08

Cancer

08-01/P

THE EFFECTS OF CD30 RECEPTOR DENSITY ON INTRACELLULAR SIGNALLING: INSIGHTS INTO ANAPLASTIC LARGE CELL LYMPHOMA TREATMENT

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CD30 is a member of the tumor necrosis factor receptor superfamily whose expression is restricted to a subset of CD45RO-positive activated T cells. Like most of the TNF receptor family, CD30 activation can result in either proliferative or apoptotic events. Signals emanating from CD30 diverge downstream of TRAF-2 & 5, leading to activation and/or induction of caspases and subsequent apoptosis. Although some TNF-R family members are very polar in their signaling potential, in general the response can be seen as a continuum with activation or proliferation at one extreme and cell death at the other. The density of the TNF-R family molecule itself may influence which of these signals predominate. For instance, TNF-R and Fas signal transduction appear to be receptor density-dependant in some instances, high receptor densities induce proliferative signals while low densities initiate apoptosis. CD30 expression is a diagnostic marker of anaplastic large cell lymphoma (ALCL). Our current studies are aimed at testing the hypothesis that the nature of the response in ALCL cells is dependant on CD30 density on the cell surface. Low receptor density allows anti-proliferative or apoptotic signals to predominate, which results in tumour regression. High receptor density allows proliferative signals to predominate leading to tumour growth. Using an ALCL cell culture model, we have shown that following siRNA-mediated knockdown of CD30, the cell's response to CD30 ligand (CD153) is skewed towards apoptosis. Measurement of Caspase 3 levels by flow cytometry indicates an increase in apoptotic potential and a decrease in proliferation. These data suggest a novel route to therapy for patients with these types of lymphoma.

08-02/P

ROLE OF 2-5A-DEPENDENT RNASE-L IN SENESCENCE AND LONGEVITY

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Senescence is a permanent growth arrest that functions as a tumor suppressor mechanism. RNase-L mediates the antiviral, antiproliferative, and proapoptotic activities of interferon, and is implicated as a tumor suppressor in prostate cancer; therefore, we examined a role for RNase-L in cellular senescence. Ectopic expression of RNase-L resulted in the induction of a senescent morphology, a decreased DNA synthesis, increased senescence-associated b-galactosidase staining, and accelerated replicative senescence. In contrast, senescence was retarded in murine embryonic fibroblasts with a targeted disruption in the RNase-L gene. Remarkably, activation of endogenous RNase-L by 2-5A transfection induced distinct senescent and apoptotic responses in parental and SV40-transformed WI38 fibroblasts respectively demonstrating cell type specific differences in the antiproliferative phenotype induced by RNase-L activation. Microarray analysis of gene expression following 2-5A transfection identified both up- and downregulated transcripts that encode known and potentially novel senescence effectors. A biphasic expression profile was observed for many 2-5A-regulated transcripts that may reflect an initial RNase-L-mediated degradation, with the subsequent attenuation of RNase-L activity required for the expression of senescence-associated genes. Replicative senescence is a model for in vivo aging, therefore, we determined if RNase-L null mice exhibited an altered lifespan. Indeed, RNase-L-/- mice survived 31.7% ($P<0.003$) longer than strain matched RNase-L+/+ mice providing evidence for a physiological role for RNase-L in aging. These findings identify a novel role for RNase-L in senescence that may contribute to its tumor suppressive function and to the enhanced longevity of RNase-L-/- mice.

08-03/P

IFN-α TREATMENT OF B16α CANCER VACCINE CELLS INDUCES CELL ASSOCIATED IL-15

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Long-term interferon-α (IFN-α) treatment converts parental B16 mela noma cells to B16α vaccine cells. Inoculation of syngeneic mice with irradiated B16α vaccine cells triggers a potent host immune response that results in the development of cancer immunity. Cancer immunity requires the function of host macrophages, T cells, and NK cells. IFN-α treatment of B16α vaccine cells induces IL-15 mRNA. Confocal microscopy of B16α vaccine cells shows that the IL-15 protein is distributed throughout the cytoplasm and nucleus. Using ELISAs, the intracellular and extracellular distributions of IL-15 have been examined. Neither parental B16 cells nor B16α vaccine cells released detectable levels of IL-15 into the supernatant fluid. However, when compared with parental B16 cells, lysates of B16α vaccine cells had 2-fold and 8-fold enhancements in the intracellular level of IL-15 at 24 h and 48 hr after treatment with fresh medium plus IFN-α. Further, B16α vaccine cells had a 2.6-fold enhancement in the amount of membrane bound IL-15 at 48 h. The intracellular IL-15, when released by lysis of inoculated B16α vaccine cells, would have a concentration of 1 ng/ml, well within the range of concentrations that have biological significance. In summary, IFN-α treatment of B16α vaccine cells may induce the truncated isoform of IL-15 tat remains cell associated. Thus, the B16α vaccine cells become packets of intracellular and membrane bound IL-15. It is envisioned that, following inoculation, the lethally irradiated B16α vaccine cells die and release their cell associated IL-15 in the milieu of a host immune response to the inoculation where it provides a potent immunostimulation of T cells, triggering the T cells to recognize B16 tumor antigens and establish cancer immunity. These studies support the potential clinical use of long-term IFN-α treated cancer cells as an immunotherapy to induce cancer immunity in patients.

08-04/P

THE ROLE OF KAPOSI'S SARCOMA-ASSOCIATED HERPES VIRUS-ENCODED VIRF-3 IN ACTIVATION OF C-MYC-MEDIATED TRANSCRIPTION

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Kaposi's sarcoma-associated herpesvirus (KSHV)-encoded nuclear protein, vIRF-3/LANA2, is constitutively expressed in latently infected B-cell lymphomas. The vIRF-3 protein was previously shown to interact with cellular IRF-3 and IRF-7 and stimulate the expression of Type I IFN genes. In our search for other potential vIRF-3 interacting proteins, we employed the yeast two-hybrid screen. We detected the association between vIRF-3 and the c-Myc suppressor, MM-1 (c-Myc Modulator-1). MM-1 is a novel tumor suppressor gene that binds to c-Myc and represses its E-box-dependent transcription activity. MM-1 bridges the association between c-Myc and TIF1β/KAP1/KRIP1, which recruits the HDAC complex including HDAC1 and mSin3A. We have confirmed the vIRF-3-MM-1 interaction using the GST pull-down assay as well as co-immunoprecipitation of endogenous vIRF-3 and MM-1 in KSHV-infected B-cell lymphomas. In a transient transfection assay with the cdk4 reporter, we observed that vIRF-3 effectively suppressed the MM-1-mediated inhibition of c-Myc. This resulted in a significant activation of cdk4 transcription. Addressing the molecular mechanism of the vIRF-3-mediated stimulation, we observed that the association between MM-1 and c-Myc is inhibited in the presence of vIRF-3 expression. DNA pull-down and chromatin immunoprecipitation assays have shown that vIRF-3 together with c-Myc form a heterodimer which is part of the enhanceosome assembled on the promoter regions of cdk4 and other c-Myc target genes. De-regulated c-myc expression is a common denominator in cancer. The c-myc gene is often implicated in a large number of human solid tumors, leukemias and lymphomas, in which chromosomal rearrangements frequently target the myc locus. Thus, in the absence of any c-myc locus rearrangements in KSHV-associated lymphomas, the effect of the vIRF-3/MM-1 association on the c-Myc-mediated transcription may represent a novel mechanism by which KSHV targets the c-Myc function and consequently de-regulates the cell growth and differentiation.

08-05/P

RELATION AMONG TNF-α, ITS RECEPTORS, IL-6, P53 AND P21 IN BREAST TUMOR GROWTH.

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The aim of this study was to characterize the expression pattern of TNF-α and its receptors, to elucidate the possible relations with other factors as IL-6, p53 and p21 (studied in previously in our laboratory in the same samples used in this study) related with the proliferation/apoptosis equilibrium in benign conditions and *in situ* and infiltrating breast cancer.

The immunoexpression of TNF-α, its receptors, as well as their relationship with proliferation/apoptosis, were studied in *in situ* and infiltrating tumors, and in benign breast lesions by means of immunohistochemistry.

The percentages of positive samples to TNF-α and TNFRII were higher in *in situ* carcinoma than in benign breast diseases, and also even higher in infiltrating than in *in situ* tumors. The percentage of positive samples to TNFRI is similar in the three groups. In the three groups of patients immunoreaction for all proteins appeared in the peripheral cytoplasm. Whereas to TNF-α, the immunostaining intensity was only significantly elevate in infiltrating tumors. For the two the receptors immunoreaction increased with the malignance. In previous study, reported in the same patients used in this study, p21 in *in situ* samples was localized in epithelial cells cytoplasm, while in other groups nuclear location was observed. Comparison of expression of TNF-α with IL-6, p53 and p21 between the three groups of breast samples (benign lesions, *in situ* -ductal and lobular- and infiltrating -ductal and lobular- tumors) was studied.

TNF-α might be an important factor in the breast cancer promotion since its proliferation/survival effects seems to be enhanced thought the increased expression of TNFRII or the inhibition of pro-apoptotic pathway of TNFRI by p21 cytoplasmic localization. This action of TNF-α could be mediated by the induction of the expression of other factors IL-6, which contributes to the tumor promotion.

08-06/P

FUNCTIONAL ANALYSIS OF AMPK ACTIVITY IN CERVICAL CANCER CELLS

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Cervical tumors are usually featured with nutrient-deprived environment. AMP-activated protein kinase (AMPK) has been well recognized as an important mediator of stress signals including hypoxia and nutrient deprivation. We previously identified gene amplification of the catalytic alpha 1 subunit of AMPK by comparative genome hybridization in cervical tumors. This study aims to investigate the effect of AMPK activity on the growth of cervical cancer cells. We also look at the involvement of LKB1 (a potential upstream kinase of AMPK) in activating AMPK. The cervical cancer cell lines, HeLa and SiHa, were cultured with addition of glucose or under glucose deprivation. Their growth was studied with addition of the AMPK activator, AICAR, at concentrations of 100μm and 1mM. The growth rates were measured by XTT assay. We also did PCR, RT-PCR and methylation specific PCR to study LKB1 gene expression. We found that in culture with glucose, HeLa cell growth was inhibited by AICAR treatment in a dose-dependent manner. On the other hand, under glucose deprivation, survival rate of HeLa cells treated with

1mM and 100µm AICAR were significantly higher at 48 hours than control without AICAR treatment. Similar results were observed with SiHa cells. No LKB1 expression was found in HeLa and SiHa. And no hypermethylation of LKB1 was detected. These data suggest that activation of AMPK inhibits cell growth in the presence of glucose while reduces cell death under glucose deprivation. It suggests that AMPK may have a dual role in controlling the cervical cancer cell growth. Moreover, the AMPK activation in HeLa and SiHa is not related to LKB1.

08-07/P

THE PROINFLAMMATORY CYTOKINE-INDUCED GTPASES HGBP-1 AND MGBP-2 CONFER RESISTANCE TO PACLITAXEL

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The guanylate-binding proteins (GBPs) are a family of pro-inflammatory cytokine-induced GTPases. The human family member, hGBP-1, was identified as one of 8 genes up-regulated in common in three different cancer cell lines as they became resistant to paclitaxel (Duan et al., 2005, *Cancer Chemother Pharmacol* 55:277-285). We have shown that the putative murine ortholog, mGBP-2, confers resistance to cytotoxicity induced by the microtubule-stabilizing drug, paclitaxel. This protection could be observed at paclitaxel concentrations from 0.005 to 15 µM (Balasubramanian et al., *Cell. Mol. Biol,*, in press). Twice as many mGBP-2-expressing NIH 3T3 cells survived treatment with 5 µM paclitaxel for 12 to 96 hours compared to nonexpressing cells. This protection does not require GTPase activity but appears to reside in the carboxy terminal α-helical region of the protein. Protection was accompanied by a higher percentage of cells with multinuclei. The paclitaxel resistance of the hGBP-1 was also examined. Using a MCF-7 breast cancer cell line engineered for tetracycline-regulated expression of hGBP-1 we found that after 24 hours of treatment with 5 µM paclitaxel the hGBP-1-expressing cells showed lower cytotoxicity (13.2 ± 0.8% dead cells) compared to controls (36.9 ± 10%). Work is underway to determine how paclitaxel induces the expression of hGBP-1 and how hGBP-1 inhibits paclitaxel-induced cytotoxicity.

08-08/P

TUMOUR CELLS FROM STAGE III MELANOMA PATIENTS ARE OFTEN RESISTANT TO GROWTH INHIBITION BY ONCOSTATIN M

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Oncostatin M (OSM) is an Interleukin-6 (IL-6) type cytokine originally described by its capacity to inhibit melanoma proliferation *in vitro*. However, OSM responsiveness is often lost in advanced stages melanoma cells. Here, the mechanisms involved in resistance to growth inhibition by OSM and IL-6 were analyzed for the first time on a large panel of metastatic melanoma cell lines (35).
For 28% of the cell lines, OSM resistance correlated with the epigenetic loss of the OSM receptor β (OSMRβ) subunit. Treatment of these cells with the histone deacetylase inhibitor Trichostatin A re-established histone acetylation in the OSMRβ promoter, expression of OSMRβ and growth inhibition by OSM. Other defects linked to OSM resistance were identified, for 31% of the cell lines, on specific signal transduction pathways such as STAT3 (Ser727 phosphorylation), PKCα/β/δ and/or AKT, explaining their co-resistance to OSM and IL-6. The use of PKCα/β/δ inhibitors indicated that these serine kinases, together with STAT3, have a crucial role in growth inhibition by OSM.
In nude mice injected with sensitive melanoma cell lines, OSM notably reduced tumour growth. Moreover, the patients whose melanoma cells were sensitive to growth inhibition by OSM and/or IL-6, and who were treated with tumor-infiltrating lymphocytes (as a potent source for these cytokines; n=13), have a mean relapse-free survival of 8 years. Those whose melanoma cells were resistant to these cytokines (n=6), have a mean relapse-free of only 15 months.
Altogether, our results suggest a role for OSM in the prevention of melanoma progression *in vitro* and *in vivo,* and that metastatic melanoma cells could escape this growth control by the loss of OSMRβ or defects on specific signal transduction pathways. We are currently validating the involvement of IL-6 type cytokines in the response to immunotherapy and to find if a specific inflammatory state could induce cytokine resistance.

08-09/O

TYK2 DEFICIENCY LEADS TO ENHANCED LYMPHOMA FORMATION IN EMU-MYC TRANSGENIC MICE

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In Eμ-Myc mice the proto-oncogene c-Myc was introduced into the heavy chain locus, such so that the mice develop B cell lymphomas. As in people, lymphomas arising in Eμ-Myc transgenic mice frequently harbor mutations. It is not known, if the so-called "second hits" modulate the outcome of immunological surveillance. To address this issue we relaxed immunological control by crossing the Eμ-Myc mice into a Janus kinase Tyk2 deficient background. In control animals (Eμ-Myc/Tyk2$^{+/-}$), we observed the following associations: Loss of p53 with low surface immunoglobulin M (sIgM) expression and accelerated disease; overexpression of Bcl-2 with an enhanced latency of disease and high sIgM (i.e. high differentiation grade); low sIgM levels and high incidence of liver metastasis. In contrast, Eμ-Myc/Tyk2$^{-/-}$ mice succumbed to their disease with shortened latency and the loss of p53 or the overexpression of Bcl-2 were irrelevant to the course of the disease. These observations strongly suggest that evolving tumours are sculptured by the immune system.

08-11/P

CONSTITUTIV STAT3 ACTIVITY OF HUMAN MELANOMA CELL LINES INCREASES WITH CELL DENSITY

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STATs (signal transducers and activators of transcription) are key mediators of cytokine signalling. Moreover, these transcription factors are also implicated in oncogenic signalling. Inappropriate activation of STATs, especially STAT3, was discovered in many tumor situations. Constitutive active STAT3 was found to prevent programmed cell death and enhance cell proliferation whereas the disruption of STAT3 signalling could block tumor growth. Constitutive active STAT3 has also been reported for melanoma tissue samples and several melanoma cell lines in vitro. We have previously shown that STAT3 is crucial for IL-6 and OSM mediated growth inhibition of melanoma cells coinciding with an up-regulation of cyclin-dependent kinase inhibitor p27/Kip1.
Consistent with our previous studies, we observed no or only very slight constitutive STAT3 phosphorylation in a panel of 20 melanoma cell lines. However, when culturing the cells at high cell density tyrosine-phosphorylated STAT3 could be found in several cell lines. The level of phosphorylation was not as high as in cells treated with IL-6 for a short time (30 min), but comparable to long-term stimulated cells. Normal human keratinocytes as well as primary fibroblasts also showed a cell density dependent STAT3 phosphorylation. In contrast, we did not observe a phosphorylation of STAT1 and STAT5. Density-dependent STAT3 phosphorylation was paralleled by a corresponding DNA-binding activity as measured by EMSA. Moreover, luciferase activities resulting from a STAT-responsive reporter gene construct increased in dependence of cell density.

We are currently investigating the mechanism underlying cell density dependent STAT3 phosphorylation: is this mediated by cell-to-cell contacts or by secretion of a STAT3 activating soluble factor? Moreover, experiments on the consequences of density dependent STAT3 activation will be addressed.

08-12/P

EXPRESSION OF SDF-1 AND A MEMBRANE-BOUND FORM OF IL-2 IN B16F10 MELANOMA CELLS INDUCES ANTI-TUMOR IMMUNITY

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SDF-1 (stromal derived factor-1) is a chemotactic factor for lymphocytes migration. The interaction between SDF-1 and its receptor, CXCR-4, also plays a crucial role in tumor metastasis. In previous study in our lab, the expression of membrane-bound form of IL-2 (mbIL-2) on B16F10 cells induced anti-tumor immunity, perhaps by direct priming of tumor antigens to CTL precursors. However, the induced anti-tumor immunity with the tumor clone was marginal, so that we attempted to produce a tumor cell vaccine expressing both mbIL-2 and SDF-1. We hypothesize that the mbIL-2 on tumor cell surface functions as a co-stimulatory molecule to tumor specific CTL and the SDF-1 may call activated CTL expressing CXCR-4 to tumor growing sites. The B16F10 transfectants with mbIL-2 and/or SDF-1 expression vectors were selected and the clones were screened for mbIL-2 expression by FACS analysis and for SDF-1 secretion by immunoblotting of culture supernatants. The B16F10 cells expressing both mbIL-2 and SDF-1 had reduced tumorigenicity and low metastatic ability compared with wild type B16F10 cells, vector transfectant, mbIL-2 clone, and SDF-1 clone. Furthermore, the mice once rejected the mbIL-2/SDF-1 tumor clone acquired antitumor immunity.

08-13/P

INTRACELLULAR GLUTATHIONE LEVELS INDICATE PERITUMORAL STROMA DEVELOPMENT AND T-CELL INFILTRATION IN PATIENTS WITH COLON CARCINOMA

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Aim: Many previous studies have discussed the relationship between the development of peritumoral stromas, successful T-cell infiltration into solid tumors, and a good prognosis for tumor-bearing patients. Predicting anti-tumor immune-responses at tumor growth sites by using peripheral blood will facilitate choosing the optimal therapeutic options for patients with solid tumors. This study determines the relationship between monocyte (Mo) intracellular glutathione (GSH) levels, and the development of the peritumoral stroma area (pTSA) and tumor mass T-cell infiltration levels. *Method:* In pre-op patients with colon cancer, intracellular GSH in $CD14^+$Mo, which was positively selected from peripheral blood mononuclear cells, was stained using Monochlorobimane (mBCI) and monitored by fluorescent microscopy. Intracellular GSH levels of these Mo were measured by means of image analysis that examined area and mean intensity of each Mo. The GSH index was determined by the following formula: GSH index = average (area x mean intensity). This index correlates well with intracellular GSH levels when evaluated by high performance chromatography. In addition, pTSA ratio was calculated using hematoxyirin-eosin staining and the number of infiltrated T-cells was counted in the tumor mass using anti-$CD45RO^+$T cells immunostaining with paraffin-embedded sections after the operation of each patient. *Results:* The patients were divided into two sub-groups: patients (n=18) whose GSH index was greater than 0.7, and patients (n=4) whose index was less than 0.7. Compared to the low GSH index groups, high index groups showed significantly broader pTSA and more T-cell infiltration in the tumor masses. *Discussion:* Present results show that patients with a higher GSH index have a broader pTSA and more T-cell infiltration into tumor masses. These results support the notion that reductive Mo with a high GSH index directs Th1-cell responses. Mo GSH indexes are a useful parameter for understanding patient's tumor status without operation, and for learning crucial information in order to choose a therapeutic strategy.

08-14/P

IMMUNOHISTOCHEMICAL STUDY OF LIF AND ITS RECEPTORS IN HUMAN BREAST CARCINOMA (IN SITU AND IN INFILTRATIVE): RELATIONSHIP WITH THE MALIGNANCE

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LIF is a member of IL-6 cytokine family that is widely expressed in different organs and associated with cancer progression. The aim of this study was to characterize the expression pattern of LIF and its receptors (LIFR and gp130), to elucidate their possible role with tumor progression in different types of breast cancer (*in situ* and infiltrating)

Immunoexpression of LIF and its receptors (LIFR and gp130) were studied in benign breast lesion, *in situ* and infiltrating tumors by Western blot and immunohistochemistry.

The percentage of positive samples to LIF was higher in *in situ* carcinoma than in benign diseases, and even higher in infiltrating tumors. The most expression to gp130 appeared in infiltrating carcinoma. LIFR was expressed in all types of breast samples studied (100 %). Infiltrating tumors showed the most intense immunostaining to LIFR and gp130; whereas immunostaining to LIF was similar in *in situ* than in infiltrating carcinoma. Comparing LIF results with LIFR and gp130 we found an association between the expression of these proteins and increasing malignancy.

Present results suggest that the development of breast tumor increases the expression of LIF, LIFR and gp130 and their expression may be positively associated with the malignancy of the tumor. Since the number of patient that expressed gp130 increase with the malignance, this receptor might be a crucial point in the development of infiltrative adenocarcinoma. In these tumors, the high expression of LIF in the epithelium, suggest an insufficient attempt to hinder cell proliferation.

08-15/O

TIR8, AN INHIBITORY MEMBER OF THE IL-1 RECEPTOR FAMILY, IN A MURINE MODEL OF COLITIS-ASSOCIATED CANCER

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The Toll-like receptor (TLR)/IL-1 receptor (IL-1R) superfamily plays an important role in inflammation and innate immunity response. Nevertheless, their excessive or inappropriate stimulation might lead to severe inflammation, thus the activity of some member of this family is tightly regulated at multiple levels. One of these regulatory factors, proposed as an endogenous inhibitor of TLR signaling, is TIR8, an orphan receptor also known as single Ig IL-1-related receptor (SIGIRR),

which presents the typical TLR/IL-1Rs intracellular domain but is the only member of the family that has a single extracellular Ig domain. TIR8 presents a distinct pattern of expression that includes epithelial tissues and sentinel dendritic cells (DC), and it plays a crucial role in tuning inflammation in the gastrointestinal tract, where is highly expressed. Indeed, TIR8- deficient mice showed a selective increase in susceptibility to intestinal inflammation, in terms of cytokine production, and a higher severity of acute and chronic colitis induced by dextran sulfate sodium (DSS). Since epidemiologic studies have revealed that chronic inflammation strongly predisposes to tumor development and progression of different forms of cancer, we investigated the role of *TIR8* in the development of colitis-associated cancer. *TIR8*- deficient and wild type mice were treated with mutagenic agent Azoxymethan (AOM) followed by three consecutive cycles of orally administrated dextran sulphate sodium (DSS) over a period of 7 days. The knockout mice showed a higher visual evidence of rectal bleeding and diarrhea, whereas the histological analysis of the intestine of these animals resulted in a superior number of proliferative lesion, expressed as adenomas and gastrointestinal intraepithelial neoplasia (GIN), as well as a higher number of lymphoid follicles in comparison with wild type. These results, together with production of inflammatory cytokines, indicated that TIR8 plays an important role in the regulation of colitis-associated cancer.

08-18/P

CONSTITUTIVE SOCS3 EXPRESSION CONFERS A GROWTH ADVANTAGE TO A HUMAN MELANOMA CELL LINE

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Growth of melanocytes and many early stage melanoma cells can be inhibited by cytokines whereas late stage melanoma cells have been reported often to be multi-cytokine resistant. We have shown previously that the transcription factor STAT3 plays a crucial role for the growth arrest of melanoma cells mediated by interleukin (IL)-6-type cytokines. We analysed the melanoma cell line 1286 resistant towards the growth-inhibitory effects of IL-6 and oncostatin M (OSM) to better understand the mechanisms leading to cytokine resistance. In spite of the expression of the receptors gp130 and OSMR at the cell surface, cytokine stimulation led only to a very weak activation of Janus kinase 1, STAT3 and STAT1. We noticed a high-level constitutive expression of SOCS3 (a STAT-inducible feedback inhibitor of Jak/STAT-signalling) that did not further increase after stimulation. Constitutive SOCS3 expression was neither affected by inhibitors of the p38 and Erk MAP kinase pathways nor by the presence of dominant negative STAT3. Importantly, upon suppression of SOCS3 by siRNA, cells became more susceptible towards OSM and IL-6: they showed an increased STAT3 phosphorylation and a dramatically increased STAT1 phosphorylation, reminiscent of the interferon-gamma-like response elicited by IL-6 in $SOCS3^{-/-}$-cells. Moreover, suppression of SOCS3 made the cells sensitive to the anti-proliferative action of IL-6 and OSM, and even control cells not stimulated with cytokine grew slower. Thus, SOCS3 expression apparently confers a growth advantage to this cell line. However, constitutive expression of SOCS3 protein does not seem to be a general phenomenon for melanoma although SOCS3 mRNA, albeit at lower levels than in the cell line mentioned above, could be detected in all ten cell lines which we have examined. There was no global difference between melanoma cells and normal melanocytes. Thus, the melanoma situation seems to differ from other reported malignancies (lung cancer, breast cancer, mesothelioma, hepatocellular carcinoma, squamous cell carcinoma of the head and neck) characterized by methylation silencing of the SOCS3 gene.

08-19/P

IMPLICATIONS OF CCR7- AND CCR10-MEDIATED LYMPH NODE METASTASIS IN TUMORIGENESIS AND IN TRAFFICKING OF TUMOR-INFILTRATING LEUKOCYTES

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Lymph node (LN) metastasis is a poor prognostic indicator in most human cancers. Overexpression of the chemokine receptor, CCR7, by B16 melanoma cells enhances LN metastasis, but the effects of such nodal metastases on tumorigenesis and host immune responses have not been explored. Herein, we demonstrated that both CCR7- and CCR10-transduced B16 melanoma cells show increased metastasis to regional draining LN following skin inoculation. While LN cells did not express significant mRNA for CCL27/CCL28 (ligands for CCR10), CCL27 protein was detected by Western blot in LN lysates, suggesting possible transport of CCL27 from skin to LN via afferent lymphatic vessels. To examine the biologic consequences of nodal metastasis, we injected CCR7-B16 cells or empty vector-transduced B16 cells (pLNCX2-B16) into ear skin or footpads of mice. Following injection of $1x10^5$ cells in ear skin, CCR7-B16 tumor formation approached 100% (vs. <10% for pLNCX2-B16-injected mice). In the footpad, injection of a greater number ($4x10^5$) of CCR7- or pLNCX2-B16 cells resulted in tumor formation of similar size in all mice. Flow cytometric analysis of tumor-infiltrating cells, however, revealed 15-, 7-, and 4-fold reductions in $CD8^+$ T cells, $CD4^+$ T cells, and $B220^+$ cells, respectively, within CCR7-B16 cell tumors compared to pLNCX2-B16 tumors. Whereas adoptively-transferred CFSE-labeled T cells from pmel-1 ($gp100_{25-33}$) TCR transgenic mice proliferated in CCR7-B16-metastatic LN, pmel-1 T cells recovered from the LN of pLNCX2-B16-injected mice did not. Interestingly, trafficking of adoptively transferred, *in vitro*-activated pmel-1 CD8+ T cells to CCR7-B16 (vs. pLNCX2-16) footpad tumors was reduced by ~50%. In summary, we confirm that overexpression of CCR10, like CCR7, is sufficient to dramatically increase LN metastasis. Moreover, CCR7-mediated nodal metastasis is associated with profound changes in tumorigenesis (depending on the site of inoculation) and in trafficking of tumor-infiltrating leukocytes.

08-20/O

EVASION OF HUMAN TUMOR-DERIVED ENDOTHELIAL CELLS FROM THE ANTIANGIOGENIC ACTIVITY OF THE INTERFERON-INDUCIBLE IFI16 GENE.

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The human IFI16 gene is an interferon (IFN)-inducible gene implicated in the regulation of endothelial cell proliferation, tube morphogenesis and proinflammatory activity. Immunohistochemical analysis demonstrated that this gene is highly expressed in endothelial cells and squamous stratified epithelia, in addition to hematopoietic tissues. Overexpression of the IFI16 protein in human umbilical vein endothelial cells (HUVEC) by an adenoviral-derived vector (ADV-IFI16) suppressed tube morphogenesis in vitro, cell cycle progression and cell growth due to induction of apoptosis. These activities appear to be mediated by interaction of IFI16 with both p53 and NF-kappaB complexes as demonstrated by transfection and co-precipitation experiments. Based on this premise, the aim of these studies was to assess the capability of IFI16 to suppress angiogenesis of tumor-derived endothelial cells (TEC) compared to HUVEC. To this purpose we used TEC lines derived from a highly-vascularized human renal carcinoma (Eck25, Eck28), a head/neck solid tumor (HN4), and a breast cell carcinoma (BTEC). These cells characterised by a constant expression of markers of endothelial activation and angiogenesis show enhanced survival and angiogenic properties. To verify how TEC react to IFI16 overexpression, we conducted in vitro experiments by evaluating cell growth, cell-cycle progression, tube morphogenesis and sensitivity to apoptosis in comparison to IFI16-overexpressing HUVEC. The data obtained demonstrate that TEC, upon IFI16 overexpression, maintain

their capability to proliferate, to form tubes when plated onto Matrigel, to progress into the cell cycle and to escape apoptosis. Altogether these results demonstrate that TEC become resistant to the antiangiogenic activity of the IFI16 gene and offer new insights into the strategies adopted by tumors to escape IFN antiangiogenic activity.

08-21/O

GRIMS: CYTOKINE DRIVEN NOVEL TUMOR SUPPRESSORS ON THE HORIZON

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We have been investigating molecular mechanisms involved in Interferon and retinoid induced tumor growth suppression. Using a genetic technique we have identified cellular Genes-associated with Retinoid-Interferon induced Mortality. Earlier, we have characterized a novel gene product GRIM-19 whose experimental inactivation promotes tumor growth. Proapoptotic effects of GRIM-19 are inhibited by viral oncoproteins. GRIM-19 inhibits the oncogenic transcription factor STAT3 for promoting apoptosis. We have recently initiated a study for defining the molecular changes associated with human renal cell carcinoma by comparing the proteomes of normal and tumor kidneys from patients using mass spectrometry. These studies identified a loss of GRIM-19 expression in the tumors. Using a number of human tumor samples we show a loss of GRIM-19 correlates with tumor growth promotion. Employing a surrogate animal model we show that downregulation of GRIM-19 promotes tumor growth in vivo. These effects are in part mediated via an inhibition of anti-STAT3 activity of GRIM-19. This is a first study that shows a mechanism by which STAT3 deregulation occurs in human tumors.

08-22/O

IDENTIFICATION OF A NOVEL GAMMARETROVIRUS IN PROSTATE TUMORS OF PATIENTS HOMOZYGOUS FOR R462Q *RNASEL* VARIANT

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RNase L is an important effector of the innate antiviral response. Mutations or variants that impair function of RNase L, particularly R462Q, have been proposed as susceptibility factors for prostate cancer. Given the role of this gene in viral defense by IFN, we sought to explore the possibility that a viral infection might contribute to prostate cancer in individuals harboring the R462Q variant. A viral detection DNA microarray composed of oligonucleotides corresponding to the most conserved sequences of all known viruses identified the presence of gammaretroviral sequences in cDNA samples from 7 of 11 R462Q-homozygous (QQ) cases, and in 1 of 8 heterozygous (RQ) and homozygous wild-type (RR) cases. An expanded survey of 86 tumors by specific RT-PCR detected the virus in eight of 20 QQ cases (40%), compared to only one sample (1.5%) among 66 RQ and RR cases. The full-length viral genome was cloned and sequenced independently from three positive QQ cases. The virus, named XMRV, is closely related to xenotropic murine leukemia viruses (MuLVs), but its sequence is clearly distinct from all known members of this group. Comparison of *gag* and *pol* sequences from different tumor isolates suggested infection with the same virus in all cases, yet sequence variation was consistent with the infections being independently acquired. Analysis of prostate tissues from XMRV-positive cases by *in situ* hybridization and immunohistochemistry showed that XMRV nucleic acid and protein can be detected in about 1% of stromal cells, predominantly fibroblasts and hematopoietic elements in regions adjacent to the carcinoma. These data provide the first demonstration that xenotropic MuLV-related viruses can produce an authentic human infection, and strongly implicate RNase L activity in the prevention or clearance of infection in vivo. These findings also raise questions about the possible relationship between exogenous infection and cancer development in genetically susceptible individuals.

08-23/P

NUCLEOCYTOPLASMIC SHUTTLING OF PERSISTENTLY ACTIVATED STAT3 ANALYZED IN SINGLE CELLS.

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The Jak/STAT signal transduction pathway plays a central role in inflammation and cancer. Interleukin-6 (IL-6) signals through the cytokine receptor gp130. Upon receptor activation, the transcription factor STAT3 (signal transducer and activator of transcription 3) is activated by tyrosine phosphorylation and translocates into the nucleus where it induces target genes. We have tagged the cytokine IL-6, its receptor gp130 and the transcription factor STAT3 with different fluorescent proteins (yellow fluorescent protein (YFP) or cyan fluorescent protein (CFP)). These fusion proteins enabled us to study ligand-binding and receptor dimerization (Giese et al. (2005) J. Cell. Sci. 118, 5129-40) as well as nuclear translocation of STAT3 (Pranada et al. (2004) J. Biol. Chem. 279, 15114-23) in single cells by the use of confocal laser-scanning microscopy and advanced photo-bleaching techniques. We demonstrated that non-phosphorylated STAT3 constitutively shuttles between the cytoplasm and the nucleus. Persistent phosphorylation of STAT3 is observed in several types of cancer. To analyze persistently activated STAT3 we cotransfected double labelled STAT3-CFP-YFP with v-Src resulting in constitutive tyrosine phosphorylation and nuclear accumulation of the fluorescent transcription factor. By bleaching selectively the YFP moiety of STAT3-CFP-YFP in one cellular compartment and by monitoring the distribution of the CFP and YFP fluorescence over time with high spatial resolution, we show that activated STAT3 constitutively shuttles between cytoplasm and nucleus. Computational evaluation of the data by model-based parameter estimations revealed that activated STAT3 shuttles more rapidly than non-activated STAT3. Since STAT3 is dephosphorylated in the nucleus and phosphorylated by v-Src in the cytoplasm, nucleocytoplasmic shuttling of STAT3 is a prerequisite for constitutive activation. We show that inhibition of nucleocytoplasmic shuttling of persistently activated STAT3 is a new target for the treatment of cancer.

08-24/P

THALIDOMIDE PLUS INTERLEUKIN-2 WITH OR WITHOUT GRANULOCYTE MACROPHAGE-COLONY STIMULATING FACTOR IN PATIENTS WITH METASTATIC RENAL CELL CANCER

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Background: Thalidomide's immunomodulatory and anti-angiogenic effects may augment the antitumor activity of Interleukin-2 (IL-2). The early efficacy and safety findings observed with thalidomide plus low-dose IL-2 therapy for the treatment of metastatic renal cell cancer (MRCC) formed the foundation for further development (Cancer, 2006). Granulocyte Macrophage-Colony-Stimulating Factor (GM-CSF) is an important cytokine for the generation and propagation of antigen presenting cells and for priming cellular immune responses. By adding GM-CSF to the thalidomide + IL-2 regimen, the objective was to improve the antitumor activity. This is an update of response

rate, time to progression (TTP) and overall survival (OS). *Patients and Methods:* Eighty-two patients (pts) with progressive MRCC (74 without prior treatment and 8 with prior treatment) were enrolled on a thalidomide + IL-2 clinical trial. Treatment consisted of an initial dose of thalidomide 200 mg which was escalated to 400 mg after the first 48 hours then given daily without an interruption. A fixed dose of IL-2 at 7 mIU/m^2 with or without GM-CSF at 250 µg/m^2, both given by subcutaneous injection, days 1-5 for the first 4 weeks, followed by a 2 week rest. A course was defined as 6 weeks. *Results:* Forty-four (54%) pts experienced disease control, including 7 (9%) complete responses, 23 (28%) partial responses, and 14 (17%) cases of stable disease. Disease progression was observed in 38 (46%) pts. The median TTP for responding and non-responding pts was 14.4 months (2.9-57.4) and 2.9 months (0.5-12.1), respectively. The median OS for responding and non-responding pts was 28.9+ months (5.9-58+) and 12.1 months (0.7-40.2), respectively. *Conclusion:* Thalidomide + IL-2 with or without GM-CSF can produce active durable responses. There is a significant delay in TTP and OS for responding pts. This data supports the further development of the regimen for this patient population who have been previously treated with molecular targeted agents such as sunitinib and sorafenib without prior immunomodulatory exposure. Furthermore, this data establishes the basis for developing pathobiologic agents in combination with immunotherapy.

08-26/P

MODULATION OF MELANOMA CELL SENSITIVITY TO THE CYTOTOXICITY OF ANTITUMOR DRUGS BY TNF-α AND IFN-α

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Recently we demonstrated the enhancement by the IFN-α and TNF-α of the cytotoxicity and apoptosis inducting activity of doxorubicin and 5-fluorouracil (5-FU) on HeLa cells. This enhancement for 5-FU correlated with the degree of bcl-2 gene expression. Last two years in Russian cancer research center some success has been achieved in the treatment of melanoma patients with combination of chemotherapy by nimustine hydrochloride (ACNU, Nidran), dacarbazine (DTIC), cisplatin and TNF-α (Alnorin). Response rates (CR and PR) was 26.3% (5/19) and 7 patiens had disease stabilization 3-15 months. In this study we investigated the possibility of modulation of nidran and dacarbazine cytotoxicity in vitro on melanoma cells by their combination with TNF-α and IFN-α. In was tested 4 melanoma cell lines. We found very low sensitivity to nidran one of this cell lines (Mel-3) as compared to other three lines (Mel-1, 2 and 4) and TNF-α significantly increased of cytotoxicity of the drug (8 times) toward this melanoma cells. TNF did not modified the sensitivity of other three cell lines. It was shown also different sensitivity of melanoma cells to the cytotoxicity of DTIC. But the cells of other line (Mel-2) had the lowest sensitivity to this drug and TNF elevated the cytotoxicity of DTIC to this cells. We are investigating what kind of differences between each melanoma cells are responsible for their different sensibility to the drugs and the mechanisms of the increasing by TNF the cytotoxicity of this drugs to melanomas.

8-27/P

LEPTIN MRNA EXPRESSION IN ADIPOSE TISSUE INDUCED BY AEROBIC TRAINING IN WALKER 256 TUMOR BEARING RATS

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Cancer related anorexia-cachexia promotes reduction of food intake, depletion of lean and fat mass, increase in plasma concentration of triacylglycerol, cholesterol, and reduction in the concentration of plasma leptin and of its gene expression in the adipose tissue. Such disruptions are aggravated by the increase in the concentration of pro-inflammatory cytokines. Recently, infiltrating macrophages have been described by our group in the adipose tissue of Walker 256 tumor-bearing rats. These cells produce both TNF-α and PGE2, which were suggested to act as mediators in the decrease of leptin production by the adipose tissue. The present investigation sought to effect of aerobic training upon the markers of cancer related anorexia-cachexia and the possible role of infiltrating macrophages in the adipose tissue upon leptin expression.

Male Wistar rats, whose initial weight body ranged from 150 to 200g, were inoculated with Walker 256 tumour cells to ($2x10^7$) in the right flank. The animals were divided in to 6 groups: S (sedentary control), SC (sedentary cancer), SPF (sedentary pair-fed), T (trained control), TC (trained cancer) and TPF (trained pair-fed). The training protocol was carried out for 6 weeks, 5 times a week 60% VO_2max. Food intake was evaluated, as well as the humid weight of the tumor and plasma concentration of triacylglycerol. Immunohistochemistry for RT1B (indicating the MHCII presence) was also carried out confirming the cells to be macrophages. The mRNA expression of leptin in the retroperitoneal adipose tissue was assessed by RT-PCR.

Aerobic training restored the cachexia affected parametric: it decreased the humid weight of the tumor by 88% and reverted the conditions of hypertriglyceridemia of group SC, reducing plasma concentration of TAG in TC group by 68%. There was no quantitative difference between SC and TC concerning macrophage infiltration. Gene expression of leptin was, however, increased in TC compared with SC.

08-28/P

FUNCTIONAL ROLE OF JAK/STAT SIGNALING IN CLASSICAL HODGKIN LYMPHOMA

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Classical Hodgkin lymphoma (cHL) is a distinct malignancy of the immune system. Despite the progress made in the understanding of the biology of cHL, the transforming events in these cells remain to be elucidated. Constitutively activated Jak/STAT pathways are one hallmark of cHL cells contributing to the malignant phenotype (Kube et al. 2001, Skinnider et al. 2002). Production of various cytokines of cHL cells are thought to be activators of the JAK/STAT pathway in an autocrine manner (Skinnider & Mak 2002).

We demonstrated that tyrphostins AG17 and AG490 affect permanently activated STAT3 and STAT6, followed by inhibition of cell proliferation of cHL cell lines and sensitization for apoptosis (Kube et al. 2001; Holtick & Vockerodt et al. 2005). In addition RNA-interference directed against STAT3 shows that this transcription factor is important for cHL cell proliferation (Holtick & Vockerodt et al. 2005, Baus et al. 2006).

Here we show the activation status of Jaks in cHL cells by immunoprecipitation and phosphoprotein immunoblotting analysis. We observed high levels of permanently activated Jak1, -2, -3 and Tyk2 despite high expression of endogenous wt-SOCS3. The inhibition of the Jaks by AG17 is in coincidence with the decrease of STAT3 and STAT6 phosphorylation leading to inhibition of cHL cell proliferation.

To identify genes that are regulated by STAT3 and thereby provide proliferation signals for cHL cells, gene expression profiles were analysed after gene specific „knock-down“ of STAT3 in cHL cells. Known STAT3 target genes were identified as well as hitherto unknown genes which are affected by STAT3 „knock-down“ and could be essential for pathogenesis of the classical Hodgkin lymphoma.

08-29/O

TUMOR SUPPRESSION BY INTERFERON REGULATORY FACTOR-1 RELIES ON DOWN-REGULATION OF CYCLIN D1

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Interferons have been ascribed to mediate antitumor effects. Interferon regulatory factor-1 (IRF-1), which is a major target gene of interferons, was identified as a tumor suppressor. Inactivation of the IRF-1 gene has been observed in certain tumors. Expression of IRF-1 inhibits cell proliferation and reverses the transformed phenotype of different tumor cells in vitro and in vivo. We show that IRF-1 can induce cell cycle arrest and inhibit transformation by influencing the G1/S transition point of the cell cycle. IRF-1 inhibits cyclin D1 expression, which is an important regulator of the G1-S phase transition. Aberrant overexpression of cyclin D1 has been linked to loss of cell cycle control and a wide variety of malignancies. Cyclin D1 reduction was accompanied by a marked decrease in retinoblastoma protein (pRb) phosphorylation on cyclin D/CDK4-specific sites, showing an early negative effect of IRF-1 on G1-S cell cycle transition. The effects of IRF-1 on cyclin D1 expression are mediated by inhibition of MEK-ERK pathway and a transcriptional repression of cyclin D1. Cyclin D1 is the key target of IRF-1 for myc/ras mediated transformation. IRF-1 mediated effects on cell cycle progression were found to be partially overridden by ectopic expression of cyclin D1. Ablation of cyclin D1 by RNA interference experiments prevents transformation by myc/ras expression. Down-regulation of cyclin D1 also prevents tumor growth in nude mice. The data demonstrate that cyclin D1 is a key target for IRF-1 mediated tumor suppressive effects.

08-30/P

IMMUNOMODULATION BY INTERFERON REGULATORY FACTOR-1 IN MOUSE TUMOR MODELS

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The transcription factor Interferon regulatory factor-1 (IRF-1) was originally identified as a protein mediating the effects of the interferon system. Besides its regulatory functions of the cellular response in host defense, e.g. establishing the antiviral state, IRF-1 was found to be a tumor suppressor. To study the tumor-suppressive effects of IRF-1 in vitro and in vivo the hepatocellular carcinoma cell line Hepa1-6 as well as the mammary adenocarcinoma cell line TS/A were stably transfected to express an IRF-1/human estrogen receptor fusion protein, which can be reversibly activated by β-Estradiol. These IRF-1/hER (IH) expressing cells, termed HepaIH and TS/AIH, showed a significant growth arrest in vitro upon activation of IRF-1. Furthermore the reversion of the transformed phenotype was shown by a decrease of anchorage independent growth in soft agar assays. Besides these direct effects on tumor cells IRF-1 is capable of mediating its antitumor effects by inducing a tumor specific immune response in the host. Upon IRF-1 activation an increase of MHC class I expression as well as an induction of various inflammatory chemokines, like Ccl5, Ccl19 and Ccl21 was shown. To evaluate whether a strong tumor specific immune response could be induced via activation of IRF-1 in our tumor models the cells were subcutaneously injected in syngenic mice bearing an implanted β-Estradiol-releasing pellet. A delay in the onset of tumor growth and a reduction in tumor size were measured in most animals, compared to control mice without pellet. Some β-Estradiol-treated mice even completely rejected the primary tumor and were also immune to a rechallenge with the parental tumor cells. An increase in the regulatory T cell compartment was found in mice with aggressivly growing tumors. The influence of β-Estradiol and IRF-1 on this parameter will be presented.

08-31/O

B-CLL CELLS EXPRESS AN ACTIVATED FORM OF NOTCH2 IN A PKC-DEPENDENT MANNER

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We have recently shown that NOTCH2 signaling is involved in the overexpression of CD23 in B-cell chronic lymphocytic leukemia (B-CLL) cells (Hubmann et al., BLOOD 2002 May 15;99(10):3742-7). NOTCH2 plays a determining role in the development/homeostasis of self-reactive CD5+ B-cells, suggesting a potential role for oncogenic NOTCH2 in B-CLL leukemogenesis. Here we show that B-CLL lymphocytes express an activated form of nuclear NOTCH2 (N2IC) irrespective of their prognostic marker profile (ie. IgVH mutational status and CD38 expression). Although the vast majority of cultured B-CLL samples lose their N2IC activity within 24 hours, DNA-bound N2IC complexes could be maintained by TPA, accompanied by an upregulation of CD23 and increased cell-viabilty. These effects are sensitive to the PKC-δ Inhibitor Rottlerin and are in many cases resistant to the γ-secretase inhibitors DAPT and compound E. Since B-CLL cells are locked in an anergic state, we next asked whether NOTCH2 modulates B-cell receptor (BCR) signaling and found that retrovirally transduced N2IC rescues the B-cell line BL41 from surface immunoglobulin M (sIgM) mediated apoptosis, a mechanism thought to prevent the uncontrolled expansion of self-reactive CD5+ B-cells. In summary, our data provide evidence that B-CLL cells express a deregulated form of NOTCH2 which may protect the malignant clone from peripheral negative selection.

08-32/P

THE PROGNOSTIC SIGNIFICANCE OF SUPPRESSOR OF CYTOKINE SIGNALING 2 (SOCS2) IN BREAST CANCER.

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Suppressor of cytokine signaling (SOCS) proteins comprise a family of eight members (SOCS 1-7 and CIS) which have initially been described as negative regulators of cytokine signaling via the Jak/Stat pathway. Recent in vivo and in vitro studies suggest that SOCS proteins are also implicated in cancer. CpG island methylation of SOCS gene loci, and consequently, inhibition of SOCS expression was observed in a variety of solid tumors and hematological malignancies, indicating that SOCS proteins might function as antioncogenes. To evaluate the role of SOCS proteins in mammary carcinoma we have investigated the mRNA expression levels of SOCS1, SOCS2, SOCS3 and CIS in a representative collection of 89 primary breast carcinomas.
We observed a negative association of SOCS1 with PR status and a trend for patients with high SOCS1 expression to worse prognosis. SOCS2 expression inversely correlated with histopathological grade and ER positive tumors exhibited higher SOCS2 levels. Patients with high SOCS2 expression lived significantly longer (108.7 vs. 77.7 months; P = 0.015) and after adjusting for clinical stage, tumor grade and lymph-node status, SOCS2 proved to be an independent predictor for good prognosis (HR = 0.45, 95%CI 0.22 – 0.9, P = 0.024). CIS expression levels positively correlated with SOCS2 expression and negatively correlated with SOCS3. However, no prognostic significance was observed for CIS and SOCS3.
This is the first report on the prognostic significance of SOCS2 in breast cancer. Taken together our results suggest that high SOCS2 expression is a feature of highly differentiated and less malignant tumors. It remains to be shown if high SOCS2 per se is causative for the differences in tumor differentiation and prolonged overall survival and whether the favorable prognostic characteristics of ER positive tumors can be attributed to some extent to higher SOCS2 expression.

08-33/P

EFFECT OF CONJUGATED LINOLEIC ACID (CLA) AND SUNFLOWER OIL ON HEPATIC GENE EXPRESSION OF TNF-α IN WALKER 256 TUMOUR CACHEXIA

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Introduction: Cachexia is a syndrome present in 50% of cancer patients associated with changes in metabolism. The changes in hormones and cytokines profile during the syndrome induce an inflammatory chronically response in effected organism. Therefore, complex interactions between cytokines and growth factors are responsible for the many effects related to the syndrome, and TNF-α plays an important role in the development of cachexia. Conjugated Linoleic Acid (CLA) consists on a group of 28 positional and geometric isomers of linoleic acid, of which the most studied are T-10, C-12 and C-9, T-11. Their biological effects are associated with anti carcinogenesis, diabetes and obesity. The role of CLA in the production of cytokines has been addressed and there are evidences that CLA diminishes TNF- α concentration in many inflammatory states. The purpose of this study was to investigate the relationship between CLA supplementation and cytokines in the liver of tumour-bearing rats. *Material and methods:* Cachectic rats were supplemented with CLA or sunflower oil for 14 days. Hepatic TNF- α gene expression was measured by PCR. *Results:* PCR analysis showed no significant differences of CLA in relation to the sunflower group and the control group, however there was a trend in the CLA group for reduced TNF-α gene expression. Statistical difference between sunflower and control group was found as sunflower showed significantly lower TNF-α gene expression in relation to control. *Conclusion:* Sunflower oil supplementation resulted in a further reduction of TNF- α gene expression than CLA supplementation.

08-34/P

ACTIVATION OF CD95L FUSION PROTEIN PRODRUGS BY TUMOR ASSOCIATED PROTEASES

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Activation of the death receptor CD95 induces apoptosis in a variety of tumor cells. However, a use of soluble agonists of CD95 in tumor therapy is prevented due to high systemic toxicity of such reagents. To achieve tumor cell-restricted activation of CD95, we developed a CD95L fusion protein format in which CD95L activity is only unmasked upon antibody mediated binding to tumor cells and subsequent processing by tumor associated proteases, such as matrix metalloprotease 2 (MMP2) and urokinase plasminogen activator (uPA). These CD95L prodrug proteins are built of a carboxy-terminal bipartite immunoglobulin single chain variable fragment (scFv)-CD95L domain, which targets the CD95L moiety to the tumor, and an amino-terminal inhibitor domain composed of the trimerized extracellular domain of CD95. Importantly, the inhibitor domain and the scFv-CD95L domain are separated by a MMP2 or uPA sensitive linker. On target negative, but MMP2 and uPA expressing HT1080 tumor cells, the CD95L prodrugs were virtually inactive. In contrast, on target antigen expressing HT1080 cells, the CD95L prodrugs showed an apoptotic activity comparable to soluble CD95L artificially activated by secondary crosslinking. CD95 activation by the CD95L prodrugs was preceded by prodrug processing. Apoptosis was blocked by inhibitors of MMP2 or uPA, by antibodies recognizing the targeted cell surface antigen or the CD95L moiety of the prodrugs. In a tumor model, local application of the prodrug reduced the growth of target antigen expressing, but not target antigen negative tumor cells, verifying targeted activation and anti-tumoral action in vivo. Taken together, CD95 activation by the prodrugs described here is a two step process requiring cell surface antigen binding and subsequent processing by endogenous proteases. A tumor localized action of the CD95L is therefore ensured by tumor restricted expression of both, the target antigen and prodrug processing proteases.

08-35/P

PATTERN OF CYTOKINE PRODUCTION IN CELL LINES DERIVED FROM PRIMARY AND METASTATIC HUMAN COLORECTAL CARCINOMAS

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The aim of the present study was to compare cytokine secretion profiles (TNF, IL-10, IL-6, IL-8, RANTES/Regulated upon Activation, Normal T-cell Expressed and Secreted, TGF-beta1/transforming growth factor-beta1) in human primary and metastatic colorectal adenocarcinoma cells, as well as normal colonic epithelial cells. We also aimed to compare cytokine response of tumor cells to SDF-1alpha (stromal cell-derived factor-1alpha) treatment. Cytokine secretions were investigated in a primary (Isreco-1) and two metastatic tumor cell lines (liver metastasis: Isreco-2, peritoneal metastasis: Isreco-3) derived from the same patient, and in a normal colonic epithelial cell line. Constitutive cytokine productions were determined in culture supernatants collected after a 24 h-incubation. For SDF-stimulation, cells were treated with 100 ng/ml of SDF-1alpha for 24 h. Cytokine levels in cell culture supernatants were measured by ELISA. Our results showed that metastatic tumor cell lines secreted significantly higher levels of IL-8 ($p<0.01$) and TGF-beta1 ($p<0.005$) than the primary tumor-derived cell line. TGF-beta1 secretion could not be detected in Isreco-1 cells. IL-8 production was significantly higher in normal colonic epithelial cells than in the tumor cell lines ($p<0.001$). Low levels of RANTES secretion were characteristic to the metastatic tumor cell lines and the normal colonic epithelial cell line. Isreco-3 and Isreco-1 cells produced low amounts of IL-6 and IL-10, respectively ($p<0.001$). The investigated cell lines secreted no TNF. SDF-1alpha induced a two-fold increase in IL-8 production in Isreco-1 cells ($p<0.001$) that could not be inhibited by pertussis toxin. Our work demonstrates distinct patterns of constitutive cytokine production in human primary and metastatic colorectal tumors. We provide evidence for differences between primary and metastatic colorectal cancer cells in cellular response to SDF-1alpha. Our findings support the hypothesis that expression of SDF-1alpha in the local tumor microenvironment may contribute to the IL-8 production by colorectal cancer cells. (OTKA grant no. T 046633)

08-36/P

THE M20 IL-1 INHIBITOR PREVENTS GVHD AND DOES NOT AFFECT GVL

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GVHD is the major complication in immunosupressed recipients of allogeneic Bone Marrow Transplantation (BMT). One of the main goals for successful transplantation of recent studies is to control GVHD, without affecting chimerism and Graft versus Leukemia (GVL) effect. The GVL effect on residual leukemia cells is very important, in order to avoid relapse in the tumor bearing recipients. Inflammatory cytokines, such as IL-1ß and TNFα, play an important role in GVHD initiation and augmentation of the disease.

The aim of our research was to study the effect of M20 IL-1 Inhibitor treatment on GVHD development and on the beneficial GVL effect in an in vivo model. The rational was that treatment with the M20 IL-1 Inhibitor may control and minimize the effects of IL-1ß and TNFα and may prevent the development of aggressive GVHD following BMT.

Treatment by M20 IL-1 Inhibitor significantly delayed the appearance of GVHD and death from GVHD - by 38d all control mice were dead, while 40 -50% of M20 injected mice were still alive. Our results show that treatment of tumor bearing mice by M20 IL-1 Inhibitor, did neither damage the beneficial GVL effect, nor affect the percent of chimerism. 90% of the treated mice did not develop leukemia as was shown by adoptive transfer experiments.

Our results showed indeed a significant decrease in the levels of inflammatory cytokines (TNFα, IL-1ß, IL-6, IFNγ) and an increase in the anti inflammatory IL-10, following treatment by M20 IL-1 Inhibitor. In conclusion, treatment by the M20 IL-1 Inhibitor reduces GVHD after BMT without damaging the beneficial GVL effect or the percent of chimerism, through a reduction in inflammatory cytokines. Therefore the M20 IL-1 Inhibitor may be regarded as a future clinical adjusts to BMT, avoiding GVHD.

08-37/O

FLUDARABINE AND ITS IMMUNOMODULATORY EFFECT ON T-CELLS IN B-CLL – MORE THAN JUST AN ANTIMETABOLITE?

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Fludarabine is a purine analogue antimetabolite and constitutes a major column of most currently used therapy regimens in B-cell chronic lymphatic leukaemia (B-CLL). Increased incidence of infectious complications and autoimmune phenomena in B-CLL patients following fludarabine therapy are indicative of an altered immune system. Since autoimmunity and anti-tumor immunity are known to be closely linked mechanisms, our goal was to elucidate the effects of fludarabine on T-cells, key players in the immunologic defense against tumor cells.
In vitro studies of peripheral blood mononuclear cells (PBMC) of B-CLL patients with fludarabine revealed highly preferential cytotoxic activity to CD8+ as compared to CD4+ T-cells. These findings were corroborated by quantitative analysis of the respective T-cell populations from patients receiving firstline fludarabine-based therapy. Changes within the T-cell compartment were rapid since they were detectable immediately after the first cycle.
PBMC from CLL patients collected prior to or after initial fludarabine-based chemotherapy were also tested for their capacity to proliferate upon stimulation by allogeneic dendritic cells. Comparison of the proliferative response of T-cell populations revealed a significant increase in proliferation of the CD8+ compartment of both *in vitro* and *in vivo* fludarabine-treated PBMC when compared to untreated PBMC from chemonaive patients.
Although CD8+ T-cell numbers are substantially reduced by fludarabine *in vitro* and *in vivo*, we demonstrate that the remaining cells are highly responsive to antigenic stimuli. This could be interpreted as a mild form of "immune-priming" in the sense of immune reorientation as has been reported for autoimmune disease after high dose chemotherapy and autologous stem cell transplantation.
Our data provide *in vitro* and *in vivo* evidence for preferential killing of CD8+ T-cells by fludarabine accompanied by an increase in the proliferative capacity of the surviving CD8+ T-cells. These findings support the concept of an immunotherapeutic role for fludarabine beside its known chemotherapeutic action.