538 Poster

Extracts prepared from primitive plant: common ladyfern (species Athyrium filix-femina) displays potent anti-cancer effects in preclinical assessments of diverse human malignant cell lines

S. Crawford[1], E. Boisvert[1], T. Herbert[1], H. Potter[1], D. Diamond[1], R. Pennareta[1], W. Sprague[1], S. Liddie[1], D. Fong[1], F. Okhiria[1]
[1]Southern Connecticut State University, Biology, New Haven Connecticut, USA

Research to be presented describes the potent anti-cancer properties of the common lady fern (plant Division Pterophyta, Family Dryoptheridaceae, species Athyrium filix-femina). This preclinical assessment has involved the determination that whole plant extracts prepared from this species (termed primiplex*™) induce cell death in diverse human solid tumor malignancies in vitro, among these, cell lines derived from cancers of the brain (glioblastoma), colon, lung, breast and several types of leukemia.

The plant extract formulations were prepared as whole plant homogenates in culture medium. Optimal dose range for the fern extract homogenate ranged from 200 microG per ml to 1mg over a time course of 24-48 hours for the preclinical assessments of anti-cancer activity in cell lines derived from several human solid tumor malignancies growing in culture either in the form of monolayers or as multicellular tumor spheroids. Cytotoxicity assays involved the standard trypan blue exclusion assay as well as survival assays to assess post-treatment culture viability. The results of these preliminary assessments suggested that the extract formulation displays potent anti-cancer properties with broad spectrum cytotoxic potential against human cancers of diverse tissue origins.

Combined treatment studies involving the use of the plant extract with standard cancer therapeutics showed that this extract may enhance the efficacy of standard treatment regimens, in that enhanced cytotoxicity was observed when the extract was combined with:

- Vinblastine in acute lymphoblastic leukemia and chronic myelogenous leukemia;
- Fluorouracil in primary and metastatic colon carcinoma
- Tamoxifen in estrogen receptor positive and negative breast cancers.

To our knowledge, this is the first documented assessment of the anti-cancer properties of this group of primitive plants. Moreover, these studies suggest that the anti-cancer properties of the primitive plant extract may be preventive as well as therapeutic. In addition, this research defines a preparative formulation of the whole plant that preserves its anti-cancer properties. Confocal laser microscopy imaging studies suggest that an important target of these plant extract preparations is the actin cytoskeleton.

*Patent pending, ™ 2008

539 Poster

The effect of the synthetic estrogen – diethylstilbestrol (des) and indol-3-carabinol(i3c) on the distribution of telomerase expression cells in lines of prostate cancer

A. Klein[1], H. Shapira[1], S. Adler[1]
[1]Meir Medical Center, Department of Medical Laboratories, Kfar Saba, Israel

Background: Expression of telomerase is an essential step in cancer cell immortalization and progression. Modulation of its expression may be beneficial for controlling cancer cell proliferation. Prostate cancer patients are treated with estrogens in order to abolish testicular androgen formation. Studies with the phytochemical Indol-3-Carbinol (I3C) have shown its anti-proliferation capabilities.

Objective: Flow cytometry was used to examine the effect of the synthetic estrogen- Diethylstilbestrol (DES) and I3C on the distribution of telomerase expressing cells in two cancer prostate cell lines (PC3 - androgen insensitive) and LNCaP

Results: Regarding fluorescence intensity of the two lines, three subgroups of cells, with low, medium and high intensities (A,B and C respectively), were demonstrated. Group C consisted of about 2% of the cells of both lines while A and B included all the rest. DES and I3C had no effect on groups A and B of both lines. While group C, although minor, was the only one, in both lines, responding to the added compounds: As to DES, concentrations of 25 and 50μM, increased the LNCaP group C to 11.8% and 6.2% of the cell population, whereas, in PC3, to 7.78 % and 4.4% respectively. Contrary to DES, I3C showed an inhibitory effect on C groups of both lines. An inhibition of 83.6% was obtained with the LNCap line treated with a combination of 25μM Des and 250μM 3IC, and 80.6% with PC3 treated with 25μM Des and 250μM 3IC.

Conclusions: The present results emphasizes an occult possible oncogenic characteristic of DES which was attenuated by I3C. We wonder whether Indol could be beneficial for treatment of prostate cancer.

540 Poster

Quercetin, luteolin and ursolic acid are potent inhibitors of proliferation in colorectal carcinoma cells: new therapeutic tools?

C.P.R. Xavier[1], C.F. Lima[1], A. Preto[2], R. Seruca[2], M. Fernandes-Ferreira[1], C. Pereira-Wilson[1]
[1]University of Minho, Department of Biology School of Sciences, Braga, Portugal; [2] University of Porto, Institute of Molecular Pathology and Immunology (IPATIMUP), Porto, Portugal

PI3K/Akt and MAPK/ERK signalling pathways play critical roles in cell proliferation and survival. Components of these pathways are often altered in colorectal carcinoma (CRC) where they increase proliferation and confer drug resistance. These pathways, therefore, have received increasing attention as targets for cancer therapy. Several dietary phytochemical constituents have been shown to have anti-cancer effects by inhibiting proliferation and inducing apoptosis in cancer cells. The aim of our study is to investigate the effect of luteolin (Lut), quercetin (Que) and ursolic acid (UA) on cell proliferation through PI3K/Akt and MAPK/ERK pathways in the CRC derived cell lines HCT15 and CO115.

Both cell lines were treated with different concentrations of compounds and significant inhibition of proliferation was observed using the BrdU incorporation assay. Western blotting analysis showed that Lut and Que inhibited ERK phosphorylation in HCT15 cells which have a RAS mutation. In CO115 cells that overexpress Akt, but have no RAS mutations, Que, Lut and UA inhibited phospho-Akt but not phospho-ERK. We find that the effect of the compounds depend on the genetic background of the cell lines. In addition, these effects were more pronounced than those obtained for wortmannin and PD-98059, reference inhibitors of PI3K and MEK, respectively. These results suggest that these compounds might be interesting to test in combination with classical chemotherapy drugs in order to enhance the effects of these drugs, and decrease chemoresistance.

541 Poster

Combination of different agents against ErbB receptors significantly reduce SKBR3 breast cancer cell line proliferation

M. Majorek[1, 3], P. Guzenda[1, 3], A. Paczkowska[2, 3], M. Lamparska-Przybysz[3], M. Wieczorek[3], A.K. Bednarek[1]
[1]Medical University in Lodz, Department of Molecular Cancerogenesis, Lodz, Poland; [2] Medical University in Lodz, Department of Medical Biotechnology, Lodz, Poland; [3] Celon Pharma, R&D Department, Lomianki, Poland

Background: The epidermal growth factor receptor (EGFR) or ErbB family consists of four closely related tyrosine kinases: ErbB-1 (EGFR), ErbB-2 (HER2), ErbB-3 (HER3) and ErbB-4 (HER4). The association of ErbB members with breast cancer development has been extensively described in literature. EGFR, ErbB-2, and ErbB-3 are frequently overexpressed in breast cancer and negative regulation of them results in an inhibition of cell proliferation and spreading. Optimal use of therapeutics targeting these receptors require further identifying the contribution of ErbB family to tumorigenesis and interactions between all their members.

This study was focused on the effect of different ErbB inhibitors on proliferation of SKBR3 breast cancer cell line. We examined antibody Herceptin®, tyrosine kinase inhibitor (TKI) erlotinib, and siRNA against ErbB member as a single agent or in combination.

Materials and methods: Flow cytometry, light microscopy, western blot and viability/cytotoxicity tests were used for evaluation of the cell growth, ErbB pathway activation and protein expression, percentage of apoptotic cells and morphological features of cell death.

Results: Our study confirmed that the downregulation and/or inhibition of EGFR, ErbB2 and ErbB3 significantly reduce proliferation of SKBR3 breast cancer cell line. This effect depends on combination of agents, doses and time of exhibition.

Conclusion: We propose that using three agents against ErbB proteins with different mechanism of action may be valuable and innovative tool in breast cancer therapy.

542 Poster

Active p53 contributes to antitumor effects of cyclin-dependent kinase roscovitine in multiple myeloma cells

V. Krystof[1], M. Paprskarova[1], M. Strnad[1]
[1]Palacky University, Laboratory of Growth Regulators, Olomouc, Czech Republic

Small molecule inhibitors of cyclin-dependent kinases (CDK) are considered to be potential anticancer agents. Our previous work recently resulted in an identification of olomoucine II, a compound structurally similar to roscovitine (CYC202, seliciclib), which currently undergoes clinical evaluation as an anticancer drug. Both roscovitine and olomoucine

II inhibit phosphorylations of the retinoblastoma protein, with a direct impact on cell cycle progression and proliferation. However, increased cellular effectivity of olomoucine II over roscovitine that probably stems from its higher affinity to CDK9, predestines this pair of compounds for comparative studies. One of the processes affected by inhibition of CDK9 is p53-dependent transcription. We therefore compared effect of both inhibitors in two multiple myeloma cell lines that differ in p53 status; RPMI-8226 bears temperature sensitive p53 (E285K), while U266 expresses completely inactive protein (A161T). When kept at 37°C, the inhibitors reduced phosphorylation of pRB and induced apoptosis in both cell lines in a dose-dependent manner, but did not influence level of p53. Conversely, p53 and Mcl-1 protein levels, as well as fragmentation of PARP were significantly changed in RMPI-8226 cultivated at 32°C. Although it was previously shown that CDK inhibitors trigger apoptosis in cell lines regardless of p53 status, we demonstrated that active p53 contributes to induction of apoptosis in multiple myeloma cells by roscovitine. The obtained data are in line with the findings that roscovitine targets not only cell cycle machinery, but also transcriptional CDKs, and that this combination is advantageous for the therapy. Currently, combination therapies to increase the potency of individual agents are often used, but with roscovitine multiple processes are targeted simultaneously.

The work was supported by grants from MSM (6198959216) and GACR (204/08/0511).

543 Poster

Updates in diagnosis and treatment of chronic myeloid leukemia

V. Musteata[1], I. Corcimaru[1]
[1]Institute of Oncology / State Medical & Pharmaceutical University "N. Testemitanu", Department of Hematology & Oncology, Chisinau, Moldova

Background: Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder, which accounts for about 15% of all leukemia cases in adults. Imatinib mesylate (STI 571) is a recently developed promising targeted treatment option for CML, but data on its efficacy and safety are still scanty. The aim of the study was to evaluate the diagnosis assertion, the short-term results and the safety of STI 571 therapy in patients with different phases of CML.

Materials and methods: Fourteen GIPAP qualified CML patients (males – 6, females – 8) at the age of 14 – 55 years have been treated with imatinib mesylate, and followed up between 2006 – 2008 at the Hematology Division of the Institute of Oncology. Cytogenetic examination of the bone marrow cells revealed Ph-chromosome and BCR-ABL p210 oncogene in all cases. However, the rate range of t(9; 22)-positive myeloid cells was 55 – 100%. Seven (50.0%) patients had been diagnosed in the chronic phase, 3 (21.4%) – in the accelerated, and 4 (28.6%) – in the acute phase of CML. Leukocyte count ranged between 6.6 - 205.0 x 10^9/l, thrombocyte count - 226,8 - 2340.0 x 10^9/l. The initial dosage of STI 571 varied between 400 – 800 mg daily, depending on CML phase. All the patients had previously failed to respond, or relapsed after the treatment with conventional chemotherapy regimens and interferon.

Results: The period diagnosis date – STI 571 starting date ranged from 1.5 to 58 months (median – 22.7 months). Complete hematologic response had been achieved in 10 (71.4%) patients within 1 – 3 months of the therapy with STI 571 ($p < 0.05$). A trend to the earlier complete hematologic response was observed in cases with chronic phase, shorter duration of CML, and lower leukocyte count ($p < 0.05$). Two (14.3%) patients with the acute CML phase have experienced clinical and hematologic improvement on the date of inclusion in the current study. The cytogenetic examination of the bone marrow cellular elements performed within 4 – 8 months of the treatment with imatinib mesylate established the decrease of Ph-positive myeloid cells up to 20 – 33%. Only 2 (14.3%) patients with acute phase failed to respond to imatinib mesylate ($p < 0.05$). Frequently registered side effects were dry mouth, angioedema, nausea, dyspepsia, abdominal pain, neutropenia, and thrombocytopenia, occurred in different combinations in 5 (35.7%) cases. Marked neutropenia developed in 3 (21.4%) patients, that required temporary cessation of treatment.

Conclusions: The combined screening for Ph-chromosome and BCR-ABL p210 oncogene is highly useful for diagnosis assertion in patients fairly suspected for CML. Imatinib mesylate may be considered as an effective and tolerable targeted medication for CML patients, even in those initially managed with conventional chemotherapy and interferon. A shorter duration of CML is associated with better response to imatinib mesylate.

544 Poster

The induction of orphan nuclear receptor Nur77 expression by n-butylenephthalide aspharmaceuticals on hepatocellular carcinoma (HCC) cells therapy

Y. Chen[1], P. Lin[2]
[1]Graduate Institute, Biotechnology, Ilan, Taiwan; [2] Graduate school, Life Science, Hualien, Taiwan

N-butylidenephthalide, isolated from the chloroform extract of Angelica sinensis, has been examined for its antitumor effects on glioblastoma multiforme brain tumors; however, little is known about its antitumor effects on hepatocellular carcinoma cells. Two hepatocellular carcinoma cell lines, HepG2 and J5, were treated with either nbutylidenephthalide or a vehicle, and cell viability and apoptosis were evaluated. Apoptosis-related mRNA and proteins expressed, including orphan receptor family Nurr1, NOR-1, and Nur77 were evaluated as well as the effect of n-butylidenephthalide in an in vivo xenograft model. N-butylidenephthalide caused growth inhibition of both the cell lines at 25 μg/ml. Further, n-butylidenephthalide-induced apoptosis appears to be related to Nur77 translocation from nucleus to cytosol, which lead to cytochrome c release and caspase-3-dependent apoptosis. N-butylidenephthalide-related tumor apoptosis was associated with PI3K/AKT/GSK3β rather than the MAPK or PKC pathway. Blockade of AKT activation enhanced proliferation inhibition and the induction of phospho-Bcl-2 and Nur77 proteins. Nur77 short interfering RNA (siRNA) blocked n-butylidenephthalideinduced apoptosis in J5 cells, and n-butylidenephthalide treatment increased luciferase activity of Nur77 in J5 cells. Administration of n-butylidenephthalide showed similar antitumoral effects in both HepG2 and J5 xenograft tumors. N-butylidenephthalide-induced apoptosis in hepatocellular carcinoma cells, both in vitro and in vivo, suggesting a potential clinical use of this compound for improving the prognosis of HCCs.

545 Poster

Design, characterization and in vitro applications of novel chemotactic peptide-based drug delivery systems against cancer

K.B. Bai[1], O. Lang[2], L. Kohidai[2], F. Hudecz[1], G. Mezo[1]
[1]Eötvös L. University, Research Group of Peptide Chemistry Hungarian Academy of Sciences, Budapest, Hungary; [2] Semmelweis University, Department of Genetics Cell- and Immunobiology, Budapest, Hungary

In the field of targeted drug delivery, numerous bioconjugates have been developed to enhance the efficiency and specificity of novel antitumor therapeutics. These kind of drug delivery system usually consist of a carrier component, a drug or drugs and targeting moieties. During the past decade, several carrier systems (e.g. liposomes, niosomes, nanoparticles, microparticles, cyclodextrins, polymers etc) have been envolved depended on the target organ. Forasmuch the receptor mediated endocytosis may provide the appropriate pathway for cellular uptake, targeting moieties have modulated the palette of drug delivery systems.

The goal of this project was to develop a targeted peptide-based drug delivery system for the treatment of cancer. Drug-conjugates consist of methotrexate as drug, GFLGC pentapeptide as enzyme-degradable spacer sequence, Tp20 (H-$[TKPPR]_4$-NH_2) as oligopeptide carrier and TKPR, For-TKPR, TKPPR, For-TKPPR as targeting peptide moieties were designed, synthesized, characterized and applied in several biological system.

Carriers with targeting moieties in branches were synthesized by solid phase synthesis using mixed Boc and Fmoc strategies. Drug molecules with enzyme-degradable spacer were attached to the carrier system in solution. The bioconjugates were characterized by analytical HPLC and ESI-MS.

In vitro biological assays such as chemotaxis, internalization and citotoxicity were investigated. The bioconjugates and their components (carrier, targeting moieties and drug-spacer) were studied on Tetrahymena pyriformis, THP-1 human tumor cell line. Cellular uptake of the fluorescent-labeled analogues was studied by flow cytometry. Most of the conjugates had advatageous chemotactic properties, they can be internalized rapidly and could trigger toxic effect on the cells.

Our results confirmed the feasibility of this novel drug targeting strategy for increasing the efficacy and specificity of chemotherapy.

Acknowledgement: This work was supported by grants from the Hungarian National Science Fund (OTKA T 043576 and T 049814), "Medichem 2" 1/A/005/2004 and GVOP-3.2.1.-2004-04-0005/3.0 and ETT 202/2006.

546 Poster

Evaluation of gonadotropin-releasing hormone analogues in mice - pharmacokinetic studies and biomarker based efficacy by mass spetrometry

T. Katsila[1], Z.D. Sofianos[1], E. Balafas[2], N. Kostomitsopoulos[2], J. Matsoukas[3], T. Tselios[3], C. Tamvakopoulos[1]
[1]Biomedical Research Foundation Academy of Athens (BRFAA), Pharmacology - Pharmacotechnology, Athens, Greece; [2] Biomedical Research Foundation Academy of Athens (BRFAA), Center for Experimental Surgery, Athens, Greece; [3] University of Patras, Department of Chemistry, Patras, Greece

Background: The study of pharmacologically active peptides is central for the understanding of diseases and the development of novel therapeutic