Methods: DNA is extracted from lymphocytes isolated from leukemia patient blood. MTT assay is used to assess cytotoxicity. Protein levels are assessed by Western blot analysis. Telomere length is assessed using qPCR. Telomerase activity is assessed using the Telomeric Repeat Amplification Protocol kit by Roche. DNA-PK autophosphorylation is determined by FACS analysis.
Results: We report that telomerase activity was present in 48% of lymphocyte samples from 24 CLL patients and that treatment with Imetelstat alone did not affect the survival of primary CLL lymphocytes *in vitro*. Nonetheless, Imetelsat increased the sensitivity of lymphocytes from CLL patients to fludarabine, independently of basal telomerase activity. Imetelstat inhibited fludarabine-induced DNA-PK autophosphorylation, a surrogate marker of DNA-PK activity, in CLL lymphocytes, to the same extent than the DNA-PK inhibitor NU7026. The effect of Imetelstat on fludarabine sensitivity was associated with a lower basal protein expression of the DNA binding subunit of DNA-PK, Ku80.
Conclusion: Our results suggest that Imetelstat can inhibit fludarabine-induced DNA-PK activity in primary CLL lymphocytes. We speculate that there may be a functional interaction between hTR and DNA-PK in primary CLL lymphocytes and conclude that Imetelstat in combination with fludarabine may be useful to decrease the tumour burden in CLL.

9205 ORAL

Mammalian Target of Rapamycin (mTOR) Activity Dependent Protein Expression and Rapamycin Sensitivity in Pediatric Acute Lymphoblastic Leukemias

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Deregulation of signal transduction pathways could be a key event in leukemogenesis. mTOR complexes with different rapamycin sensitivity are central mediators of several signaling pathways, regulate cell proliferation, survival and protein translation. The mTOR pathway has recently attracted a lot of attention as a potential target in oncological therapy, including hematological diseases. However, limited data exists about the activity of mTOR in lymphoid malignancies, especially in pediatric acute lymphoblastic leukemia (ALL), representing nearly one third of all pediatric cancers.
We characterized the expression of mTOR activity dependent phospho-proteins by ELISA (pmTOR, p4EBP1, pS6) in human leukemia/lymphoma cell lines, isolated peripheral mononuclear cells, T- and B-cells, and lymphoblasts from childhood ALL patients. Expression was measured before and during therapy and at relapses during a minimum of a 2-year follow-up of 22 patients. Phospho-protein levels and clinical data were statistically evaluated. The effect of rapamycin treatment on apoptosis and the amount of mTORC1/C2 complexes was measured by flow cytometry and immunocytochemistry in cell lines and short-term cultures.
Cell lines exhibited increased pS6 (2.4−8-fold) and p4EBP1 (62.5–72.5-fold) protein levels. Statistical analysis of more than 80 ALL samples and non-leukemic bone marrow/blood samples showed that p4EBP1 expression was significantly higher (20−58-fold) in ALL samples at diagnosis; the decrease of p4EBP1 expression followed the effectivity of chemotherapy in all patients. We also found that mTOR activity was significantly higher at diagnosis in the samples of patients with worse prognosis, both in B- and T-ALL; p4EBP1 expression was retained and increased above day 0 level at relapses. In vitro rapamycin treatment induced apoptosis in cell lines and in short-term ALL cultures only when p4EBP1 expression concomitantly decreased. In vitro results also suggest that Rictor/Raptor expression correlates with rapamycin resistance in lymphoma/leukemia cells.
Our results suggest that mTOR activity is elevated in ALL cells, which can be monitored by measuring p4EBP1 by ELISA. p4EBP1 may be an important marker for identifying patients with poor prognosis at diagnosis, following p4EBP1 expression could help in the earlier detection of relapse during the therapy; and it may also be useful for the selection of patients who may benefit from rapalog tratment.
Supported by OTKA K81624, K68341

Poster Presentations (Sun, 25 Sep, 14:00−16:30)
Haematological Malignancies and Myeloma

9206 POSTER

Redox-sensitive P73-related Pro-apoptotic Effect of the Polyphenolic-rich Aronia Melanocarpa Juice on Human Acute Lymphoblastic Leukemia Cells

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Background: Natural products derived from plants have received considerable attention as potential cancer chemopreventive and chemotherapeutic agents over few decades. On the basis of epidemiological and animal studies, it has been recurrently reported that diets rich in fruits and vegetables are associated with a reduced rate of cancer mortality. Natural products rich in polyphenols have been shown to have strong chemopreventive properties in different types of cancer cells. Moreover, the polyphenol-induced cytotoxic effect appears to target specifically cancer cells.
Aronia melanocarpa also known as black chokeberry is a shrub native from North America. *Aronia melanocarpa* juice (AMJ) is one of the richest sources of natural polyphenols. AMJ has been shown to have numerous health benefits, including cardioprotective, hepatoprotective and antidiabetic activities. Several *in vitro* and *in vivo* studies indicate that *Aronia melanocarpa* extracts have also antiproliferative effects against colon cancer cells. The aim of the present study was to determine whether AMJ inhibits proliferation of the human acute lymphoblastic leukaemia cells, and if so, to identify the underlying molecular mechanism in particular the role of reactive oxygen species (ROS).
Material and Methods: Human acute lymphoblastic leukemia Jurkat cell line, human primary lymphoblastic leukemia cells and normal human primary T-lymphocytes were used in the study. MTS assay, Cell cycle phase distribution and Apoptosis analysis were performed to study the effect of AMI on proliferation, cell cycle and apoptosis respectively. The formation of ROS was determined by staining with dihydroethidine (DHE). Western blot experiments were performed to detect p73, Cytochrome c, Cyclin B1, Caspase 3 and UHRF1 in Jurkat cells.
Results: We have found that AMJ inhibited cell proliferation and induced cell cycle arrest in G_2/M phase leading to apoptotis. These effects are associated with an upregulation of the tumour suppressor p73 and cleaved caspase 3, and a downregulation of cyclin B1 and UHRF1. AMJ significantly increased the formation of ROS associated with the release of cytochrome c into the cytoplasm. Treatment with intracellular ROS scavengers prevented AMJ-induced apoptosis and upregulation of p73 and active caspase 3 expression. Moreover, it was found that AMI selectively killed the primary lymphoblastic leukemia cells without effecting normal human primary T-lymphocytes.
Conclusion: These findings indicate that AMJ exhibits potent anticancer activity through a redox-sensitive mechanism in the p53-deficient Jurkat cells. In addition, AMJ exerted a strong pro-apoptotic effect in human primary lymphoblastic leukemia cells but not in human normal primary T-lymphocytes. In conclusion, these results suggest that AMJ has chemo-preventive and chemotherapeutic properties against acute lymphoblastic leukemia by selectively targeting lymphoblast-derived tumour cells.

9207 POSTER

Evaluation of Effect of Caffeic Acid Phenyl Ester on Acute T-Lymphoblastic Leukemia Cells by Mitochondria and Peroxisome

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Background: More than 70% of chemotherapeutic agents currently used for treatment of leukemia have been derived from natural sources. Leukemia mainly can be classified as acute and chronic and for the acute leukemia treated with current agents, 5 years survival rates have been around 40−50%. There have been new therapy models for specific targets in the last decade. New targeted drugs have been mostly combined with classical drugs. But, classical chemotherapy agents are still the main treatment for leukemia. Complications associated with classical drugs have brought forth new researches to develop new cancer treatment agents. Caffeic acid phenethyl ester (CAPE) is the active compound that has wide spectrum effects such as antioxidant, anti-inflammatory, anti-viral, carcinogenetic and anti-cancer. PPAR-gamma plays a key role in atherosclerosis, inflammation, obesity, diabetes, immune response and