell in 5-10 ml blood. We have utilized this method to examine a group of laboratory pigs (MeLiM strain) with advanced hereditary melanoma for the presence of circulating tumour cells. The primary aim of our study was to assess the applicability of pyrosequencing to monitor dissemination of tumour cells in different clinical stages of the disease and for studying the response of experimental therapy by devitalization technique. Since the porcine tyrosinase gene has not been sequenced, PCR oligonucleotide primers were derived from gene segments conserved among different animal species. Homology of amplified DNA fragments with the sequence of tyrosinase gene was confirmed by restriction enzyme digestion. Blood samples from eight animals with advanced melanoma and from five non-melanoma control animals were examined. Tyrosinase mRNA was detected in five samples (62%) from animals with melanoma. Nonmelanoma animals gave negative results. Supported by grants GACR-523/98/0229, LF1-206019-2