G-CSF mobilized peripheral blood and then cultured for 14 days on fibronectin-coated chamber slides with IMDM supplemented with 10% fetal bovine serum and 10% horse serum; at day 0, different growth factors were added to the media, including SCF (100 ng/ml), SCGF (100 ng/ml), and VEGF (50 ng/ml). Cells of endothelial lineage were identified by immunocytochemistry, flow cytometry, and electron microscopy. Additionally, gene expression patterns of the freshly isolated AC133+ cells as well as of the cultured were analyzed by RT-PCR. Within 1–2 hours of culture in these conditions, AC133+ cells became adherent. In the presence of VEGF alone or in combination with SCF, the adherent cells did not proliferate whereas the stimulation with SCGF and VEGF resulted in an up to 8-fold higher cell number after 14 days of culture. In those proliferating cultures, a round non-adherent cell population occurred within 6–7 days of culture, and was transferred to fresh chamber slides to again become adherent. Phenotypic analysis of the adherent cells grown in the presence of SCGF and VEGF revealed that the vast majority displayed characteristics of the endothelial lineage including the expression of CD34, VE-Cadherin, vWF, FLK-1/KDR, TIE-2, and Ulex europaeus agglutinin-1. Furthermore, electron microscopic analysis showed structures similar to Weibel-Palade bodies which are found exclusively in vascular endothelium. These data indicate that the AC133+ cell population contains precursors not only with haematopoietic, but also with endothelial potential. The functional role of circulating EC in vivo was assayed in tumour-bearing mice and will be presented at the meeting.

361 ORAL

Genomic instability in bronchial lavage specimens from individuals with no evidence of lung cancer: An early detection marker?

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Genomic instability (GI) is now considered to be a hallmark of cancer. Analysis of microsatellite markers in tumour tissue compared to its normal counterpart has become the most widely used method to determine genomic instability in the form of microsatellite alterations (MA) or loss of heterozygosity (LOH). We have studied 90 bronchial lavage specimens with 12 microsatellite markers from individuals with suspected lung cancer. GI was found in 15/43 (35%) patients with lung cancer, however, GI was also found in 11/47 (23%) patients with no cytological or radiological evidence of bronchial neoplasia; of whom 9 or 11 individuals had evidence of MA/LOH and two were found to have LOH alone. On comparing LOH with MA based on the cytology review, we found that the prevalent type of instability in specimens with cytological evidence of malignancy, is LOH. In contrast, the individuals with negative cytology show a preponderance of MA (Fisher,s exact P = 0.01). A statistical correlation was found between GI and individuals who smoked more than 2 packs/day (P = 0.02) and in the lung cancer patients (P = 0.009). Using current diagnostic techniques, the detection of lung cancer usually occurs late in the disease when it is beyond effective treatment. Thus, increased attention on earlier detection and intervention management is therefore imperative.

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362 ORAL

Tumour cells can eliminate amplified genes

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Highly malignant tumours are often characterised by the presence of an amplified proto-oncogene providing the cells with a proliferation advantage. Aggressive neuroblastomas (NBs) and their cell lines often show a substantial increase of copies of the MYCN gene, which encodes for a nuclear phosphoprotein involved in proliferation control. NB cell lines are usually characterised by the occurrence of morphologically distinct cell types. The neuronal cells (N-cells) show a neuronal expression pattern and phenotype, whereas the so-called flat cells (F-cells) cease to express neuronal markers and have a more fibroblastoid-like morphology. Our aim was to further characterise the nature of the F-cells with special focus on the genetic features, the proliferative activity and the expression pattern of different antigens. For these purposes, we used fluorescence-based in situ hybridisation techniques, BrdU incorporation and immunocytochemistry in five NB cell lines. F-cells were shown to have a markedly decreased MYCN copy number or even to completely lack amplification and the amplified gene copies were shown to be entrapped by micronuclei. In contrast to the N-cells, F-cells showed a reduction or lack of MYCN expression, a decreased proliferation rate and lack of bcl-2 expression. However, they up-regulated MHC class I molecules and expressed b-galactosidase, an enzyme linked to cellular senescence. Based on these results, we conclude that NB cells in vitro can loose their malignant properties, a process which is accompanied by elimination of the amplified oncogene and which allows the cells to senesce. Moreover, MYCN containing micronuclei were also observed in tumour cells infiltrating the bone marrow and in tumours after exposure to cytotoxic agents.

363 ORAL

Inhibition of NF-kB activation confers sensitivity to TNFa by impairment of cell-cycle progression in human glioma cells

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Tumor necrosis factor a (TNFa) has been shown to exert cytotoxic or cytostatic effect on tumor cells, but the susceptibility varies among the different cells. TNFa activates a transcription factor, nuclear factor kappa B (NF-kB), which induces a wide variety of genes and causes pleiotrophic responses. In this study, the relationship between the susceptibility to TNFa and the activation of NF-kB was investigated in six human malignant glioma cell lines. Cell proliferation analysis revealed that only one cell line, SK-MG-1, was sensitive to TNFa and that the other five including U-251MG were resistant. On electrophoretic mobility shift assay, TNFa strongly activated a subtype of NF-kB, p50–p65 heterodimer, in all the resistant cell lines tested. However, this activation was weak in the sensitive cell line, SK-MG-1. Activation of NF-kB by TNFa in the resistant cell lines resulted in significant increases of a reporter gene expression driven by NF-kB site, suggesting a possibility that activation of p50–p65 confers resistance to TNFa. To test this hypothesis, a stable cell line which expresses an inducible dominant negative NF-kB (p65 DN) protein was established in one of the TNFa resistant cell lines, U-251MG. In the established clone, induction of p65 DN protein decreased TNFa-dependent increase in the DNA binding of p50–p65 heterodimer and NF-kB-dependent reporter gene activity. While no growth inhibition of this clone was observed by TNFa treatment, induction of p65 DN together with TNFa resulted in a significant decrease in cell number. Cell-cycle analysis revealed that this growth inhibition was due to an impairment of cell-cycle progression. These results indicate that the active NF-kB complex, such as p50–p65 heterodimer, plays a crucial role for the progression of cell-cycle in malignant glioma cells. The refractoriness to TNFa treatment could be prevented by inhibiting the NF-kB activation.

364 POSTER

Death receptors in etoposide treated lymphoma cells

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Purpose: Apoptosis induction of tumor cells is a potential aim in cancer therapy. Since tumor cells could be resistant to apoptosis inducing agents, it is a question, how we can circumwent this problem. How many pathways are available in a particular tumor cell population using one inducer?

Methods: target cells (HT58 NHL cell line, in vitro) were treated with etoposide; basic methods were immunocytochemistry, flow cytometry and RTPCR

Results: Etoposide activated three different apoptotic signaling pathways in a human B-cell lymphoma (HT58) line, which were distinguished upon time-kinetics and the participants. The first wave of cell death is rapid (~4 h), does not require new proteins, and dependent on caspases. The second wave is slower (1–3 days), still caspase-dependent. The cells express FasR and FasL, and the former appears on the cell membrane. The third wave (3–5 days) of etoposide induced apoptosis became caspase independent. The role of other newly identified death receptors (DR3, 4, 5, TRAIL) is still uncertain, still the target lymphoma cells express all of them.

Conclusion: A commonly used cytostatics, etoposide, can induce three different pathways in lymphoma cells of a well established in vitro line, suggesting the heterogeneity of tumor cells towards a potentially apoptosis inducing agents.

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