stage ($p<0.05$). In 3 cases cytokeratin positive dendritic-like cells were detected.

Conclusions: IM cell separation with cytocentrifugation and immuncytochemical labelling is a useful method in the detection of circulating colon cancer cells and clusters. Further studies are in progress to reveal the significance of the cytokeratin negative cluster cells and the dendritic-like cells. Our results showed an association between the size of circulating cell clusters and the TNM stages. Our human data are supported by the animal models about the importance of the circulating cell clusters.

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Detection of circulating tumour cells in the peripheral blood of colorectal cancer patients using cytokeratin 20 RT-PCR and immuncytochemistry

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Background: The detection of circulating tumour cells and micrometastases may have important therapeutic and prognostic implications. Our objective was to compare the two gold stndard techniques, such as RT-PCR and immuncytochemistry (ICC), of circulating tumour cell detection in clinical samples with advanced disease.

Methods: 30ml of peripheral blood was obtained from 11 Dukes D colorectal cancer patients at two separate times within a month. The first 5ml after puncturing the skin were discarded. Five ml of the sample was used for mRNA isolation and subsequent RT-PCR with cytokeratin 20 primers. Twenty ml was subjected to immunmagnetic tumour cell enrichment with positive selection of cytokeratin 7/8 expressing cells on high-gradient magnetic columns (Carcinoma Cell Enrichment Kit, Miltenyi Biotech, Germany). The enriched cell fraction was cytocentrifuged on poly-L lysin coated slides. The cytospins were immuncytochemically labelled using a pancytokeratin antibody (DAKO, MNF116) and biotin-streptavidin-peroxidase technique (DAKO, LSAB2). In spiking experiments the sensitivity of both assays were in the 1 cell/ml range.

Results: Of the 22 samples in this study, 19 (86.36%) showed concordance between the two methodologies. The 3 disparity cases were due to RT-PCR positivity, but ICC negativity. With using RT-PCR 9/22 (40.90%) samples were positive, while with ICC 6/22 (27.27%) samples showed positive results. In 5 cases (45.45%) with RT-PCR and in 4 (36.36%) with ICC the result of the second sample conflicted with the first one.

Conclusion: Our results indicate that both RT-PCR and immunmagnetic enrichment with subsequent ICC are sensitive and reliable methods for circulating tumour cell detection. The concordance rate in our study is high, but because both techniques have significant limitations it may be advisable to use them complementary in further investigations. Even in advanced staged patients with a high probability of circulating tumour cells, multiple sampling can increase the risk of positive finding.

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Damage-induced Bax N-terminal change and translocation to mitochondria occur regardless of cell fate

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Resistance to chemotherapy is the major obstacle to the successful treatment of neuroblastoma. A model system for the investigation of drug-resistance in vitro is described, exploiting two NB cell lines, SH-EP1 and SH-SY5Y, derived from the same parental background. These subclones show no difference in their sensitivity to the DNA damaging agent cisplatin, but have very different responses to the microtubule stabilising agent paclitaxel; SH-EP1 cells are sensitive, whilst SH-SY5Y cells are resistant. The protein product of the tumour suppressor gene p53 is stabilised to the same extent in SH-SY5Y cells following exposure to cisplatin, which readily engage apoptosis, as in those exposed to paclitaxel, which do not. Stabilised p53 is active in SH-SY5Y cells following paclitaxel exposure as reflected by the transcriptional upregulation of the cyclin dependant kinase inhibitor, p21WAF-1, a downstream effector of p53, after both drug treatments.

The pro-apoptotic Bcl-2 family protein Bax is latent in healthy cells and requires activation by drug-damage signals. Exposure of an epitope in the N-terminus of Bax was observed in both NB cell lines following both types of drug induced damage. This N-terminal exposure occurred to the same extent in settings of drug resistance as in those of drug sensitivity. The exposure of the N-terminus of Bax occurred in the cytosol, and was followed by the translocation of Bax to the mitochondria, again irrespective of cell fate. The exposure of the N-terminus of Bax was also observed following detachment of NB cells into suspension. Thus the N-terminal changes in Bax represent a reversible response to disparate types of damage, and do not commit the cell to death. A model for the activation of Bax by drug-induced damage in NB cells is suggested that must require a second signal, after N-terminal epitope exposure and mitochondrial translocation, which is needed to commit the cell to apoptosis. This damage-induced second signal is suggested to be abrogated in SH-SY5Y cells after treatment with paclitaxel. Lack of the full activation of Bax may represent a novel method of drug resistance in NB cells.

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A new approach for the detection of control sites in DNA

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Purpose: The concept of the consensus sequence of DNA control sites is well established. We have examined the variation of individual sequences about the consensus sequence. With the rapidly rising volume of reported DNA sequences, there is lively interest in automatic methods for detecting control sites or other short specific sub-sequences. We have developed an approach by which the statistical analysis of a reference set of sequences of a particular type of site allows one or more equations to be defined. If such an equation is satisfied by a new sequence then it is highly likely that the sequence corresponds to a site of the particular type. The definition of the equations makes use of the properties of the eigenvalues and vectors of the covariance matrix of suitably encoded sequences. In particular, the existence of one or more zero eigenvalues implies the existence of one or more such equations.

Methods: The approach is illustrated with the sequences of 173 promoters recognized by human RNA polymerase II. Many of these promoters are of particular interest in oncology and the database includes sequences for growth factors (e.g. GM-CSF, erythropoietin, various interleukins etc.), oncogenes and tumour viruses among others. Sub-sequences of 25 bases around the TATA box were extracted. Two bits were used to encode each base and the covariance matrix of the resulting 50 variables was determined. The eigenvalues and eigenvectors of the covariance matrix were calculated.

Results: The eigenvalues of the matrix ranged from 0.787 down to 0.035. This eigenvalue of 0.035 (almost zero) means that there is an equation which is (almost) satisfied by all the promoters in the dataset. A new sequence which (almost) satisfies this equation may be regarded as a putative promoter.

Conclusion: We have shown that promoter sequences contain correlations of such a nature that a rule can be derived which may be applied to detect other putative promoters. This regularity, not previously described, may be a common or indeed universal feature of sets of sequences with a common function.

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Measurement of tumor blood flow using colored dye extraction microspheres in two rat tumor models

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A technique that can measure tumor blood flow easily, accurately and economically is required to study tumor angiogenesis and angiogenesis inhibition. Using dye extraction colored microspheres, we measured tumor blood flow in Sato lung carcinoma (SLC) and ascites hepatoma LY80 in rats. Colored microspheres were infused into tumor-bearing rats via a catheter in the left ventricle. After removal of the tumor and the liver, the tissue samples were dissolved, and the microspheres were isolated. Dye was extracted, and the dye concentration was quantified by spectrophotometry. The dye concentration per gram of tumor was compared with that per gram of liver as follows (AU = absorbency units): [AU per gram of tumor]/[AU per gram of liver] x 100 = (%). Tumor blood flow corrected for wet weight was calculated as follows: [blood flow to tumor] = [AU per gram of tumor] x [reference withdrawal rate]/[AU per gram of reference blood]. Tumor blood flow rate was divided by tumor weight to yield ml/Emin-1g-1. The tumors were also examined histologically, and casts of the tumor vasculature were prepared with silicone rubber. Blood flow 2 weeks after transplantation was equivalent to 1/10 and 1/2 at 1 week in SLC and LY80 tumors, respectively (SLC, $P = 0.009$, $n = 10$; LY80, $P = 0.05$, $n = 10$). These decreases in tumor blood flow were associated with underlying pathological and vascular change. Blood flow in LY80 tumors negatively correlated with tumor volume ($P = 0.009$, $n = 10$). We concluded that the colored microsphere method,