activated in melanoma cells and is up-regulated after contact with B-1 cells; (2) treatment with either Gö6976 or PD98059 during co-cultivation of B-1 with melanoma cells inhibited the increased metastatic potential induced by B-1 cells; (3) previous treatment of melanoma cells with Gö6976 but not with PD98059 before their co-cultivation with B-1 cells inhibited this effect, and (4) inhibition of ERK activity in B1 cells prevents their capacity to increase the metastatic potential of melanoma cells.
Conclusion: ERK activity in B-1 cells is important for increasing the metastatic potential of murine melanoma cells.
Financial support: FAPESP.

344 POSTER

Effects of cancer cachexia on heart function

J. Springer[1], S. Palus[1], A. Hilmann[1], M. Rauchhaus[1], V.C. Flach[1], K. Hartmann[1], T. Braun[1], K. Schmidt[2], J.M. Argiles[2], S.D. Anker[1]. *[1]Charité Campus Virchow, Divsion of Applied Cachexia Research, Berlin, Germany; [2]Universitat de Barcelona, Department de Bioquimica i Biologia Molecular, Barcelona, Spain*

Background: Cachexia is a very common co-morbidity in cancer patients which drastically reduces quality of life and survival. These patients also develop shortness of breath of unknown reason. Here we assessed the cardiac function in terminally ill rats with cancer.
Methods: Rats (weight approx. 195 g) were inoculated intra-peritoneally with 10^8 AH-130 hepatoma cells (n = 15) or saline (sham, n = 8). Heart function (high resolution echocardiography) was assessed before inoculation and on day 11 of the 14-day protocol. Weight and body composition (NMR-scan) were assessed on day 0 and day 14 after sacrifice (without tumour).
Results: Animals with cancer displayed severe cachexia (164±5 g vs sham: 258±5 g, $p < 0.0001$) and lost fat (−9.1±0.9 g vs sham 9.2±1.1 g, $p < 0.0001$) and lean tissue (−19.6±3.3 g vs sham 42.2±2.6 g, $p < 0.0001$). Heart weight was reduced in cancer animals (515.5±15.8 mg vs 751.9±9.0 mg; $p < 0.0001$). Heart function was significantly impaired in tumour rats compared to sham and compared to pre-inoculation (see table).

	Tumor		Sham	
	Day 0	Day 11	Day 0	Day 11
LV ejection fraction (%)	79±3##	64±2***	78±3	81±2
Fractional shortening (%)	50±3##	36±2*	48±3	51±3
LVD dia (mm)	6.47±0.11	6.28±0.25	6.24±0.25	6.38±0.13
LVD sys (mm)	3.27±0.19##	4.06±0.23*	3.23±0.11	3.12±0.21
PWT dia (μm)	1588±61	1452±78*	1566±59	1732±74
PWT sys (μm)	2598±126###	2032±110***	2508±159	2885±152
LV Vol dia	273±11	251±13	256±28	280±15
LV Vol sys	65±8##	110±6*	83±10	89±5
LVSV (μl)	208±11	141±11*	172±21	190±14
LVmass (mg)	451±16	413±12***	437±25	529±12

LV: left ventricular, D: diameter, dis: diastole, sys: systole, PWT: posterior wall thickness, Vol: volume, SV: stroke volume. *: $p < 0.05$, ***$p < 0.001$ tumour vs sham (t-test). #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ tumour day 0 vs tumour day 11 (paired t-test).

Conclusion: Cardiac function is severely impaired in terminally ill rats with cancer cachexia. The nature of these impairments is comparable to processes in chronic heart failure and hence ACE-inhibition and beta blocker approaches in cancer cachexia may be useful.

345 POSTER

A novel statistical method for analyzing standard tumor growth curves

C. Murayama[1], M. Isozaki[1], A. Shintani[2], A. Kamijo[3], Y. Maeda[3], Y. Ohizumi[4], H. Kobayashi[1], S. Sadahiro[3]. *[1]Tokai University, Clinical Pharmacology, Isehara, Japan; [2]Vanderbilt University, Biostatistics, Nashville, USA; [3]Tokai University, Surgery, Isehara, Japan; [4]Tokai University, Radiation Oncology, Isehara, Japan*

We previously reported that combining preoperative chemo-radiotherapy with oral UFT and intraoperative electron-radiation therapy (IORT) significantly reduced local recurrence and improved prognosis for clinical T3−4Nx rectal cancer (Sadahiro et al., EJSO 2004, Hepato-gastroenterology 2007). A new oral fluoropyrimidine (S-1) has recently shown promise in treating metastatic colorectal cancer with a response rate of 35%. We now present preclinical results concerning the combined therapeutic effect of S-1 and radiotherapy evaluated by standard growth curve analysis methods in a murine model using human colon xenografts. We studied four groups (Control, S-1 only, Radiotherapy only, and S-1 plus Radiotherapy) over a period of 43 days to assess treatment effect on tumor growth time (TGT). Table shows synergistic and radio-modifying measurements of treatment effect when comparing TGT for 3-fold and 5-fold increases in tumor volume. In both cases, therapeutic benefit was observed by combination of S-1 and radiotherapy treatments. Our results showed a synergistic effect for 3-fold growth TGT, but only an additive effect at 5-fold growth TGT assessment. This discrepancy illustrates a fundamental problem with growth curve analysis methods in that results vary depending upon the chosen tumor magnification level. Tumor control assay methods (e.g. TCD_{50}) are more reliable, but the increased time required for data collection and associated costs are often prohibitive. Given these limitations, we are developing novel statistical methods for assessment of treatment effect using all measurements collected for standard growth curve analysis procedures. In this result, our novel statistical approach constructed from the bootstrap method and R for analysis of standard tumor growth curve data points supported an additive effect at 5-fold growth TGT assessment.
Conclusion: In preclinical studies, novel statistical methods using all data points are robust and eliminate problems associated with choosing just one tumor magnification level.

	Synergy ratio		Radio modifying factor		Chemo modifying factor	
	mean	95%CI	mean	95%CI	mean	95%CI
3x#	1.33	1.08−1.58	2.13	1.28−2.98	1.47	1.12−1.82
5x##	1.06	0.73−1.39	1.19	0.18−2.19	1.09	0.6−1.58

#TGT = time required for the initial tumor volumes to grow to 3-times original size. ##TGT = time required for the initial tumor volumes to grow to 5-times original size.

346 POSTER

The role of N-myc downstream regulated gene 1 (NDRG1) in human prostate cancer cells

F. Fares, T. Chosha. *Carmel Medical Center-Technion Faculty of Medicine, Molecular Genetics, Haifa, Israel*

Background: NDRG1 is a member of the N-myc down-regulated gene family, which belongs to the α/β hydrolase superfamily. This gene was previously identified as an up-regulator of cellular differentiation, and was found to be down regulated during colon, breast and prostate tumor progression. NDRG1 is regulated by several factors including androgen, p53 and N-myc. The biological function of NDRG1 and the physiological relevance of its role in the cellular context remain elusive. To clarify the functional role of NDRG1 in prostate cancer cells, we over-expressed the NDRG1 in three prostate cancer cell lines with different differentiation levels.
Methods: Normal trophoblast cells were used in order to clone the NDRG1 cDNA. The coding sequence of NDRG1 was amplified by PCR and cloned into the eukaryotic expression vector, PCDNA 3.1. The plasmid was transfected into human prostate cancer cell lines; LNCaP (well differentiated), DU145 (moderately differentiated) and PC3 (poorly differentiated). Expression of NDRG1 was detected in the transcription and translation levels. The differentiation factors p21 and cytokeratin 8/18 were detected by western blotting.
Results: All the three cell lines expressed NDRG1. High levels were detected in poorly and moderately differentiated whereas low levels were found in the well differentiated cell line.
The cDNAs from all cell lines were used as templates for amplification of the NDRG1 by PCR. Sequencing results of NDRG1 showed that there are no mutations in the coding sequence of the gene.
To investigate the role of NDRG1 in the prostate cancer cell lines, the cells were transfected with molk vector and with NDRG1 expression plasmid. The expression of NDRG1 in the cloned cells was tested by RT-PCR and further confirmed by Western blot.
The NDRG1 transfected cells were examined for the rate of proliferation in comparison to molk transfections. The results revealed that there were no significant differences from the control cells. However, it was found that over-expression of NDRG1 up-regulated p21 and c8/18, therefore causing or progressing differentiation in cancer cell lines.
Conclusions: In this study we showed that human prostate cancer cells expressed basal levels of NDRG1 and that there are no mutations in the coding sequence of the gene. The NDRG1 CDNA was cloned into eukaryotic expression vector. Over expression of NDRG1 by transfection studies into human prostate cancer cells induced the expression of the differentiation markers; p21 and c8/18. However overexpression of NDRG1 did not influence the proliferation rate of the cells. Further studies are needed in order to clarify the role of NDRG1 in prostate cancer cells.