accompained by an important apoptotic fraction of all cell lines after treatment with either of extracts. The sub-G1 accumulation of the target cells reached values greater than 25% at 17–34 μl/ml *uman*[b] and at 14–28 μl/ml *uman*[c], depending on the individual cell line.
Conclusion: Two investigated aqueous-ethanol extracts showed significant cytotoxic activity on all neoplastic cell lines, after 72 h of continuous treatment, and point to the need for further characterization of the extracts, their phytochemical analysis, to provide the information about the compounds responsible for the antitumor action of investigated herbal mixtures.

225 PUBLICATION

Inhibition of the endothelin-1/endothelin A receptor axis by green tea polyphenol Epigallocatechin-3-Gallate in ovarian carcinoma

F. Spinella[1], L. Rosano[1], V. Di Castro[1], S. Decandia[1], G. Elia[1], A. Albini[2], P. Natali[3], A. Bagnato[1]. [1]*Regina Elena Cancer Institute, Molecular Pathology Laboratory, Rome, Italy;* [2]*National Institute for Cancer Research and Center of Advanced Biotechnology, Genoa, Italy;* [3]*Regina Elena Cancer Institute, Immunology Laboratory, Rome, Italy*

Background: Green tea polyphenols are reported to possess anti-cancer properties. However, the molecular mechanisms leading to tumor growth inhibition are not fully understood. The endothelin A receptor (ET_AR)/endothelin-1 (ET-1) autocrine pathway is overexpressed in ovarian carcinoma and triggers tumor growth, survival, neoangiogenesis, and invasion indicating that ET_AR-inhibitory agents may be of therapeutic value. In the present study, we investigated whether green tea polyphenol epigallocatechin-3-gallate (EGCG) could act by inhibiting ET-1/ET_AR axis and signaling pathway in ovarian carcinoma cell lines.
Materials and methods: The effects of EGCG and green tea on ET_AR-mediated actions were tested by RT-PCR, Northern and Western blot, ELISA, chemoinvasion assay and immunohistochemical analysis in HEY and OVCA 433 ovarian carcinoma cell lines and in HEY xenografted nude mice.
Results: EGCG and green tea treatment inhibited ET_AR and ET-1 expression, at mRNA and protein levels. These effects resulted in reduction of the basal and ET-1-induced cell proliferation and invasion. Remarkably, EGCG treatment resulted in a reduction of basal and ET-1-induced mediators of angiogenesis, such as cyclooxygenase (COX)-1, COX-2, prostaglandin E_2, vascular endothelial growth factor (VEGF) expression and matrix-metalloproteinase activity. The EGCG-induced inhibitory effects were associated with a reduction of ET_AR-dependent activation of the p42/44 and p38 mitogen-activated protein kinases and phosphatidylinositol-3-kinase pathway. Finally, tumor growth was significantly reduced by oral administration of green tea *in vivo*. This effect was associated with ET-1, ET_AR, and VEGF mRNA and protein expression reduction, as well as with a decreased in the microvessel density and proliferation index.
Conclusions: These results provide a novel insight into the mechanism by which EGCG, affecting multiple ET_AR-driven pathways may inhibit tumor growth suggesting that EGCG may be useful in preventing and treating ovarian carcinoma.
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226 PUBLICATION

Investigation of some quinols and epoxyquinols as potential antitumor agents

Z. Zizak[1], T. Kop[2], B. Solaja[2], T. Stanojkovic[1], Z. Juranic[1]. [1]*Institute of Oncology and Radiology of Serbia, Experimental Oncology, Belgrade, Serbia and Montenegro;* [2]*Faculty of Chemistry, Belgrade, Serbia and Montenegro*

Background: The search for new antitumor agents is the imperative in modern oncology. The aim of this work was to investigate the antiproliferative activity of several newly synthesized quinols and epoxyquinols against five human tumor cell lines *in vitro*.
Material and methods: Stock solutions of investigated compounds were dissolved in DMSO at concentrations of 10 mM, and afterwards diluted by nutrient medium to various final concentrations. Target cells used were malignant human breast adenocarcinoma MDA-MB-361 and MDA-MB-453, cervix carcinoma – HeLa, melanoma – Fem-x and myelogenous leukemia – K562 cells. Normal human peripheral blood mononuclear cells (PBMC) were used as control cells. Antiproliferative activity of investigated compounds was assessed indirectly, measuring cell survival in standard, 72 h MTT test. In order to determine the mode of HeLa cell death induced by the investigated compounds, microscopic examination of morphological characteristics of acridine orange and ethidium bromide stained cells was performed.
Results: Investigated quinols and epoxyquinols exerted a dose dependent antiproliferative action towards investigated cell lines with good selectivity in their action to tumor cells in comparison to normal immunocompetent cells. Concentrations inducing 50% decrease in cell survival (IC50) obtained from three independent experiments, and on mononuclear cells, were given on table.

	IC50[μM]							
Cell lines	TK4	TK21	TK22	TK23	TK24	TK25	TK26	TK27
HeLa	1.5	47.7	90.7	8.4	49.6	5.5	35.0	3.9
Fem-x	1.2	81.4	> 100	8.2	70.6	5.1	54.7	4.6
MDA-MB-361	3.4	37.7	93.6	9.4	36.4	6.2	28.9	4.7
MDA-MB-543	5.6	53.9	> 100	23.7	43.6	9.4	35.2	16.3
K562	1.3	42.6	92.6	5.7	36.1	3.7	26.7	1.0
PBMC−PHA	16.6	> 100	46.4	> 100	19.0	66.3	19.3	
PBMC+PHA	16.3	> 100	> 100	54.1	> 100	20.0	> 100	18.9

Microscopic examination of the mode of direct cell death induced by the most active compounds, epoxyquinols TK4, TK23, TK25 and TK27, 24 h after continuous agents action in concentrations 2 × IC50, showed morphological appearance of apoptosis (condensed and/or fragmented nuclei).
Conclusions: Results obtained showed that investigated compounds, especially epoxyquinols 4β, 5β-epoxy-10β-hydroxy-17β-propionyloxy-1-estren-3-one (TK4), 4β,5β-epoxy-10β,17β-dihydroxy-1-estren-3-one (TK23), 4β,5β-epoxy-10β,17β-dihydroxy-17α-(phenylmethyl)-1-estren-3-one (TK25) and 17α-butyl-4β,5β-epoxy-10β,17β-dihydroxy-1-estren-3-one (TK27) could be promising agents for the treatment of human tumors, and are candidates for further analyses on experimental animals, *in vivo*.

227 PUBLICATION

The inhibitors of hydroxy-methyl-glutaryl-CoA (statins) induce cell growth arrest and apoptosis in osteosarcoma cell lines

J.M. Garcia-Castellano. *Hospital De Gran Canaria Dr Negrin (Research Unit), Las Palmas De Gran Canaria, Spain*

Osteosarcoma (OS) is an aggresive bone tumor of children and adolescents. The introduction of neoadjuvant chemotherapy has increased the fraction of patients who can cured to about 70%. Nevertheless, subsequent clinical trials of a variety of new treatment have all failed further improve survival. In order to progress in the treatment of OS we have tried to identify new pathways, like the mevalonato pathway, which may be exploited therapeutically.
For this purpose rat (UMR-106) and human (HOS, SaOS, U2OS) OS cell lines were grown under standar conditions. The parameters studied after administration of simvastatin at differents doses and times, with or without mevalonate, FPP, GGPP, FTI or GGTI were: cell growth rate, cell viability, morphologic changes, apoptotic response, cell cycle alterations, p53 and p27(Waf1/Cip1) protein expression and cell motility.
We observed that statins induced: 1. a decreased in cell growth rate; 2. an increased in the number of no-viable cells; 3. morphological alterations characterized by cell rounding and cell detachment from the substrate; 4. a p53-independent apoptotic response, dependent of the mevalonate pathway; 5. cell growth arrest in G1 and G2/M phases, dependent of of an increase in the p27(Cip/Kip/Waf) and decrease wound assay.
In conclusion, Statins, at leasti n vitro, are useful agent in the treatment of osteosarcome. These drugs are able to decrease cell proliferation, induce cell death by apoptosis and affect the cell motility. At present, we are evaluating the *in vivo* effect of these drugs in the osteosarcoma growing in nude mouse.

228 PUBLICATION

The expression of plakoglobin correlates with a favourable outcome of breast cancer patients

H. Bühler[1], E. Mahnke[2], B. Duvnjak[1], G. Schaller[3]. [1]*Ruhr-Universität Bochum, Medical Center Marienhospital, Herne, Germany;* [2]*Martin-Luther Krankenhaus, Gynecology, Berlin, Germany;* [3]*Ruhr-Universität Bochum, Gynecology, Bochum, Germany*

Background: Plakoglobin = γ-catenin is an important protein of cellular adhesion structures in epithelia. It is part of the desmosomal plaque as well as of the adherens junctions. Together, both structures account for more than 90% of total cellular adhesion. During the metastatic process cell-cell adhesion has to be broken before tumor cells are able to disseminate. Since plakoglobin is part of both important adhesive structures it might be a main candidate for downregulation during dedifferentiation and malignant transformation. In a retrospective study we have determined the plakoglobin

expression in breast carcinomas and correlated with the clinical outcome of the patients.
Methods: 86 specimens were tested so far for plakoglobin by means of immunohistochemistry and the expression scored separately for membrane, cytosol, and nucleus. Mean plakoglobin values were evaluated for the two groups of surviving and deceased tumor patients.
Results: In a 15 years follow-up the ratio surviving/deceased was 2.2 for membrane, 1.6 for nucleus, 1.2 for cytosol, and 1.4 for overall staining. All patients with an intense staining of either membrane or nucleus are still alive, in contrast to about 40% survival for low membrane or low nuclear staining and 12% survival for low both.
Conclusion: In conclusion, we found a close correlation of conserved plakoglobin expression in the tumor with 15 years overall survival, in particular for membraneous and nuclear staining.

229 PUBLICATION

A molecular analysis by gene expression profiling reveals BIK/NBK overexpression in sporadic breast tumors of Mexican female samples

N. Garcia[1], H. Astudillo-de la Vega[2], F. Salamanca[1], I. Alvarado[2], M. Perez[3], A. Silva[3], D. Arenas[1]. *[1]IMSS CMN Siglo XXI, Unidad de Invest. Medica en Genetica Humana, Mexico, D.F., Mexico; [2]IMSS CMN Siglo XXI, Unidad de Invest. Medica en Enf. Oncologicas, Mexico, D.F., Mexico; [3]IMSS CMN Siglo XXI, Departamento de Oncologia, Hospital de Oncologia, Mexico, D.F., Mexico*

Background: Breast cancer is the second cause of death in Mexican women over 35 years of age. At molecular level, changes in many genetic networks have been reported as associated with this neoplasia. To analyze these changes, we determined gene expression profiles of tumors from Mexican women with breast cancer at different stages and compared these whit those of normal breast tissue samples.
Material and methods: ^{32}P-radiolabeled cDNA was synthesized by reverse transcription of mRNA from fresh sporadic breast tumor biopsies as well as normal breast tissue. cDNA probes were hybridized to microarrays and expression levels registered using a phosphorimager. Expression levels of some genes were validated by real time RT-PCR and immunohistochemical assays.
Results: We identified two subgroups of tumors according to their expression profiles, probably related with cancer progression. Ten genes unexpressed in normal tissue were turned on in some tumors. We found consistent high expression of *Bik* gene in 14/15 tumors with predominant cytoplasmic distribution.
Discussion: Recently, the product of the *Bik* gene has been associated with tumoral reversion in different neoplasic cell lines, and was proposed as therapy to induce apoptosis in cancers including breast tumors. Even though a relationship between genes, for example those from a particular pathway, can be observed through microarrays, this relationship might not be sufficient to assign a definitive role to *Bik* in development and progression of the neoplasia. The findings herein reported deserve further investigation.

Poster presentations (Mon, 31 Oct)
Molecular predictive assays (including: genetics, genomics, molecular diagnostics, prognostic factors, proteomics)

230 POSTER

Enhanced sensitivity of human lymphoblastoid cell lines with heterozygosity for a mutation in BRCA1 or BRCA2 towards the DNA-damaging agent cisplatin

L. Delgado[1,2], G. Grotïuz[2], R. Fresco[1], D. Lens[2], G. Sabini[1], M. Musé[1]. *[1]Hospital de Clinicas, Oncología Clínica, Montevideo, Uruguay; [2]Hospital de Clínicas, Básico de Medicina, Montevideo, Uruguay*

Background: heterozygous carriers of BRCA1 or BRCA2 germline mutations exhibit a high risk of developing breast and other cancers. The loss of the wild-type allele is frequently observed in the primary breast and ovarian tumours in these susceptible patients. Previous studies suggest that homozygous mutations in BRCA1/2 (BRCA−/−) result in impaired DNA damage repair and response to genotoxic damage. However, it is unclear if heterozygosity for BRCA1/2 mutations (BRCA±) have any phenotypic effect.
Material and methods: to assess whether heterozygous mutations in these genes are associated with modified sensitivity to the genotoxic anticancer agent cisplatin, we performed an *in vitro* chemosensitivity assay on human lymphoblastoid cell lines developed from a BRCA1 heterozygote carrier (GM13705), a BRCA2 heterozygote carrier (GM14622) and two BRCA1/2 competent (BRCA+/+) individuals (GM14453 and GM14454), using the MTT assay. The concentration of drug that reduced the number of viable cells to 50% (IC50) after 24 hours of exposure was calculated by logarithmic regression model. Results were derived from at least six independent sets of triplicate experiments.
Results: GM13705 (IC50: mean = 5.2 μM, s.d. = 1.9) and GM14622 (IC50: mean = 6.2 μM, s.d=1.5) cell lines were significantly more chemosensitive than the BRCA-competent GM14453 cell line (IC50: mean = 15.3 μM, s.d. = 8.0) (p = 0.0012 and 0.0026 respectively). Also, GM13705 (IC50: mean = 5.0 μM, s.d. = 1.9) and GM14622 (IC50: mean = 6.4 μM, s.d. = 1.7) cell lines were more chemosensitive than the BRCA-competent GM14454 cell line (IC50: mean = 19.1 μM, s.d. = 8.0) (p = 0.0002 and 0.0017 respectively).
Conclusions: cells containing a heterozygous mutation in BRCA1 or BRCA2 are more sensitive to the genotoxic agent cisplatin. These findings suggest that heterozigote cells are not phenotypically normal. Carriers of a single defective copy of BRCA1 or BRCA2 would have a higher risk for the induction of mutations and development of secondary tumours when exposed to DNA-damaging agents.

231 POSTER

Quantitative and qualitative analyses of plasma DNA in colorectal cancer patients as prognostic tools

E. Leo[1], F. Belli[1], G. Gallino[1], M. Frattini[2], G. Bonfanti[1], M. Vitellaro[1], E. Poiasina[1], A. Vannelli[1], L. Battaglia[1], D. Balestra[2], S. Signoroni[2], M. Pierotti[2]. *[1]National Cancer Institute, Colo-Rectal Surgery, Milano, Italy; [2]National Cancer Institute, Department Of Experimental Oncology, Milano, Italy*

Background: A high level of cell-free circulating DNA both in plasma and in serum has been reported in several tumoral models at the time of surgery. Starting from this evidence, we would like to verify whether high levels of cell-free DNA in plasma may predict the presence of colorectal cancer.
Material and methods: We analyzed 70 patients with primary colorectal cancer. Plasma samples were obtained at the time of surgery and after 4, 10 and 16 months in patients follow-up. The cell-free circulating DNA in plasma was quantified by the Dipstick method. Tumor and plasma samples were characterized for K-Ras mutations and $p16^{INK4a}$ promoter hypermethylation. Tumor specimens were also investigated for CD31 immunohistochemical staining.
Results: In all patients the cell-free DNA levels in plasma are significantly higher at the time of surgery in comparison with healthy donors (about 25 times higher). In addition, we found that colon cancers release more DNA than tumors with a rectal location and that the levels of cell-free DNA are related to angiogenesis. The CEA value of this cohort of patients was altered in about 40% of cases. Moreover, our data show that cell-free DNA levels decreased 4 months after surgery. Ten and sixteen months after surgical intervention, cell-free DNA plasma quantities decreased progressively in tumor-free patients. By contrast, patients who developed recurrences or metastasis showed a concomitant increasing plasma DNA level. All our data are statistically significant.
Conclusions: Our preliminary data confirm that plasma DNA levels:
i. are significantly higher in all patients with colorectal cancer,
ii. decrease progressively in tumor-free patients,
iii. increase in patients with recurrence of metastasis.
Thus, we suggest that the quantification of plasma cell-free DNA may represent an useful tool for diagnostic and monitoring of colorectal cancer.

232 POSTER

Epidermal growth factor receptor as a predictor of tumor downstaging in locally advanced rectal cancer patients treated with preoperative chemoradiation

K. Jun-Sang[1,4], L. Shengjin[2], K. Jin-Man[2,4], C. Moon-June[1,4], Y. Wan-Hee[3,4], S. Kyu-Sang[2], Y. Seung-Gu[1], K. Jae-Sung[5]. *[1]Chungnam National University, College of Medicine, Radiation Oncology, Daejeon, South Korea; [2]Chungnam National University, College of Medicine, Pathology, Daejeon, South Korea; [3]Chungnam National University, College of Medicine, General Surgery, Daejeon, South Korea; [4]Chungnam National University, Cancer Research Institute, Daejeon, South Korea; [5]Seoul National University, Radiation Oncology, Seoul, South Korea*

Background: This study examined whether the expression of epidermal growth factor receptor (EGFR) can predict tumor downstaging to preoperative chemoradiation in patients with locally advanced rectal cancer.