Conclusions: We explored transcriptomic data from three different microenvironments involved in colorectal cancer progression. Levels of stromal chemokines TGFB2 AND NTF3 seems to be important in the progression of colorectal carcinoma and depicted a higher proangiogenic microenvironment in liver metastasis.

579 Cytotoxic effects of resveratrol on imatinib sensitive and resistant K562 chronic myeloid leukemia cells

G. Can[1], Y. Baran[1]. [1]*Izmir Institute of Technology, Molecular Biology and Genetics, Izmir, Turkey*

Background: Chronic myelogenous leukemia is a hematological malignancy resulting from translocation between chromosome 9 and 22 that generates BCR/ABL protein. BCR/ABL protein has constitutive tyrosine kinase activity involved in cell growth, differentiation and evasion of apoptosis. Imatinib is the first target specific agent that specifically binds to the ATP binding pocket of the BCR/ABL. However, resistance to imatinib is the major problem of CML patients. Resveratrol is a naturally produced phytoalexin, mostly synthesized in red gapes. It has anti-oxidant, cardioprotective, anti-inflammatory, and anti-tumour activities.
Aims: In this study, we aimed to examine apoptotic effects of resveratrol on imatinib sensitive and resistant K562 cells and determine the mechanisms of resveratrol-regulated cell death.
Methods: Antiproliferative effects of resveratrol were determined by XTT cell proliferation assay. Apoptotic effects of resveratrol on K562 and K562/IMA-3 cells were determined through changes in caspase-3 enzyme activity, loss of mitochondrial membrane potential (MMP), and apoptosis by caspase-3 colorimetric assay kit, JC-1 MMP kit, and Annexin V-FITC, respectively. On the other hand, expression profiles of BCR/ABL in response to resveratrol were analysed by RT-PCR.
Results: IC50 values (drug concentration that inhibit cell proliferation 50% comparing to untreated controls) of resveratrol were calculated as 85 and 122 μM in K562 and K562/IMA-3 cells, respectively. There were 1.91, 7.42 and 14.73-fold increases in loss of MMP in 10, 50, and 100 μM resveratrol applied K562 cells. The same concentrations of resveratrol resulted in 2.21, 3.30, and 7.65-fold increases in loss of MMP in K562/IMA-3 cells. Caspase-3 enzyme activity results showed that there were 1.04, 2.77, 4.8-fold increases in K562 and 1.02, 1.41, 3.46-fold in K562/IMA-3 cells increases in response to the same concentrations of resveratrol, respectively. There were 4 and 3.7-fold increases in apoptotic K562 and K562/IMA-3 cell populations in response to 100 μM resveratrol as compared to their untreated controls. RT-PCR results showed for the first time that resveratrol downregulated expression levels of oncogenic BCR/ABL gene in a dose-dependent manner in both imatinib sensitive and resistant K562 cells.
Conclusion: The results of this study may suggest potential use of resveratrol in both responding chronic phase CML and from patients with primary and/or acquired resistance to imatinib.

580 Isolation and characterization of a new human lung cancer cell line derived from a metastatic limph node

A.M. Sánchez-López[1], M.I. Aroca Siendones[1], P. de la Cueva[1], H. Navarro[1], R. Gomez[1], M.J. Cuevas[2], M. Cava[2], J. Esquivias[2], A.I. Sáez[1]. [1]*Tumour Banks of the Andalusia Network, Coordination office, Granada, Spain,* [2]*San Cecilio Hospital, Anatomical pathology, Granada, Spain*

Background: Lung cancer is currently the most frequently diagnosed cancer and the most common cause of cancer mortality in males (American Cancer Society, 2007).
Low passage tumour cell lines are a powerful model to cancer investigation (Blanco-Aparicio et al, 2005; Moneo et al, 2007). Specifically lung cancer cell lines serve as an invaluable tool for medical science (Gazdar et al, 2010) because the most part of these types of cell lines are very representative of the tumour specimen from which they are derived (Wistuba et al, 1999).We report the isolation and characterization of a new human low passage lung tumour cell culture, from a lung cancer resistant to therapies.
Material and Methods: Tumoural fresh sample was obtained from Tumour Bank of San Cecilio Hospital belong to the Tumour Banks of the Andalusia Network, by axilar limph node biopsy from 54-year-old men diagnosed with a metastasic tumour originated by primary lung tumour resistant to chemotherapy and radiotherapy.
Sample was processed and placed in culture with specific media until their generation was achieved. The culture was analysed to determine growth parameters by MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay and was characterized in various phases of generation for lineage and cycle markers by immunohistochemistry and apoptosis cells rate by ISOL® (in situ oligo ligation). Morever, telomerase activity was quantified by qPCR using telomerase repeat amplification protocol and we performed cytogenetic analysis by G-bands and spectral kariotyping.
Results: After incubation for a few weeks, cells grew as firm adherent monolayer with polygonal and epithelial-like morphology, some round and floating cells, and a few multinucleated cells and the doubling time was 48 hours in complete media with 10% FBS. Moreover cells grew in culture media with low serum concentration with 1% FBS.
Immunohistochemistry study showed positive staining for vimentin, cytokeratins, CD44, EGFR, p53, Ki67, Ciclin D1 and B-catenin. Non apoptotic cells were found in any passage. The study for telomerase activity was positive and the cytogenetic in early passage, showed recurrent chromosomal aberrations in lung cancer, with simple and complex structural rearrangements, which was confirmed in later passage.
Conclusions: These results indicate that the features of this cell line are closely similar with a lung malignant tumour, and therefore a good tool for cancer investigation.

581 Characterization of anaplastic astrocytoma xenografts derived from cancer stem cells in nude mice

O. Zarnescu[1], F.M. Brehar[2], C. Bleotu[3], R.M. Gorgan[2]. [1]*University of Bucharest Faculty of Biology, Animal Biology, Bucharest, Romania,* [2]*Clinical Hospital "Bagdasar-Arseni", Neurosurgery, Bucharest, Romania,* [3]*"Stefan S Nicolau" Institute of Virology, Antiviral therapy, Bucharest, Romania*

Background: The cancer stem cell hypothesis proposes that tumours contain a small subset of cancer cells, the cancer stem cells, which constitute a reservoir of self-sustaining cells with the exclusive ability to self-renew and maintain the tumour. The aims of the present study were to investigated the morphology and growing pattern of xenografts derived from cancer stem cells isolated from anaplastic astrocytoma.
Material and Methods: Tumours from a patient with grade III glioma (anaplastic astrocytoma) were mechanically and enzymaticaly dissociated and growth in neural stem cell expansion medium to generate neurospheres. The *in vivo* tumourigenic potential of tumour spheres was assayed by intracranial injection of 2 X 10^5 glioblastoma-derived stem cells into the right striatum of Balb/c nude mice. Tumour growth was monitored *in vivo* by serially sectioning the xenograft brains at two and three months postinjection. Double immunofluorescence for human nestin and PCNA were performed.
Results: Astrocytoma xenografts have a small size, indicating a slow growing rate. The xenografts have also a low density cells population which resembles the original tumour-anaplastic astrocytoma. Tumour cells were identified either associated with the white matter tracts of alveus hippocampus or spread in the hippocampus. The shape and size of xenografts varied depending on the mouse. Xenografts obtained from tumour stem cells derived from anaplastic astrocytoma have showed a great number of cells positive for both PCNA and the human nestin. Moreover, distribution of cells positive for human nestin showed diffuse, infiltrative pattern in host brain.
Conclusions: Taken together, these results suggest that anaplastic astrocytoma contains cancer stem cells that are able to propagate and can reconstitute the original human tumour *in vivo*.
This work was supported by Project No. 41–035/2007.

582 Expression of GSE24.2 prevents DNA damage in X-linked dyskeratosis congenita cells

C. Manguan-Garcia[1], R. Machado-Pinilla[1], L. Sastre[1], A. Mentzer[2], B.W. Gu[2], L. Pintado-Berniches[1], P.J. Mason[2], R. Perona[1]. [1]*Instituto de Investigaciones Biomédicas Madrid. CIBERER, Modelos Experimentales de Enfermedades Humanas, Madrid, Spain,* [2]*Washington University School of Medicine Campus, Division of Hematology Department of Internal Medicine, Washington DC, USA*

Dyskeratosis congenita is rare X-linked recessive disease characterized for presenting mucotutaneus abnormalities, increased cancer susceptibility and bone marrow failure. At the cellular level dyskeratosis congenita cells have short telomeres and premature senescence. We have previously found that an internal domain of dyskerin (GSE24.2) rescues telomerase activity in X-linked dyskeratosis congenita (X-DC) patient cells. Here we have used F9 mouse cell lines expressing the most common mutation found in X-DC patients, A353V, and found that expression of GSE24−2 is able to induce a recovery in telomerase activity in this F9 X-DC mouse model, by increasing the mTR and mTERT RNA levels. SnoRNA levels are not affected. Moreover, a peptide containing the GSE24.2 sequence is also able to directly rescue telomerase activity. F9 X-DC mouse cells show increased DNA damage and expression of GSE24.2 is able to protect from such damage. Further, F9 X-DC mutant cells are more sensitive to DNA damaging agents and GSE24.2 expression rescued both global and telomeric DNA damage. This data indicates that use of GSE24.2 either as a cDNA vector, or as a peptide, could be a suitable approach for therapy of X-DC patients in which by rescuing telomerase activity and DNA damage, may protect from induced cell death or senescence.
Supported by FIS project numbers: PI081485 and CIBER de Enfermedades Raras.