with TRAIL in combination with subtoxic concentrations of a synthetic methyl jasmonate derivative (J7) sensitized TRAIL-mediated apoptosis in HepG2 cells.
Material and Methods: HepG2 cells were incubated with TRAIL, J7, caspases inhibitor and ROS scavenger NAC. Growth activity was assessed using a MTT assay, the effects of apoptosis induction by TRAIL and J7, and the regulatory mechanisms were studied by DAPI staining, flow cytometry, caspase assay kits and Western blot analyses.
Results: Combined treatment with J7 and TRAIL induced rapid apoptosis in TRAIL-resistant HepG2 cells and effectively induced Bid cleavage and down-regulation of IAP family proteins, leading to the activation of caspases, and cleavage of poly(ADP-ribose) polymerase. The reactive oxygen species (ROS) were significantly up-regulated in cells following exposure to TRAIL and J7, indeed, the pre-treatment of ROS scavenger by NAC attenuated J7 plus TRAIL-induced apoptosis. Furthermore, pretreatment an ERK-specific inhibitor, PD98059, and a p38-specific inhibitor, SB203580, showed increased sub-G1 phase DNA content and activation of caspases in TRAIL and J7 induced apoptosis.
Conclusions: The use of TRAIL in combination with subtoxic doses of J7 may provide an effective therapeutic strategy for safely treating some TRAIL-resistant hepatoma cancer cells.

492 Role of Bcl-2 family members on cold stress-induced cell-death in multidrug resistant leukemic cells

M. Lencina[1], D. Cerezo[1], A.J. Ruiz-Alcaraz[1], P. Garcia-Peñarrubia[1], E. Martin-Orozco[1]. [1]*University of Murcia, Biochemistry and Molecular Biology B and Immunology, Murcia, Spain*

The family of Bcl-2 proteins plays a key role as regulators of apoptosis. In healthy cells these proteins are maintained in a basal latent state, but in response to apoptotic stimuli they become activated through a variety of mechanisms involving post-translational modification or transcriptional activation. Here, we have undertaken the study of Bcl-2 family members involved on cold stress-induced cell death on resistant leukemic cells. Thus, we have focussed on Bcl-xL and Bcl-2, as anti-apoptotic members and Bax (belonging to the multi-domain or Bax subfamily) and Bad (belonging to the BH3-only subfamily) as pro-apoptotic members. By using western-blot techniques, we have found that acquisition of MDR phenotype by leukemic cells is accompanied by changes on the expression of Bcl-2 family members. Specifically, Bcl-xL levels diminish on MDR versus sensitive cells, while Bcl-2 protein expression increases on resistant cells in comparison to their sensitive counterparts. Furthermore, silencing experiments demonstrate the protective role of Bcl-xL on leukemic cells. Additionally, alterations in subcellular location have been observed for Bcl-2 proteins. These events lead to an increase on the outer mitochondrial membrane permeability followed by the translocation of cytochrome c and other apoptogenic factors. Together, these findings demonstrate that during the process of drug resistance, leukemic cells undergo alterations on Bcl-2 family members expression, among others. This fact could explain viability differences on sensitive *versus* resistant leukemic cells under stress conditions.

493 Gene expression profiles in resveratrol-induced cell death in acute promyelocytic leukemia cells

Z. Cakir[1], G. Can[1], G. Saydam[2], F. Sahin[2], Y. Baran[1]. [1]*Izmir Institute of Technology, Molecular Biology And Genetics, Izmir, Turkey*, [2]*Ege University, Hematology, Izmir, Turkey*

Background: Resveratrol, (3,5,4′-trans-trihydroxystilbene) is a natural product found in plant constitutes such as grape skin. It has shown significant cytotoxic and apoptotic effects on various types of cancer cells with no harm to normal healthy cells. Resveratol inhibits tumour initiation, promotion, and progression. However the mechanisms of resveratrol induced cell death is not well-known. We have previously showed cytotoxic and apoptotic effects of resveratrol on acute premyelocytic leukemia (APL) cells. APL is a hematological malignancy characterized by increased number of clonal population of hematopoietic progenitor cells.
Aims: In this study, we aimed to show molecular mechanisms of resveratrol induced apoptosis by examining the changes in expression profiles of human cancer signalling pathway genes in HL60 APL cells exposed to resveratrol.
Methods: Effective concentrations of resveratrol on HL60 cells were determined by XTT cell proliferation assay. Total RNAs were isolated from HL60 cells exposed to 10 and 50 μM resveratrol, converted to cDNA, and changes in expression levels of 84 genes involved in apoptosis, metastasis, angiogenesis, invasion, adhesion, tumour supressors, and transcription factors by PCR array.
Results: Resveratrol has shown antiproliferative effect on HL60 cells in a dose dependent manner. There were 15 and 45% decreases in cell proliferation in response to 10 and 50 μM resveratrol in HL60 cells as compared to untreated controls, PCR array results demonstrated that there were more than 3-fold increase in expression levels of 24 and 36 genes in HL60 cells treated with the same concentrations of resveratrol as compared to control, respectively. On the other hand, there were 6 genes whose expression levels were decreased more than 4-fold in response to 10 and 50 μM resveratrol, respectively. We observed significant increases in expression levels of p53 in a dose-dependent manner which is not detected in control group. The most significant increases were observed in apoptotic genes (e.g. Bax, Tert), and decreases were observed in antiapoptotic genes (e.g. Bcl-2). Although, there were increases in expression levels of certain growth factor, antiapoptotic and metastatic genes, our whole data demonstrated that these concentrations of resveratrol inhibited cell growth and induced apoptosis in HL60 cells.
Summary and Conclusions: In this study the mechanisms of resveratrol-induced apoptosis were demonstrated in detail. This *in vitro* data by being supported with clinical data may open the way of the potential use of resveratrol for acute premyelocytic leukemia patients.
This study was supported by Turkish Society of Hematology.

494 Clinical significance of lymphangiogenesis in molecular types of invasive breast cancer

M. Raica[1], P.N. Gaje[1], A.R. Ceausu[1], A.M. Cimpean[1]. [1]*Victor Babes University of Medicine and Pharmacy, Histology, Timisoara, Romania*

Background: In last years, there were accumulated a lot of data that support the crucial role of lymphangiogenesis in the development of lymph node metastases (LNM) in breast cancer. The expression of lymphangiogenic molecules and lymphatic microvessel density (LMVD) in molecular types of breast cancer were less investigated. In the present work we investigated the relationships between LMVD, VEGF-C and VEGFR-3 expression, molecular types, and clinico-pathological factors of prognosis in breast cancer.
Material and Methods: There were studied 106 patients with invasive breast cancer and 49 had LNM. Cases were stratified according the molecular classification, based on the immunohistochemical expression of estrogen receptor, progesterone receptor, HER2 protein, cytokeratin 5, p53, Bcl-2 and epidermal growth factor receptor. Additional slides were stained for VEGF-C, VEGFR-3, and D2−40. VEGF-C and VEGFR-3 were scored using an intensity/percent-based system, and LMVD was estimated using Weidner's method. Lymphatic vessels (LVs) were counted in the peri- and intratumoural areas.
Results: VEGF-C was strong or moderate positive in 64 cases, and weak and negative in 42 cases. Significant correlation was found with LNM and grade of the tumour (G), but not with the molecular type of carcinoma, excepting for the HER2 type. LMVD was evaluated in the peri- (LVs found in all the cases, range 0.6 to 15.3/×200) and intratumoural (LVs found in 57 cases, range 0 to 7.6/×200) area. Intratumoural but not peritumoural LMVD correlated with LNM, VEGF-C. No correlation was found between LMVD and tumour stage and grade. Higher LMVD were found in HER2 and Luminal B types, and lowest in basal-like carcinoma. Overexpression of VEGF-C was associated with VEGFR-3 expression in the endothelium. Additionally, VEGFR-3 was expressed by tumour cells in 52% of the cases and in 26% by stromal cells. A strong correlation was found between VEGFR-3/VEGF-C expression and LNM.
Conclusion: Our data showed that a differential expression of VEGF-C and VEGFR-3, and different values of LMVD are found in the molecular types of breast cancer. This suggests the prognostic role of these markers and indicates their potential use as targets for antitumour therapy.

495 Cancer-associated adipocytes exhibit an activated phenotype and contribute to early breast cancer invasion in vitro and in vivo

B. Dirat[1], L. Bochet[1], M. Dabek[1], D. Daviaud[2], S. Dauvillier[1], S. Le Gonidec[2], G. Escourrou[2], P. Valet[2], C. Muller[1]. [1]*Institute of Pharmacology and Structural Biology, Cancer Biology, Toulouse, France,* [2]*INSERM U858 I2MR, Team AdipOlab, Toulouse, France*

Early local tumour invasion in breast cancer results in immediate proximity of cancer cells to mature adipocytes, but the role of these cells in tumour progression has been poorly studied. Using a 2D co-culture system, we demonstrate that tumour cells co-cultivated with mature adipocytes exhibit an increase in invasive capacities *in vitro* and *in vivo*. In turn, adipocytes cultivated with cancer cells exhibit delipidation and decreased adipocyte markers associated to the occurrence of an activated phenotype marked by over-expression of proteases, including MMP11, and pro-inflammatory cytokines (IL-6, IL-1b), IL-6 playing a key role in the adipocyte-dependent pro-invasive effect. The phenotypic changes of adipocytes are observed in human breast tumours using immunohistochemistry and qPCR in a series of adipocytes isolated from adipose tissues obtained either from tumourectomy or mammoplasty. The tumours of larger size and/or with lymph nodes involvement exhibit the higher levels of IL-6 in cancer-associated adipocytes. This new bidirectional crosstalk between adipocytes and breast tumour cells might explain the poor prognosis of breast cancer in obese women that frequently exhibit extended tumours at diagnosis.