POSTER ÖZETLERİ
[POSTER ABSTRACTS]
**P-001 - EVALUATION OF SERUM IMMUNOFIXATION ELECTROPHORESIS RESULTS IN KONYA REGION**

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**Introduction:** Multiple myeloma (MM) is a malignant plasma-cell disorder caused by an uncontrolled proliferation of monoclonal plasma cells in the bone marrow. MM is characterized by malignant plasma cell hyperplasia, monoclonal immunoglobulin (M protein) in the serum and urine, which can lead to anemia, renal insufficiency, wide-spread bone lesions, hypercalcemia and serious recurrent infections. Multiple myeloma is increasing abnormal plasma cells produce abnormal proteins and that useful not fight infections.

In addition to history taking and physical examination, the diagnostic work-up for multiple myeloma comprises clinical chemistry, cytogenetic analysis of bone marrow, and radiological investigation to detect bone changes. We aimed to evaluate three years IFE results of our laboratory.

**Material and Methods:** In this study, serum IFE levels of totally 706 individuals (372 females and 334 males) who admitted to our faculty with different complaints between 01.08.2013 and 01.08.2016 were assayed. Serum IFE analysis was performed using Helena SAS-I and SAS-II devices. Statistical analysis was performed using SPSS version 21.0.

**Results:** We examine serum IFE studies from our laboratory; The number of female patients 372 (52.7%) was higher than the number of male patients 334 (47.3%). The average age of female patients (63,17) was significantly lower than male patients (65,04). The result of the evaluation of 497 patients serum IFE results were normal. The remaining 309 patients were seen in the monoclonal band. The most common monoclonal band we identified was IgG kappa with 48%. IgM lambda is a monoclonal gammopathy of rare species with 3%.

**Discussion and Decision:** The follow cases IFE results were accordance with the literature. Because of its great versatility, potentially high sensitivity, ease to perform and customize, and relatively low cost with no requirement for expensive instrumentation, IFE remains a valuable tool for both clinical diagnostic testing and research.

**Keywords:** Immunofixation electrophoresis, Konya region, Monoclonal gammopathy

**Age and MGUS Type**

<table>
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<th>Type</th>
<th>Mean</th>
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The result of the evaluation of 497 patients serum IFE results were normal. The remaining 309 patients were seen in the monoclonal band. The most common monoclonal band we identified was IgG kappa with 48%. IgM lambda is a monoclonal gammopathy of rare species with 3%.

**Age and Sex**

<table>
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<th>Mean</th>
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The number of female patients 372 (52.7%) was higher than the number of male patients 334 (47.3%). The average age of female patients (63,17) was significantly lower than male patients (65,04).
P-002 - EFFECT OF MESENCHYNAL STEM CELLS, TUMOR INFILTRATING LYMPHOCYTES AND CANCER STEM CELLS IN NEUROBLASTOMA

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Introduction: Cancer stem cells are thought to play a central role in tumor initiation, progression and recurrence. The aim of this study investigate the interaction between neuroblastoma cancer-initiating cells and the elements of the tumor microenvironment which is tumor infiltrating lymphocytes and mesenchymal stem cells.

Methods: isolated from fresh tissue of neuroblastoma surgical material were perfomed single-cell suspension and cultured. CD133+ stem cells were isolated with magnetic insulation from cultured neuroblastoma cancer cells. by using AIMV migration method proved accessing to the migration of tumor infiltrating lymphocytes from fresh tissue fragments to medium and expanded with GCSF and IL-2. Mesenchymal stem cells were isolated with CD54+, CD90+ magnetic insulation. The isolated cells, tumor infiltrating lymphocytes and cisplatin were seeded in multi-well Plates in single quad binary and ternary various combinations. Then the cells viability measured at 24 and 48 hours. Mann Whitney U testi test was used for the comparison of the nonparametric outcomes. P < 0.05 was considered statistically significant.

Results: A total of 20 neuroblastoma samples obtained from patients who are 2 to 168 (mean 39) months old were evaluated with cell culture. Tumor infiltrating lymphocytes and mesenchymal stem cells protect the neuroblastoma cells effect of cisplatin was observed. Tumor infiltrating lymphocytes has no effect on neuroblastoma stem cells but mesenchymal stem cells protect the neuroblastoma stem cells effect of cisplatin was observed.

Conclusion: In this study, interaction with neuroblastoma cancer stem cells and tumor microenvironment cells was investigated ex vivo for the first time. Protection of mesenchymal stem cells form cytotoxic effects of cisplatin the neuroblastoma stem cells effect of cisplatin was observed.

Keywords: Neuroblastoma, cancer Stem Cells, Mesenchymal Stem Cells

Note: This study was supported by Turkish Pediatric Oncology Group Assosiation

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P-003 - IN VITRO EFFECT OF FLUBENDAZOLE ON NEUROBLASTOMA STEM CELLS

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Aim: Despite the development of new treatment options, the prognosis of high-risk neuroblastoma patients remains poor and more than half of NB patients have disease recurrence. Cancer stem cells (CSC) are thought to be responsible for cancer initiation, drug resistance and metastasis so various therapeutic agents have been studied to eliminate this subgroup of cells. Based on this issue we aimed to evaluate the in vitro effect of Flubendazole (FB) on cell proliferation, apoptosis and DNA damage of CD133+ N-myc amplified NB cells.

Methods: Kelly (Nmyc positive) NB cells were cultivated in RPMI medium supplemented with 10% FBS, 1% penicillin/streptomycin, 1% L-glutamine. CSCs were isolated with ferritin coated CD133 monoclonal antibodies at magnetic field. CSCs were incubated with 300 and 500 mM FB for 24 hours. Flow cytometric analysis of cell proliferation was performed by BrdU, apoptosis levels were determined according to cleaved-PARP and DNA damage levels were detected by H2AX expression.

Results: FB increased anti-apoptotic effect in a dose dependent manner (14,7% and 42,5%). However, DNA damage of cells did not show a significant difference (6,9% and 3%). Cell proliferation was less in 500 mM FB treated group (47,8%) compared to 300 mM FB treated group (29,1%). Cell proliferation of control group was 26,9%.

Conclusion: FB showed anti-apoptotic effect on NB CSCs. However it did not cause significant anti-proliferative effect. We suggest that FB should be studied in combination with anti-proliferative agents in further studies.

Keywords: Neuroblastoma, Cancer Stem Cells, Flubendazole
### Cell Proliferation, Apoptosis and DNA Damage Levels of FB treated NB CSCs

<table>
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<tr>
<th></th>
<th>Cell Proliferation (BrdU)</th>
<th>DNA Damage (H2AX)</th>
<th>Apoptosis (cleaved-PARP)</th>
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<tr>
<td>Control</td>
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<td>7.2</td>
<td>4.1</td>
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<tr>
<td>300 mM FB</td>
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<tr>
<td>500 mM FB</td>
<td>29.1</td>
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*Flow cytometric analysis of BrdU, cleaved-PARP and H2AX expression after 300 and 500 mM FB treatment on CD133+ NB stem cells.*

### P-004 - AUTOPHAGY INHIBITION ENHANCES PALLADIUM (II)-BARBITURATE COMPLEX - INDUCED APOPTOSIS IN PROSTATE CANCER

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**Introduction:** Palladium (Pd) (II) complex were shown to have significant anti-tumor activities against different types of cancer cells. Chloroquine (CQ) as an inhibitor of autophagy is used prosurvival and resistance mechanism against cancer cells. Therefore, the combination of these two may be a realistic strategy for new treatment modality.

**Materials and Methods:** The androgen-sensitive human prostate adenocarcinoma cell line LNCaP and the human normal prostate cell line PNT1A were treated with different concentration of Pd (II) complex (1.56-100µM), CQ (0.6-40µM) alone or in combination with CQ. Viability was detected by MTT and ATP viability assays at 24 h and 48 h. Flow cytometry was used to determine the mode of cell death (apoptosis/necrosis/autophagy) responsible for the cytotoxicity in combination treatments. This results were confirmed with fluorescence images using triple staining method (Hoechst 33343, Annexin V, and Propidium iodide). Formation of acidic vesicular organelles (AVOs) was observed by fluorescence microscopy using acridine orange staining. Finally, protein expression levels associated to autophagy and cell death were determined by immunoblotting method and Luminex assay.

**Results:** The combination of CQ (5 µM) and Pd (II) complex (12.5 µM) at 48 h has enhanced cytotoxic activity resulted from the induction of apoptosis (indicated by the presence of pyknotic nuclei, increased the Annexin-V (+) cells and over expression of pro-apoptotic proteins). Importantly, the addition of CQ resulted in the suppression of autophagy that might have contributed to the enhanced cytotoxicity. In addition, PI3K/AKT/mTOR-related protein expression were altered after combination treatments. Treatment with Pd (II) complex alone resulted in increased AVOs formation, but the effect was potentiated by CQ-pretreatment.
**Discussion:** The combination of Pd (II) complex and CQ enhances apoptotic cell death, possibly via the inhibition of autophagy. This combination may be regarded as a novel and effective approach for the treatment of prostate cancer.

**Keywords:** Autophagy, Prostate Cancer, Apoptosis

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**P-005 - INHIBITION OF THE WNT SIGNALLING PATHWAY SENSITIZES BREAST CANCER STEM CELLS TO PD(II) COMPLEX-INDUCED APOPTOSIS**

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**Introduction:** Cancer stem cells (CSC) are a rare cell population found in the tumor and responsible for drug resistance. Wnt signaling pathway is re-activated in CSCs and has an essential role in cell survival, self-renewal and proliferation. Therefore, elimination of these cells along with the other cancer cells is crucial for the successful treatment. In the present study, we evaluated the mechanism of cell death induced by palladium(II) complex, \( [\text{PdCl(terpy)}](\text{sac}) \cdot 2\text{H}_{2}\text{O}] \), synthesized at the Uludag University and its combination with niclosamide which is an inhibitor of Wnt signaling pathway in breast cancer stem cells.

**Material and Methods:** Breast cancer stem cells (CD44+/CD24- cells) were propagated from parental MCF-7 cells and allowed to form mammosphere structures. After then, cells were pre-treated with Niclosamide (at the dose 1,5 μM) for 24h followed by exposure to Pd(II) complex (50μM) for 24 and 48h. Altered expression of proteins associated with stemness (Oct-4 and Bmi-1), Wnt signalling (LRP6, p-LRP6, Axin1, Naked2, Dvl3, Wnt5a/b, β-katenin) and cell death (Fas, PARP, Bax, kaspaz 8, p-c-Jun, p-SAPK/JNK, RIP1, Atg5, Beclin1, LC3II, p62/SQSTM1) were analyzed with western blotting.

**Results:** It was shown that the combinatorial treatment decreased the LRP6, p-LRP6, Dvl3, Axin1, β-katenin levels and increased the Naked2 (Wnt antagonist) levels compared with the effect of complex or Niclosamide alone, implying the inhibition of Wnt signalling in a most effective manner. Stemness related proteins were also decreased. Apoptosis was induced via the activation of caspase 8 and cleavage of PARP as well as activation of stress-related proteins (JNK and c-Jun). Inhibition of necroptosis and autophagy was also observed.
Discussion: Inhibition of Wnt signalling resulted with the enhanced apoptotic activity possibly due to suppression of autophagy and necroptosis. Therefore, this combination may be regarded as an effective approach to eliminate breast CSCs although in vivo experiments are required for the proof-of concept.

Keywords: Breast cancer stem cell, Wnt Signalling, Targeted therapy, Apoptosis

This study was supported by TUBITAK (The Scientific and Technological Research Council of Turkey) for the project that was numbered 212T147.

P-006 - TARGETING EPIGENETIC REGULATION OF HISTONES WITH VALPROIC ACID LEADS TO APOPTOSIS IN BREAST CANCER STEM CELLS

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Introduction: Cancer stem cells (CSCs) are a small subpopulation of cells within the tumor and lead to disease relapse because of an acquired resistance to apoptosis. In particular, epigenetic changes play a crucial role in the regulation of stemness and also have been implicated in the development of drug resistance. Therefore, in the present study, we focused on the cytotoxic and apoptotic activity of valproic acid (VPA) as an inhibitor of histone deacetylases (HDACs) against breast CSCs.

Material and Methods: Elevated expression of stemness markers were determined by western blotting in mammospheres (MCF-7s, cancer stem cell-enriched population) propagated from parental MCF-7 cells. Anti-growth activity of VPA was determined via ATP viability assay. The sphere formation assay (SFA) was performed to assess the inhibitory effect of VPA on the self-renewal capacity of MCF-7s cells. Acetylation of histon H3 was detected with ELISA assay. Cell death mechanism was determined via fluorescence microscopy (Hoechst dye 33342 and Propidium iodide staining), M30 (apoptosis) and M65 (primary or secondary necrosis) ELISAs and cytofluorimetric analysis (caspase 3/7 activity and annexin-V-FITC staining).

Results: VPA exhibited anti-growth effect against both MCF-7 and MCF-7s cells in a dose (0.6-20 mM) and time (24, 48, 72h) dependent manner. As expected, MCF-7s cells were found more resistant to VPA than MCF-7 cells. It was observed that VPA prevented mammosphere formation at lower doses (2.5 and 5 mM) in which acetylation of histon H3 was increased. VPA also increased the M30 and M65 levels at the same doses and secondary necrosis (late stage of apoptosis) was evidenced by nuclear pyknosis with propidium iodide staining positivity, annexin-V-FITC positivity and caspase 3/7 activation.

Discussion: Our results suggested that inhibition of HDACs sensitizes breast CSCs...
to apoptosis and targeting the epigenetic regulation of histones may hold significant promise for successful treatment of breast cancer.

Keywords: Breast cancer stem cell, Epigenetic regulation, Histone Acetylation, Apoptosis

P-007 - INVESTIGATION OF THE CYTOTOXIC AND APOPTOTIC EFFECTS OF COMBINATION OF THE HISTONE DEACETYLASE INHIBITOR AND WNT PATHWAY INHIBITOR ON LUNG CANCER CELL LINES

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2Uludag University, Faculty of Medicine, Department of Medical Biochemistry, Bursa, Turkey

Background and Aim: Epigenetic changes including histone modifications play an important role in the carcinogenesis. In this study, cytotoxic and apoptotic effects of histone deacetylase inhibitor valproic acid (VPA) in combination with Wnt pathway inhibitor niclosamide was investigated on lung cancer cell lines A549 and H1299.

Materials and Methods: Cytotoxic effects of VPA (500 µM) and niclosamide (0.08, 5 µM) combination was determined on A549 and H1299 cell lines after 72 h treatment by SRB and ATP cell viability assays. Nuclear morphology and plasma membrane integrity were visualized via Hoechst 33342, Propidium Iodide fluorescent staining for 72h. The change in mitochondrial transmembrane potential was evaluated using the cationic fluorescent indicator JC-1. Oxidative stress parameters were detected by H2DCFDA staining and flow cytometry.

Result: The combination of VPA with niclosamide dramatically decreased the viability, relative to either compound alone in A549 and H1299 cell lines. Also, the combination further increased mitochondrial membrane depolarization in cells. Moreover, combination treatment was increased oxidative stress dramatically in both cell lines, has been showed by flow cytometry and H2DFCA staining.

Conclusion: The combination of VPA and niclosamide enhances apoptotic cell death thorough disturbance of mitochondrial membrane potential and oxidative stress. Therefore, this novel combination deserves further attention for proof of concept in the treatment of lung cancer.

Keywords: Investigation, Cytotoxic, Apoptotic

This study is supported by University of Uludag with a project number of OUAP(F)-2015/15.
P-008 - GENOTOXIC ACTIVITY OF PALLADIUM(II) SACCHARINATE COMPLEX OF TERPYRIDINE ON BREAST CANCER STEM CELLS

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²Uludag University, Faculty of Arts and Sciences, Department of Chemistry, Bursa, Turkey
³Uludag University, Medical School, Department of Clinical Biochemistry, Bursa, Turkey

Introduction: In the present study, we determined the potential DNA-damaging effect of palladium(II) complex, [{PdCl(terpy)}(sac)-2H2O], synthesized at the Uludag University on MCF7 breast cancer cells and mammospheres (cancer stem cells-enriched population) derived from MCF7 cells.

Material and Methods: First, MCF7 cells and mammospheres were treated with Pd(II) complex (50 µM) for 12, 24 and 48 hours. The results of comet assay were analyzed by using Microsystem Comet Software Program (Argenit, Istanbul, Turkey) and comet length, tail length, percentage of DNA in the comet tail and head, Genetic Damage Index (GDI) and percentage of damaged cells (PDC) parameters were calculated.

Results: Based on the analyses, it has been found statistically significant increase in comet length, tail length and percentage of DNA in MCF7 cells in a time dependent manner compared to mammospheres (p<0.001, p<0.001 and p<0.005, respectively). The percentage of DNA in head significantly decreased in MCF7 cells (p<0.005) and percentage of damaged cells and genetic damage index were also found more higher when compared to mammospheres (p<0.0001 and p<0.0001, respectively).

Discussion: Results indicated that MCF7 cells were more sensitive to complex than mammospheres. Hence, novel approaches are urgently needed to also target CSCs.

Keywords: Genotoxic, Activity, Palladium(II)

This study was supported by TUBITAK (The Scientific and Technological Research Council of Turkey) for the project that was numbered 212T147.
Conclusion: These data suggest that m-MDSCs derived from metastatic 4T1 tumor-bearing mice are able to confer EMT/CSC phenotype on tumor cells, while g-MDSCs are more potent in inducing epithelial phenotype and proliferation in tumor cells.

Keywords: Breast cancer, Metastasis, Myeloid-derived suppressor cells, EMT plasticity

P-010 - INFLAMMATORY CYTOKINE NETWORKS MODULATE HOST IMMUNE RESPONSES, GENERATING A PERMISSIVE MICROENVIRONMENT FOR METASTASIS

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Aim and Background: Establishment of pre-metastatic niche in distant organs is thought to be an important step during metastasis. Previous studies from our team and others established that tumors with activated inflammatory cytokines display aggressive epithelial mesenchymal (EMT) and cancer stem cell (CSC) phenotype.

Material and Method: In order to investigate the link between inflammation and breast cancer metastasis in syngeneic mouse model, we chose metastatic and non-metastatic murine tumors. 4T1 murine mammary tumor isolated from a BALB/c mouse has been characterized as metastatic while EMT6 as non-metastatic. To compare cytokine secretion between EMT6 and 4T1 cells, we collected conditioned media and performed cytokine antibody array. We also utilized flow cytometry analysis to check the infiltration of myeloid derived suppressor cells (MDSC) in tumor sites.

Results: Our data showed that metastatic 4T1 tumor cells highly express inflammatory cytokines and growth factors, compared to non-metastatic EMT6. Mouse transcriptome analysis showed 781 genes that are differentially expressed between 4T1 and EMT6 tumors. We found a significant accumulation of both mMDSC and gMDSC in bone marrow, spleen, lung and tumor of 4T1 tumor-bearing mice. To examine whether 4T1 tumor secreted soluble factors can enhance metastasis in vivo, we intravenously injected EMT6 cells into mice with or without pre-treatment of conditioned medium (CM) from 4T1 tumor cells. It showed that injection of 4T1-CM significantly enhanced the metastatic ability of EMT6 tumors in lungs.

Conclusion and Discussion: These studies would provide an evidence that tumor secreted cytokines are capable of modulating early immune responses and establishing a pre-metastatic niche through recruitment of MDSCs and the reciprocal interactions of tumors and MDSCs facilitating metastatic steps such as dissemination and colonization in secondary organs.

Keywords: Breast cancer, Cytokines, Myeloid-derived suppressor cells, Premetastatic niche
P-011 - ANTI-METASTATIC EFFECT OF SINAPIC ACID IN PC-3 HUMAN PROSTATE CANCER CELL LINE

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Introduction: Prostate cancer is one of important health problems diagnosed in men in many populations. Patients with prostate cancer has a good prognosis when it does not spread to other organs. However, the survival rate of patients sharply declines when it metastasizes. The treatment of the disease is often difficult due to metastasis. The aim of the study was to investigate the anti-metastatic effect of sinapic acid, is a phenolic compound and found in various vegetables and fruit species, in the PC-3 human prostate cancer cells.

Materials and Methods: Cytotoxic effect of sinapic acid was determined by using XTT assay. Total RNA isolation of control and dose groups (IC_{50} dose of sinapic acid) was conducted using TRIzol Reagent. Expressions of important genes in metastasis including MMP-2, MMP-9, TIMP-1, TIMP-2, CDH1 and CDH2 were investigated in control and dose groups by qPCR.

Results: IC_{50} dose of sinapic acid was detected as 1 mM for 72h in PC-3 cells. According to qPCR results, a significant increase in the expressions of TIMP-1 and CDH1 genes, and a significant decrease MMP-9 gene were observed in the dose group, compared with the control group cells.

Discussion: It is thought that sinapic acid demonstrates anti-metastatic activity by regulating expression of important genes in metastasis on PC-3 cells. Furthermore, more detailed studies should be conduct to illuminate molecular mechanism of anti-metastatic activity of sinapic acid on prostate cancer.

Keywords: Metastasis, PC-3 cells, Prostate Cancer, Sinapic acid

P-012 - THE COMBINATION OF FERULIC ACID AND GEMCITABINE AFFECTS METASTASIS IN PC-3 HUMAN PROSTATE CANCER CELLS

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²Department of Medical Biology, Faculty of Medicine, Pamukkale University, Denizli, Turkey

Introduction: Prostate cancer, the second causing of cancer-related death in men, can metastasize to the bone, lung and liver. Metastasis, associated with the aggressiveness of tumors and high mortality rate, is a multistep process. Ferulic acid (FA) is known as an abundant phenolic compound found in various fruit and vegetables. In this study, we aimed to determine the effects of FA and gemcitabine used either as a single agent or as a part of combination treatment in prostate cancer therapy, on metastasis in PC-3 human prostate cancer cell line.

Materials and Methods: The cytotoxic effects were determined by using XTT method after the treatment with FA, gemcitabine and combination of both of them. Total RNA was isolated with TRIzol Reagent. Expressions of genes are important in metastasis including MMP-2, -9, TIMP1, 2, CDH1, CDH2, COL4A3, VEGFA and HIF1A were evaluated in four groups by qPCR.

Results: The IC_{50} doses of FA and gemcitabine were found to be 300 μM and 50 μM for 48h in PC-3 cells, respectively. For determination of combination effect, PC-3 cells were treated with <IC_{50} doses (200 μM FA and 35 μM gemcitabine). When compared with the control group, qPCR results showed a significant decrease in the expressions of MMP-2 and VEGFA genes; whereas, TIMP-1 gene expression was decreased in the FA treatment group. After the treatment with gemcitabine, the expression of TIMP-1, TIMP-2 and CDH1 genes were significantly elevated, and the expression of MMP-2 and VEGFA genes were significantly downregulated. Furthermore, combination of FA and gemcitabine significantly increased expression of TIMP-1, TIMP-2 and CDH1 genes with higher fold change compared with other groups.

Discussion: In conclusion, it is thought that combination of FA and gemcitabine affected expression of metastasis genes with higher level compared with the single treatments in PC-3 cells.

Keywords: Ferulic acid, Gemcitabine, Metastasis, PC-3 cells, Prostate Cancer
P-013 - IDENTIFYING AND TARGETING NON-CODING RNAS TO INHIBIT LUNG METASTASIS IN TRIPLE NEGATIVE BREAST CANCER

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Introduction: Triple negative breast cancer (TNBC), the most aggressive breast cancer subtype, has high incidence rate of lung metastasis. Not only protein coding transcripts, but also noncoding transcriptome, such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), have active roles in cancer progression and metastasis. Additionally, lncRNAs can act as sponges for miRNAs. Here, we aimed i) to construct the first mRNA-miRNA-lncRNA network controlling metastasis in TNBC, and ii) to prevent lung metastasis by targeting identified central candidate genes.

Material and Method: We established primary tumor and human-in-mouse (HIM) and mouse-in-mouse (MIM) lung metastasis models using TNBC cell lines in nude and Balb/c mice, respectively. We visualized both primary and metastatic tumors using in vivo imaging system, harvested tumors and performed both RNA and small RNA sequencing. We obtained differentially expressed miRNAs, mRNAs and lncRNAs between primary and metastatic tumors. Using several bioinformatics tools, we did enrichment analyses, miRNA target predictions, and network construction.

Results: 45 and 91 miRNAs were differentially expressed between primary and metastatic tumors in HIM and MIM models, respectively. Moreover, 1127 and 3350 mRNAs, and 85 and 111 lncRNAs were differentially expressed in HIM and MIM models, respectively. DAVID bioinformatics tool showed significant enrichment of metastasis-related processes in metastatic tumors.

Discussion: Our study deciphered important candidates mediating lung metastasis in TNBC. Currently, we are integrating these 3 layers of data together with target predictions to construct the first mRNA-miRNA-lncRNA network controlling metastasis in TNBC. Identified central candidates will be tested in in vitro and in vivo metastasis assay models. Ultimately, our study will uncover lncRNAs that can be used as potential targets and/or biomarkers in breast-to-lung metastasis.

Keywords: Long non-coding RNA, Lung Metastasis, microRNA, Network Analysis, Triple negative breast cancer

This study is approved by Animal Ethics Committee of Bilkent University with decision number 2014/39. This study is supported by TUBITAK-CNRS Bilateral Grant with project number 214S364.
P-014 - INCREASED EXPRESSION OF TGF-β1 AND TSLP IN HIGHLY METASTATIC BREAST CANCER CELL LINES

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Aim: We performed proteomic analysis of exosomes derived from conditioned medium of breast cancer cells metastasized to vital organs such as brain, liver and heart. We found 1855 peptides in exosomes. Of these peptides, 225 of them, which corresponded to 83 proteins, were significantly altered in metastatic cell lines compared to non-metastatic cells. To support these results, we investigated the expression of two proteins which found higher in metastatic cell lines; Transforming growth factor beta (TGF-β1) and Thymic stromal lymphopoietin (TSLP). TGF-β1, a secreted protein, promotes cancer invasion and metastasis by inducing epithelial-to-mesenchymal transition. TSLP induces growth and metastasis of breast and pancreatic cancers through activating CD4+ T cells, inducing Th2-skewed immune responses and production of immunosuppressive factors.

Materials and Methods: We previously isolated liver, brain and heart metastatic cells of 4T1 murine breast carcinoma and named them as 4TLM, 4TBM and 4THM, respectively. In addition, we used non-metastatic 67NR breast cancer cells. In order to measure TGF-β1 levels, we treated the cell’s supernatants with acid and base as recommended by the ELISA kit protocol; however, this protocol was unsuccessful. Afterwards, we used OASIS HLB 6cc (200 mg) Extraction Cartridges to concentrate and dissociate the proteins. TSLP levels measured directly from the conditioned mediums using ELISA.

Results: Depending on the subset of metastatic cells TGF-β1 levels were 6-13 fold higher compared to non-metastatic cells. Metastatic cells secreted markedly higher levels of TSLP (>15 fold).

Conclusions: Given the previous findings, our results suggests that increased levels of TGF-β1 and TSLP in exosomes of metastatic breast cancer cells mediate metastatic process and further studies targeting these molecules are required to determine possible therapeutic values of TSLP and TGF-b antagonists.

Keywords: breast cancer, metastasis, TGF-β1, TSLP

This study was supported by TUBİTAK Grant no: 115Z286.

P-015 - COMPARISON OF MIGRATION AND INVASIVE PROPERTIES OF SW-620 AND HT-29 COLON CANCER CELLS

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Introduction: Colon cancer is the third leading disease of death in the world wide especially industrial countries as well as developing ones. Current therapeutic approaches for colon cancer generally have variable efficiency, develop metastasis and drug-resistance, and have high toxicity to normal tissues. The aim of this study to clarify effects of phenolic compounds on metastatic and invasive properties of different colon cancer cell lines.

Material and Methods: SW-620 cells and HT-29 cells were grown in Leibovitz’s L-15 Medium and McCoy’s 5a medium, respectively and supplemented with 10% fetal bovine serum and 2 mM glutamine. Cytotoxicity of tannic acid on SW-620 cells and HT-29 cells were determined with Alamar blue and IC50 value was calculated. The effects of tannic acid on proliferation, invasive potential and metastasis of cancer cell lines were analyzed by wound healing, colony formation and matrigel chamber assays, respectively.

Results: In this study, we found that TA was found potential inhibitor against SW-620 than HT-29 and inhibited proliferation of both cell lines a concentration-dependent manner with an IC50 of 7.2 μM and 37.6 μM. TA treatment of cells led to a significant 90% and 85% decrease in motility and metastasis of PC-3, respectively. In addition, tannic acid treatment of SW-620 and HT-29 cells inhibits migration 82% and 73%, respectively as well as 73% and 55% inhibition were observed in invasive potential of cells.

Conclusions: These results show that tannic acid has more inhibitory action on the proliferation, invasive and migration properties of colon cancer cell line SW-620 than HT-29 cells. SW-620 cells has the highest migration potential in all colon cancer cells. Hence it was shown that tannic acid have protective capacity upon the colon cancer.

Keywords: Colon cancer, SW-620, HT-29, Tannic acid, migration

This work was supported by a grant from Selcuk University-BAP, Project No: 14401031, TURKEY.
P-016 - ANALYSING OF SRP9 GENE EXPRESSION AND CORRELATION WITH CLINICOPATHOLOGIC PARAMETERS IN HUMAN BREAST CARCINOMA

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Aim: Recently, post-transcriptional modification events, such as A-to-I RNA editing, catalysed by ADAR, has been revealed a new player in cancer, by changing the nucleotide sequence of target RNAs and introducing A-to-I/G “mutations”. Analysis, comparing genome and transcriptome sequencing revealed a generalized increase of A-to-I RNA editing events in metastatic breast cancer; particularly, the signal recognition particle 9 kDa (SRP9), involing in protein secretory pathways, showed a high frequency of adenosine-to-guanosine transition, which alters its protein sequence. Interestingly, when analysed by gene-expression array, the lobular breast cancer metastasis showed an upregulation of ADAR1 mRNA. From this point of view, the purpose of this study is to determine SRP9 gene expression levels in breast cancer tissue and to research its relation with clinical findings related to breast cancer by presenting the connection of these data with general increase in pre-mRNA modifications related to AID in breast cancer pathogenesis and their results. This study was supported by Istanbul University BAP unit; Project No: 34625.

Methods: The expression levels of SRP9 gene (mRNA) in tumor and corresponding adjacent normal tissue samples obtained from 50 breast cancer patients were analyzed by ‘Quantitative Real Time-PCR’ and 2-ΔΔCT method and statistically evaluated by ‘Independent-t test’ to investigate the possible role of SRP gene in breast cancer. The comparision between the changes in SRP9 gene expression levels with clinical parameters were statistically analyzed by ‘chi-square test’ to investigate any possible relations.

Results: We found that there was a decrease of SRP9 gene expression levels in 18 (36.0%), increase in 23 (46.0%) and no change in 9 (18%) of the tumor tissue samples belonging to patients. There was an association between the increase of SRP9 gene and ER, PR expression (positive) respectively; (p=0.001*) and (p=0.009*).

Conclusion: According to our findings, SRP9 gene could have a potential role in increased expression of ER and PR in human breast carcinoma.

Keywords: ADAR1, Post-Transcriptional Modifications, SRP9 mRNA, Clinopathological Parameters, Breast Cancer Tissue
P-017 - EFFECTS OF TRPV1 AGONISTS AND ANTAGONISTS ON PROLIFERATION OF METASTATIC BREAST CARCINOMA CELLS

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Introduction: Transient Receptor Potential Cation Channel Subfamily V member 1 (TRPV1) or as known as capsaicin receptors mostly found on peripheral and central nervous system. There are few studies examining the expression and role of TRPV1 channels in cancer cells. The purpose of this study was to determine the effects of TRPV1 agonists and antagonists on survival and proliferation of metastatic breast carcinoma.

Materials and Methods: In this study, heart (4THM) and brain (4TBM) metastatic cells of 4T1 breast carcinoma cells which originally obtained from spontaneously formed breast cancer in a Balb-c mouse were used. First of all, expressions of TRPV1 on these cell lines were determined by western blot. A Balb-c mouse brain cortex was used as a positive control. Afterwards, 4THM and 4TBM cells were cultured on 96-well plate (500 and 1500 cell per well) and then treated with different concentrations (0.1 μM, 1 μM, 10 μM) of TRPV1 agonists (Resiniferotoxin, Capsaicin, MSK-195) and antagonists (Capsazepine, AMG-9810). Changes in cell proliferation were determined by using WST-1 after 72 hours incubation.

Results: Among TRPV1 agonists Capsaicin was the most effective and Resiniferatoxin was the least effective agent in inhibition of cell proliferation. Capsaicin dose-dependently inhibited cell proliferation in both 4THM and 4TBM cells. Surprisingly, TRPV1 antagonist AMG-9810 at 10 μM concentration markedly suppressed proliferation of metastatic cells. The effects of the other antagonist, Capsazepine was somewhat similar to AMG-9810 but the anti-proliferative effect was less marked.

Conclusion: Our findings show that TRPV1 channels are present on metastatic breast carcinoma cells and regulate cell proliferation. Further studies are required to determine the mechanisms of TRPV1-induced alterations on cell proliferation.

Keywords: AMG-9810, breast cancer, capsaicin, cell proliferation, TRPV1

This study was supported by TUBITAK-COST action, Grant No: 115S943.

P-018 - THE EFFECTS OF PI3Kα AND PI3Kβ INHIBITORS ON MIP-2 AND KC SECRETION FROM METASTATIC BREAST CARCINOMA

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Background: Phosphoinositide 3-kinases (PI3Ks) play key regulatory roles in many cellular processes including cell survival, proliferation, differentiation of cancer cells. Class I PI3Ks are composed of a p110 catalytic subunit, of which there are 4 isoforms (p110α, p110β, p110δ, and p100γ), and a p85 regulatory subunit. MIP-2 is a member of the CXC chemokine family, the mouse homologue of CXCL2, activates CXCR2 inducing angiogenesis and tumor progression. KC is a mouse homologue of CXCL1 and involved in the processes of angiogenesis, arteriogenesis, inflammation, wound healing, and tumorigenesis. Intracellular mechanisms involved in MIP-2 and KC secretion from cancer cells are not entirely known. The goal of this study is to determine the role of PI3Kα and PI3Kβ on MIP-2 and KC secretion from breast carcinoma cells metastasized to brain (4TBM) and heart (4THM).

Materials and Methods: BYL719 is a selective PI3Kα inhibitor, and TGX-221, a potent, selective, and cell-permeable inhibitor of PI3K p110β were used. 4THM and 4TBM cells were treated with 0,1-10μM doses of BYL719 and TGX221. Changes in MIP-2 and KC levels and cell proliferation were determined using Elisa and WST-1 respectively.

Results: BYL719 treatment dose dependently suppressed proliferation of 4TBM and 4THM cell when cells were seeded as 500 cells/96 well plate. Secretion of MIP-2 and KC was markedly suppressed by 10μM BYL719. A partial inhibitory effect was also observed at doses of 1 μM. These effects were more pronounced in 4TBM cell line. TGX221 did not effect cell proliferation and MIP-2 secretion both cell lines. On the other hand TGX221 effectively suppressed KC secretion in 4TBM cells.

Conclusion: These results demonstrate that increased PI3-Kα activity might be involved in MIP-2 and KC secretion.

Keywords: Breast Cancer, MIP-2, BYL719, TGX221, PI3-K
P-019 - MUTATIONAL ANALYSIS OF AMINO TERMINAL DOMAIN OF INSULIN RECEPTOR SUBSTRATE-1 (IRS1) GENE IN GLIOBLASTOMA MULTIFORME PATIENTS

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Aim: In the recent studies about cancer-diabetes relations, Insulin Receptor Substrate (IRS) proteins that are mediator protein family and have pivotal roles in insulin signal pathway have become the focus of attention. Because IRS-1 is widely expressed in human tissues and has important role in insulin signaling, it is most studied member of this protein family. Due to becoming one of targeted protein and gene in both cancer and diabetes studies; genomic changes, expression/activation profiles and functional status of IRS-1 have been studied by a lot of researchers. We have detected some mutations in carboxy terminal domain of IRS-1 in our previous study. In this study, we want to also determine presence or absence of mutation in PH (pleckstrin homology) and PTB (phosphotyrosine binding) regions that are located in amino terminal domain of this protein.

Material and Methods: In this study, we have isolated and sequenced genomic DNAs from tumor samples of 28 Glioblastoma Multiforme tumors and 6 control tissues were obtained by autopsy and we looked for the presence or absence of mutations in the region PH and PTB domains.

Results: On the basis of our results, we detected p.A124S heterozygote changes in PH domain in 1 patient and p.G234G, c.702G>A heterozygote changes in PTB region of IRS1 in 1 of 28 patient samples compare to the controls.

Discussion and Conclusion: PH and PTB regions have a pivotal role in activation of IRS1 and diversity of signaling. Therefore, genomic changes in these regions may lead to tumorigenesis. Our results suggest that heterozygote changes in these regions of IRS1 may be involved in the modulation of IRS1 functions and could be relevant to Glioblastoma Multiforme.

Keywords: Insulin Substrate Proteins, IRS, Cancer, Glioblastoma Multiforme

P-020 - ASSESSMENT OF LOW DOSE EFFECT OF DOXORUBICIN ON APOPTOSIS AND MULTIDRUG RESISTANCE IN MCF-7 CELLS

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Introduction: As breast cancer is the most common cancer type amongst women worldwide, natural and synthetic drugs have been continuously developed and used as chemotherapeutic agents. It has been proved that many cancer types arise as a result of malfunctioning genes coding for anti-apoptotic proteins, apoptosis inhibitors or tumor suppressors. Doxorubicin (DOX) is an anthracycline based chemotherapeutic agent which inhibits topoisomerase II activity and halts replication by intercalating to the DNA helix. Alongside with various types of cancer, DOX is clinically used in the treatment of breast cancer. Multidrug resistance (MDR) is defined as simultaneous resistance towards different drugs which do or do not demonstrate structural resemblance and have different effects on their molecular targets. P-glycoprotein is a membrane protein coded by ABCB1 (MDR-1) gene which acts as an ATP-dependent pump and has a role on efflux of numerous drugs including DOX.

Methods: In this study MCF-7 breast cancer cells were treated with varying doses (50 nm-20 μM) of DOX and cell viability was determined using SRB assay. Subsequently, Tali® assay was performed to define the percentage of apoptosis and MDR assay was carried out to investigate the drug efflux level of DOX in MCF-7 cells.

Results: Cell viability decreased significantly following application of DOX (IC50=10 μM). The population of apoptotic cells were slightly elevated with treatment of 50, 200 and 800 nm of the drug and apoptotic cell ratios were measured to be 6.3, 10 and 17.7 %, respectively. Additionally, efflux of DOX was shown to be dramatically increased when MCF-7 cells were treated with same doses of the drug.

Conclusion: It was demonstrated that at low doses of DOX treatment, cell viability was decreased only partially through apoptosis and the dramatic increase detected in DOX efflux may contribute to the reduced apoptotic response in MCF-7 cells.

Keywords: Apoptosis, Breast Cancer, Doxorubicin, Multidrug Resistance
P-021 - GOLD NANOPARTICLE-SIRNA MEDIATED NF-κB SILENCING IN PROSTATE CANCER

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Background and Aim: Nuclear Factor kappa B (NF-κB) is a well-known transcription factor which is activated in hematological malignancies and solid tumors. We found NF-κB overexpression using gene expression microarray in our previous studies about B-CLL after ionizing radiation exposure and prostate, papillary thyroid, breast cancers. Inhibition of NF-κB activation may allow selective killing of cancer cells. Based on this hypothesis, we aimed silencing NF-κB2 gene via gold nanoparticles functionalized with siRNA (small interfering RNA) in androgen-dependent prostate cancer cell line, NE-1-8.

Materials and Methods: Proliferation assay was performed using xCELLigence RTCA DP Real Time Cell Analyzer to determine the optimal cell concentration. The cells were transfected with gold nanoparticle-siRNA at a concentration of 2 nM, 4 nM and 8 nM at the 25th hour. Untransfected cells (cells only) were used as a control. The 24-hours post-transfection effect of gold nanoparticle-siRNA on NF-κB2 and apoptosis related genes (Bcl-2 and Bax) were determined by quantitative real-time PCR. IC50 value was calculated by cytotoxicity assays performed on the xCELLigence RTCA DP.

Results: We observed decreasing concentrations of cells transfected with gold nanoparticle-siRNA at a concentration of 8 nM, 4 nM and 2 nM from the 33rd, 39th and 45th hours respectively, whereas there was no change in cells only. NF-κB2 gene expression levels were decreased (1,352 folds in 2 nM, 3,063 folds in 4 nM) after transfection. Gene expression changes of Bcl-2 and Bax showed increased apoptosis with increasing molarity. Cytotoxicity assays determined IC50 value as 2.26974E-009 M.

Discussion and Conclusion: Our results suggest that gold nanoparticle-siRNA mediated NF-κB2 silencing may be an effective gene therapy for prostate cancer treatment. Further studies including modifications of gold nanoparticles for targeting tumor cells in tissue cultures and xenograft models for showing systemic effects of functionalized gold nanoparticles are needed.

Keywords: Cancer, Prostate Cancer, NF-κB, siRNA Delivery, Gold Nanoparticles
P-022 - SYNTHESIS OF PLGA COATED MAGNETIC NANOPARTICLES FOR CO-DELIVERY OF CANCER DRUG AND VITAMIN E TPGS

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Introduction: Magnetic nanoparticles (MNPs) which are used for targeted delivery of anticancer drugs under external magnetic field; maximize the efficacy of the drug, minimize its side effects and reduce systemic toxicity. Vitamin E TPGS, being a water-soluble derivative of natural Vitamin E has advantages in drug delivery such as extension of the half-life of the drug in plasma and enhancement of the cellular uptake of the drug. In the current study PLGA coated magnetic nanoparticles were designed for co-delivery of anti-cancer drug Doxorubicin and vitamin E TPGS.

Materials and Methods: Magnetic nanoparticles were synthesized by co-precipitation method. Doxorubicin and vitamin E TPGS loaded magnetic PLGA nanoparticles were produced by nanoprecipitation method. Synthesized nanoparticles were characterized by using FTIR, zeta potential, VSM, DLS, TGA, TEM analyses. Drug loading and release profiles were studied in vitro. Internalization of nanoparticles by MCF-7 cells was visualized under light microscope.

Results: According to FTIR and TGA analysis, Doxorubicin and vitamin E TPGS were loaded in PLGA coated MNPs successfully. DLS results confirmed that the size of the nanoparticles were in nano-scale. Moreover, the nanoparticles were superparamagnetic according to VSM results. Drug loading efficiency was high and loaded drug could be released in sustainable form. Finally, it was shown that the nanoparticles could be internalized into MCF-7 cells successfully.

Conclusion: PLGA coated magnetic nanoparticles could be successfully loaded by Doxorubicin and vitamin E TPGS. Internalization studies confirmed that these nanoparticles can be taken up by MCF-7 cells. These results showed that PLGA coated magnetic nanoparticles can be a suitable drug carrier for co-delivery of Doxorubicin and vitamin E TPGS.

Keywords: Magnetic Nanoparticles, Targeted Drug Delivery, Vitamin E TPGS

P-023 - THE CYTOTOXIC EFFECTS OF GREEN SYNTHESIS SILVER NANOPARTICLES ON MCF-7 CELL LINE

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In this study, silver nanoparticles (AgNPs) was obtained by using green synthesis method in the presence of Pomegranate (Punica granatum L). In this method, an aqueous extraction of Pomegranate (Punica granatum L) was mixed with AgNO3 for AgNPs synthesis. The resulting Ag NP’s cytotoxic effect have been investigated by the help of MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) test. After 24 hour incubation period at 20 mg/ml Ag NPs, IC50 of human breast cancer cell line (MCF7) were found to inhibit the proliferation. These results showed that Ag NPs supplemented with pomegranate have revealed the growth suppressive properties.

It was thought that Ag NPs supplemented with pomegranate may help the antiproliferative effect on MCF7 cell line.

Keywords: MCF-7, Pomegranate (Punica granatum L), MTT, Green Synthesis
P-024 - 6-(4-METYLPHENYL-8-(4-CHLOROPHENYL)IMIDAZO[1,2-A]PYRAZINE: A MOLECULE FOR TELOMERASE INHIBITION

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Objective: Imidazopyrazine derivatives have been studied for their curative effects on some diseases like cancer and neurological problems; also, some of these molecules have been patented. Primary human cells exhibit limited replicative potential but the cancer cells divided indefinitely with passage in culture. This immortality is mainly a result of telomerase activity. We investigated the possible telomerase inhibitor effect and possible mtDNA damage action of two imidazopyrazine derivatives 6-(4-Methylphenyl-8-(4-chlorophenyl)imidazo[1,2-a]pyrazine and 6-(4-Methylphenyl-8-(4-methoxiphenyl)imidazo[1,2-a]pyrazine.

Material and Methods: Telomerase activities were measured by the PCR-ELISA based TRAP method and mtDNA damage assays were achieved by quantitative PCR. We used zebrafish as a model organism for our research.

Results: In the application of 6-(4-Methylphenyl-8-(4-chlorophenyl)imidazo[1,2-a]pyrazine (C19H14N3Cl), it was determined that they inhibit telomerase activities to a statistically significant degree. There were no significant differences among the groups in terms of mtDNA damage or copy number.

Conclusion: Cancer cells which can be divided limitlessly due to telomerase activation may lose this characteristics through inhibition of telomerase enzyme. According to these results, it was found out that this compound has the probability to be used as an anti-cancer agent as a result of detailed studies. All zebrafish applications were approved by the Ethical Committe of the Mehmet Akif Ersoy University (27.01.2014/ 57 and 24.02.2015/114).

Keywords: Anticancer drugs, mtDNA damage, Imidazopyrazine derivatives, Telomerase inhibition

P-025 - UKRAIN INHIBITS THE PROLIFERATION AND INDUCES APOPTOSIS OF BREAST CANCER CELLS COMPARED WITH TAMOXIFEN AND DOCETAXEL

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Introduction: Ukrain is the trade-marked name of a semi-synthetic substance derived from the plant Chelidonium majus and promoted as a drug to treat of various cancer types. In this study, we investigated for the first time the effect of anticancer agent ukrain and compared with tamoxifen and docetaxel on MCF–7 (ER+, PR+, HER-2-) and MDA MB-231 (ER–, PR–, HER-2-) cell lines.

Material and Method: A real-time cell analyzer (xCELLigence, Roche Diagnostics GmbH, Penzberg, Germany) was used to evaluate the effects of different doses of ukrain (12.5, 100 µM), Tamoxifen (12.5, 100 µM) and Docetaxel (12.5, 100 nM) on the proliferation of both cell lines and determined IC50 for each drug. Cell blocks were prepared from cultured cells treated with drugs and formalin-fixed paraffin-embedded breast cancer cells were examined histopathologically using Haematoxylin&Eosin staining method. In addition, the expressions of Ki-67, Bel-2, BAX, and cyclin-D1 were assessed immunohistochemically. Statistical analysis was performed GraphPad Prism version 6.05 (GraphPad Software, Inc, CA, USA).

Results: Ukrain inhibits the proliferation in both cell lines time and dose dependent manner. IC50 for Ukrain, Tamoxifen and Docetaxel in MDA-MB–231 and MCF–7 cells were 75 µM at 48h and 34 µM at 111h; 50 µM at 45h and 40 µM at 41h; 32 nM at 60h and 43nM at 40h, respectively. As a results of histopathologic and immunohistochemical analysis, Ukrain decreases Ki-67, cyclin-D1 and increases BAX/Bcl-2 ratio in both breast cancer cells.

Conclusions: The results of this study suggest that Ukrain decreases cell viability of breast cancer cells and induces apoptosis compared with Tamoxifen and Docetaxel. It has been observed more effective for MDA MB–231 compared with MCF–7, thus supporting its use as a therapeutic agent for the treatment of reseptor negative breast cancer.

Keywords: Docetaxel, MCF-7, MDA MB-231, Tamoxifen, Ukrain
P-026 - EFFECT OF CDDO-ME, TAMOXIFEN AND DOCATAXEL ON MCF-7 AND MDA MB 231 CELL LINES

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**Introduction:** Bardoxolone methyl (CDDO-me; methyl-2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oate) is semi-synthetic triterpenoid. CDDO-me acts as an activator of the Nrf2 pathway and an inhibitor of the NF-κB pathway. It also has been used in traditional medicine as an anticancer and antiinflammatory agent. We have investigated the therapeutic efficacy of CDDO-Me, Tamoxifen and Docataxel in MCF–7 (ER+, PR+, HER-2-) and MDA MB-231 (ER-, PR-, HER-2-) cell lines.

**Material and Method:** A real-time cell analyzer (xCELLigence, Roche Diagnostics GmbH, Penzbeerg, Germany) was used to evaluate the effects of different doses of CDDO-me (10-200nM for MCF-7; 0,1-10 µM for MDA MB-231), Tamoxifen (12,5-100 µM) and Docetaxel (12,5-100nM) on the proliferation of both cell lines and determined IC50 for each drug. Cell blocks were prepared from cultured cells treated with drugs and formalin-fixed paraffin-embedded breast cancer cells were examined histopathologically using Haematoxylin&Eosin staining method. In addition, the expressions of Ki-67, Bcl-2, BAX, and cyclin-D1 were assessed immunohistochemically. Statistical analysis was performed GraphPad Prism version 6.05 (GraphPad Software, Inc, CA, USA).

**Results:** CDDO-me inhibits the proliferation in both cell lines time and dose dependent manner compared with Tamoxifen and Docataxel. IC50 for CDDO-me, Tamoxifen and Docetaxel in MDA-MB-231 and MCF-7 cells were 27nM at 65h and 82nM at 43h; 50 µM at 45h and 40 µM at 41h; 32 nM at 60h and 43nM at 40h, respectively. As a result of histopathologic and immunohistochemical analysis that CDDO-me decreases Ki-67, cyclin-D1 and increases BAX/Bcl-2 ratio in both breast cancer cells.

**Conclusions:** CDDO-me has been observed more effective for MDA MB-231 compared with MCF-7 and also has been more influential in both cell lines compared with docetaxel. CDDO-me may be helpful for the therapies of breast cancer according to our findings.

**Keywords:** CDDO-me, Docetaxel, MCF-7, MDA MB-231, Tamoxifen
P-027 - INVESTIGATION OF THE EFFECT OF POMEGRANATE EXTRACT AND PLATINUM NANOPARTICLE COMBINATION ON MCF-7 CELL LINE

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The present study aims to develop an easy and eco-friendly method for the synthesis of platinum nanoparticles using extracts from the medicinal plant, Punica granatum and evaluation of its anticancer properties. The various parts of P. granatum were screened and the root extract was found to have the highest potential for the synthesis of nanoparticles. The shell extracts were able to quickly reduce Pt⁺ to Pt⁰ and stabilized the nanoparticles. The synthesis of nanoparticles was confirmed by UV–Visible spectrophotometry and further characterized using Zeta sizer, Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), transmission electron microscope (TEM) and X-ray diffraction (XRD). The studies of phytochemical analysis of nanoparticles indicated that the adsorbed components on the surface of nanoparticles were mainly flavonoid in nature. Furthermore, nanoparticles were evaluated as cytotoxic against various cancer cell lines and 25 to 100 μg/mL nanoparticles showed good toxicity. The IC50 value of nanoparticles was found to be 25 and 50 μg/mL against MCF-7 cell lines, respectively. Additionally, the apoptotic effect of synthesized nanoparticles on normal and cancer cells was studied using trypan blue assay analysis. The results indicate the synthesized nanoparticle ability to kill cancer cells compared to normal cells.

Keywords: MCF-7, Pomegranate (Punica granatum L), Platinum nanoparticles, MTT, Green Synthesis

P-028 - INHIBITING CERAMIDASE ENZYME PROMOTES CELL DEATH IN HUMAN BREAST CANCER: AN IN VITRO STUDY

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Background: Acid ceramidases are enzymes with a vital role in metabolizing ceramide to sphingosine-1-phosphate that is an antiproliferative metabolite in ceramide pathway. Inhibition of exogenous ceramides with ceramidase inhibitors lead to augmented ceramide levels in cells in turn lead to cell cycle arrest and apoptosis.

Objective: Our study aimed at targeting ceramide metabolic pathway to induce apoptosis in human breast cancer cell line (MCF7) and we examined the antiproliferative and apoptotic activities of ceranib-2, an inhibitor of human ceramidase, on this cell line including with morphological changes caused by ceranib-2.

Material and Methods: Cytotoxic effects of ceranib-2 on MCF7 cells was detected via MTT test system. Dilutions prepared from the stock solution (in DMSO) of ceranib-2 was applied on MCF7 cells (1x10⁴ cells/well) for 24 hours at 37 °C and 5% CO₂ in air. The plates were read on ELISA reader (ELx808), at wavelength of 540 nm (n=3). For detecting the structural alterations IC50 concentration of ceranib-2 was applied on MCF7 cells for 24 hours. Treated cells were stained with Alexa fluor-488 phalloidine and acridine orange and observed under confocal microscope.

Results: Viability percentages and IC50 (13μM) value were determined. Morphological alterations detected on our confocal micrographs were damaged cytoskeleton as hole formation, shrinked cells and fragmented and condensed nuclei as apoptotic hallmarks.

Discussion: According to our results, ceranib-2 caused structural changes in MCF7 cells morphology.

Conclusion: We can conclude that ceranib-2 showed high cytotoxicity on MCF7 cancer cells in low concentrations and may be encouraging in designing of pharmaceutical products helpful in cancer treatment.

Keywords: Cytotoxicity, MCF7, Confocal microscopy
P-029 - APOPTOTIC EFFECT OF PRUNUS SPINOSA FRUIT EXTRACT ON HT-29 COLON CANCER CELL LINE

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Introduction: Colon cancer is the death-causing type of cancer, seen on both men and women. Natural compounds are mostly used lately and have shown prevention, inhibition, and dilatory effects of different cancer types. In this study, it was aimed to investigate apoptotic effect of water extract of Prunus spinosa L. (Rosaceae) herb fruit traditionally known as Güvem or Çakal Ereği, on HT-29 colon cancer cell line.

Materials and Methods: Apoptotic effect on cell proliferation of different concentrations (44 g/ml, 88 mg/ml, and 4500 mg/ml) of water extracts of P. spinosa fruits on HT-29 cell line was investigated for 24 and 48 hours by using EB / AO (ethidium bromide /acridine orange) coloring method. The cells were morphologically evaluated and live, dead, necrotic and apoptotic cells were detected to differentiate the apoptotic effect on cell proliferation.

Results: It was shown that water extracts of P. spinosa fruits inhibited cell proliferation as the concentration and time increase and showed cytotoxic effect after 24 and 48 hour treatment. Cell death was significantly increased for both treatment period and for all tested concentrations compared to control (p<0.01; p<0.001). Probit analyze reports have shown IC50 values as 159.3 and 123.8 µg/ml for 24 and 48 hours treatment periods respectively.

Discussion and Results: The results showed that P. spinosa fruit extract inhibited cell proliferation depending on concentration and time increase and has a cytotoxic and apoptotic effect on HT-29 colon cell line. In the other investigations an anthocyanin compounds obtained from Prunus sp. also have cytotoxic effect on GLC, NCL-H460, A549 cancer cell lines. Further studies obtaining metabolites by using different extraction methods, and detecting activeness of metabolites might enhance economical value of Prunus sp and might have a potential to take part in anticancer pharmaceutical industry as a natural medicine.

Keywords: Prunus spinosa, Colon Cancer, EB/AO, Apoptosis, HT-29

P-030 - EFFECT OF 4-AMINOPYRIDINE ON PACLITAXEL ACTIVITY IN MCF-7 CELL LINE

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Introduction and Purpose: Paclitaxel is a clinically proven antineoplastic agent that induces arrest at G2/ M phase. However its use is limited due to its side effects. There is a need for alternative methods to increase its effectiveness at low doses. It has been shown that pharmacological or genetic block of Kv channels reduces proliferation. 4-aminopyridine(4- AP) is a blocking agents in use. This study aimed to determine changes that may occur in the activity of paclitaxel in MCF- 7 breast cancer cell line preincubated with 4- aminopyridine.

Materials and Method: MCF-7 cells were grown in DMEM supplemented with 10% FBS, 2 mM L-glutamine and 100 U penicillin/streptomycin in 5% CO2 at 37 °C. Cells were seeded at 10000 cells/well into a 96-well plate and incubated overnight. Cells were incubated with 4- amino pyridine or paclitaxel or both for 24 hours and MTT assay was performed.

Results: MCF- 7 cells were incubated with low concentration (6 nM- 8 nM) of paclitaxel for 24 hours. A reduction by % 20 ±3 in cell viability was detected. Incubation with 4 mM and 5 mM concentrations of 4- AP for 24 hours caused % 20± 2 reduction in cell viability. After 24 h preincubation with specified concentration of 4- AP cells were incubated with same doses of paclitaxel for 24 h. Viability assay results showed no increase in paclitaxel activity. Conversely, an increase in cell proliferation of %15± 3 was observed.

Conclusion: There are two probabilities for this effect: 4- AP antagonized the cytotoxicity of paclitaxel by preventing MCF- 7 cells from entering the G2/ M phase, or Ca++ entry (by P2X7 receptors) upon blockage of Kv channels might have interfered with paclitaxel binding to tubuline.

Keywords: 4- Aminopyridine, MCF- 7 Breast Cancer Cell Line, Paclitaxel.
P-031 - INVESTIGATION OF ANTICANCER POTENTIAL OF ANTIHISTAMINE DRUG EBASTINE ON U266 MULTIPLE MYELOMA CELL LINE

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Introduction: A new drug discovery and development process is very difficult, time consuming and costs approximately over $2 billion. However, reposition of drugs increases success rate of the treatment and can save time and money. Multiple myeloma (MM) is a hematological malignancy which is characterized by the uncontrolled growth of plasma cells in the bone marrow. This study shows that a second generation antihistamine drug Ebastine has a significant anticancer effect on MM U266 cell line.

Aim of Study: The aim of this study was to investigate anticancer potential of EBA on U266 MM cell line.

Materials and Methods: Dose and time response studies were performed with fluorescence-based CellTiter Blue Cell Viability Assay (Promega, USA). Caspase-3 levels were detected with PE Active Caspase-3 Apoptosis Kit (BD, USA) and performed on BD Accuri C6 flow cytometer.

Results: The anticancer effect of EBA was found to be time (12, 24, 48 hours) and dose (1-100 µM) dependent (IC50 15.97 ± 0.3 µM at 24 hour). Caspase-3 is one of the most important members of caspase family of cysteine proteases that plays a significant role in apoptosis. Active caspase-3 is used as a marker for cells undergoing apoptosis. To evaluate the apoptotic effect of EBA, cells were treated with 15 µM drug for 24 h. There was a significant increase in Caspase-3 activity of EBA treated cells.

Conclusion: Our results show that EBA has potent growth inhibitory and apoptotic effect on U266 cell line.

Keywords: Cancer, Multiple Myeloma, Anticancer Drug, Antihistamine, Drug repurposing

P-032 - IN VITRO ANTICANCER ACTIVITY OF CHLORPROMAZINE ON 266 MULTIPLE MYELOMA CELL LINE

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Introduction: Multiple Myeloma (MM) is a cancer of plasma cells. MM accounts for 1% of all types of cancer and 13% of all hematological malignancies. Approximately three thousand people each year are diagnosed with MM in Turkey. MM has high morbidity and very low survival rates. Chlorpromazine (CPZ) is a phenothiazine and a marketed antipsychotic medication which is listed as an essential drug by the World Health Organization. It is primarily used to treat psychotic disorders such as schizophrenia, bipolar disorder, attention deficit hyperactivity disorder, nausea and vomiting, anxiety before surgery.

Aim of Study: The main goal of this study was to investigate the anticancer potential of CPZ on U266 MM cell line.

Materials and Methods: CellTiter-Blue Cell Viability Assay (Promega, USA) was used to analyze growth inhibitory effect of CPZ. Then, PE Active Caspase-3 Apoptosis Kit (BD, USA) was used to confirm the apoptotic effect of CPZ. Time- and dose-response studies were performed with nine doses of CPZ in the range of 1 to 100 µM and at 3 time points (12, 24, 48 h). For the apoptosis study, 20 µM CPZ was used.

Results: CPZ showed dose- and time-dependent inhibitory effect on cell viability. IC50 of CPZ was found as 22 ± 2.5 µM. Also, the increase in PE Active Caspase-3 fluorescence signal after treatment indicates that CPZ exerts its cytotoxic effect on cells via inducing apoptosis.

Conclusion: Our results show that CPZ has potent growth inhibitory and apoptotic effect on U266 cell line.

Keywords: Cancer, Multiple Myeloma, Chlorpromazine, Drug Repurposing
P-033 - COMPUTATIONAL ANTI-CANCER AGENT REPURPOSING FOR PANCREATIC CANCER

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Pancreas cancer is one of the known cancer types within late diagnosis and high mortality rates. Despite this fact, there is not sufficient literature information on prevention of disease, early diagnosis or efficient therapy strategies. On the other hand, investments including money, time and labor have gradually increased to fulfill demand of pharmaceutical industry. Elevated studies and improvements on omic technologies and their computational based analysis provide opportunities for further investigations such as drug repurposing.

This study aims to determine anti-cancer agent candidates for pancreatic cancer at transcriptome level.

4 transcriptome datasets (GSE19650, GSE16515, GSE22780, GSE32676) were acquired from Gene Expression Omnibus and chosen according to having healthy versus disease state. Each dataset was statistically analyzed independently to identify differential expressed genes (DEGs) with threshold p-value<0.01. All datasets were normalized and implemented in the affy and limma package of R/Bioconductor. Hub proteins were determined according to two metric (betweenness and degree) thru cytohubba plugin in CytoScape. Drugs were determined via DGIdb. Their significance was calculated via hyper geometric distribution. FDA approved anti-cancer agent list were arranged from National Cancer Institute lists for comparison.

Consequently, 16510 DEGs were determined as a combination of all datasets. We have found interactions between 509 genes & 2489 drugs which also covers the current pancreatic cancer drugs. 65 drugs were found as new candidate anti-cancer agents against pancreatic cancer although there are already used for other cancer types and some are also reported as candidates of pancreatic anti-cancer agents, recently. After statistical analysis, we have reduced the drug number based on threshold (p-value<0.001). Moreover, we have determined the hub protein-drug interactions. 9 drugs including Bosutinib, Dasatinib, Enzalutamide etc. were found in interaction with hub proteins.

Briefly, candidate anti-cancer agents were reported based on the computational analysis to investigate the effects on pancreatic cancer cases.

Keywords: Drug Repurposing, Pancreatic Cancer, System Biomedicine
P-034 - PRODUCTION OF ORGANIC NANOPARTICLES BY USING NANOPOROUS MEMBRANES

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Background: The spatial and temporal control of the release of pharmaceuticals at the site of where they act is a key requirement for the therapeutic use of a drug. One method for realizing this objective is to create drug-loaded nanoparticles made out of biodegradable polymers. We present here an alternative strategy based on the use of a nanoporous membrane that separates the two liquids. In this work, we wanted to understand how to obtain nanoparticles under which conditions by using nanoporous membranes. We believe this knowledge can help to find most efficient way to produce the particles for different needs.

Methods: Two liquids, a feed solution and a receiver solution, are separated by a nanoporous polycarbonate track-etched (PCTE) membranes. The feed solution is pumped through the membrane into the receiver solution. The feed solution contains biopolymers such as chitosan, collagen and alginic acid brown algea. The receiver solution contains 1mM NaOH. According to these parameters, we aimed to find the ideal pore sizes and production conditions. The particles are illustrated by SEM.

Results: Collagen nanoparticles with 0.4-2 μm diameter; algea nanoparticles with 2 μm diameter; chitosan needles with 0.2-3 μm diameter and 0.3-3 μm length are observed. Conclusions: According to our findings, it is shown that different pore sizes affect the shape and length of the collagen particles. Particles that passed through big pores (10 μm) were sphere-shaped, in contrast to particles which were rod-shaped at small pores (0.2 μm). Moreover, small pores lead to particles with 2-3 μm in length and 0.2-0.5 μm in width. Big pore sizes lead to particles with 1 μm radius. Different experimental conditions, such as pore sizes and velocity affected the features of the chitosan nanoparticles. It was shown that high velocity (100 μm/min) and low pore size (0.2 μm) lead to not only fibrilles (0.5 μm-3 μm in length) but also sphere-shaped particles (0.1 μm-1.5 μm). Chitosan is the only biomaterial that shows needles and sphere-shaped particles together.

Keywords: Nanoparticles, chitosan, collagen, alginic acid brown algea
P-035 - IS IT USEFUL ADIPOSE DERIVED MESENCHYMAL STEM CELLS INDUCED BY THYMOQUINONE IN THE TREATMENT OF LUNG CANCER?

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Introduction and Objective: Lung cancer is the most common cancer type and traditional therapies cannot provide effective treatment. Therefore, developments of targeted and effective cell therapies are very important. Human adipose tissue derived mesenchymal stem cells (hAD-MSCs) have the following properties: natural migration mechanism of cancerous tissue; easy to obtain, reproduce and genetic modifications; express to therapeutic molecules easily, and they don’t generate an immune response when compared to other cellular therapy vehicles in cancer. However, hAD-MSCs can support cancer cells via paracrine factors; moreover they can increase migration, proliferation, angiogenesis, and metastasis of cancer cells. Thus, studies that eliminate the supporting features of MSCs to cancer cells are of great importance.

Thymoquinone inhibits expression of paracrine factors released from MSCs associated with cancer development.

In this study we aimed that co-culture of stimulated MSCs via thymoquinone and lung cancer cells in vitro determine their interaction and to support the development of cancer treatments with stem cells.

Materials and Method: hAD-MSCs are stimulated by thymoquinone. After that, stimulated hAD-MSCs and lung adenocarcinoma A-549 cancer cells are co-cultured in vitro. Interaction between cells is determined by the change in the level of interleukin-8; vascular endothelial growth factor and MMP-2, MMP-9 metalloproteinase detected by ELISA assay. After interaction of cancer cells with stem cells; wound healing, colony formation and MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) assays indicated migration, invasion and proliferation capacities of cancer cells are performed.

Results: Co-culture of lung cancer cells and stimulated hAD-MSCs will be demonstrated that expression level of above paracrine factors and proliferation, migration, invasion capacity of cancer cells compared to co-culture of lung cancer cells and without stimulation of hAD-MSCs.

Discussion and Conclusion: To identify the interaction between stimulated hAD-MSCs and cancer cells will be supported that produce new approaches about cellular therapy of lung cancer as well as other cancers.

Keywords: Lung cancer, adipose tissue derived mesenchymal stem cells, thymoquinone, paracrine factors, targeted cancer therapy
6. MULTİDİŞİPLİNER KANSER ARAŞTIRMA KONGRESİ

6. Multidisipliner Kanser Araştırma Kongresi, Konya

P-036 - CONCENTRATION DEPENDENT EFFECTS OF ALOE VERA LECTIN AND A. VERA LEAF AQUEOUS EXTRACT ON THE IN VITRO GROWTH OF DIFFERENT HUMAN CANCER CELL LINES

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Malignant transformation is known to be associated with various changes in cell glycosylation, therefore lectins, which have the ability to bind to specific carbohydrates on cell surfaces, can be useful in distinguishing cell differentiation and metastasis.

Aloe vera extracts have been reported to prevent or regress tumour growth. However, it is not well documented whether the lectins purified from Aloe species are responsible for the antitumour activity.

In the present study, we aimed to compare the concentration dependent effects of A. vera lectin (Aloctin), with A. vera leaf skin aqueous extract on the in vitro growth of some human cancer cell lines.

Aqueous extract was prepared from the leaf skin of the fresh plant leaves and Aloctin was isolated from this extract by ammonium sulphate precipitation and cyanogen bromide CNBr-Sepharose 4B-ovalbumin-affinity chromatography. Cytotoxicity experiments were done using human colorectal carcinoma HCT116, promyelocytic leukemia HL-60, chronic myeloid leukemia K562 cells using MTT assay. 5-fluorouracil (5-FU) and Imatinib (IM) were tested as positive controls. The cytotoxic effects were evaluated by comparing the cytotoxic concentration that provides 50% inhibition of cell growth (IC50). Induction of apoptosis and necrosis were monitored by flow cytometry using the Annexin V-FITC/PI kit.

Aloctin showed significantly strong cytotoxic effect on all cells tested, while A. vera extract have no effect on cells at the same concentrations. Apoptosis and necrosis were’nt detected as possible mechanisms of cytotoxicity. Positive controls, 5-FU and IM strongly inhibited the cell proliferation, as expected.

The anticancer potential of A. vera extracts is available in many publications. Since antitumour or cytotoxic effects of some lectins from other sources are also reported in literature, this research provides insight to the effects of the compounds responsible of the cytotoxic effects of A. vera.

Keywords: Aloe Vera, Cytotoxicity, Lectin

P-037 - CYTOTOXICITY AND STABILITY STUDIES ON INCLUSION COMPLEX OF QUERCETIN WITH METHYL-β-CYCLODEXTRIN

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Introduction and Aim: Quercetin (Qu) is one of the most effective plant originated antioxidant. Despite the potential usage in cancer treatment, stability problems and the scarcity of cellular bioavailability limit its applications. Cyclodextrins (CD) are the cyclic oligomers of glucose. They can form inclusion complexes with the drug molecules and change the physicochemical properties of the drugs. These changes may increase the therapeutic potential of the drugs. The aims of study were evaluation the impact of methyl-β-cyclodextrin (M-β-CD) on the cytotoxic effects of Qu and investigation of stability of Qu/M-β-CD complexes.

Materials and Methods: All Qu/M-β-CD (L1-L3-L4-SS) complexes were synthesized using varies methods and kept at 4±1°C, 25±1°C and 40±1°C during...
the 3 months. The stability was evaluated with particle size (PS), zeta potential (ZP) and Qu amount analysis. Cytotoxicity assay of the complexes were performed on HeLa and SKOV-3 cells. Hoechst staining and flow cytometry analysis were also performed.

Results: L3 (concentration range: 400μg/mL–195μg/mL) was found to be a potent cytotoxic complex on HeLa cells after incubation time. On the other hand, 235μg/mL of L4 treatment was decreased the cell viability of SKOV-3 cells after 24 h. The flow cytometry studies showed that the percentage of early apoptotic cells was found to be the highest value when L3 compare the other complexes by HeLa cells. The amounts of Qu in L3 were decreased 12-20 % after 3 months at different storage conditions.

Discussion and Conclusion: Cytotoxic effects of the complexes varies depending on the cell type. L3 complex has a potent cytotoxic effect on HeLa cells, while L4 complex is cytotoxic on SKOV-3 cells suggesting that the synthesis method used might be an effective role on the ability of cytotoxicity of complexes. L3 stored at 4±1°C in 3 months were the most ideal complex according to PS, ZP and Qu amount in stability test.

Keywords: Cytotoxicity, Inclusion Complex, Methyl-β-Cyclodextrin, Quercetin, Stability

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**P-038 - OXADIAZOLE DERIVATIVES AS A POTENTIAL GLUTATHIONE S-TRANSFERASE INHIBITORS**

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Introduction and Aim: In the treatment of cancer, over production of Glutathione S-transferase is a one of the important problem. Because, this enzyme inactivate the chemotherapeutic agents and induce the drug resistance¹. Nowadays, studies on inhibitors of these enzymes are progressing and for this, variety of chemical compounds are synthesized. For this reason, the structure-based relationships of these oxadiazoles in inhibiting the activity of hGST P1 (human pi-class glutathione S-transferase enzyme) based on inhibitors were studied in this study.

Materials and Methods: Oxadiazole-group containing compounds (1a, 1b, 1c, 1d and 1f) were chosen and elucidated based on the quantum chemical parameters²a,b. The various quantum mechanical parameters such as dipole moment, global hardness (η) and etc. were calculated at DFT/B3LYP/6-31G* basis set by using Gaussian09 program. Molecular docking²c was also carried out with these compounds in hGST P1 to obtain lead compound according to active site of the hGST P1.

Result: The proposed compounds were achieved using the above the human pi-class glutathione S-transferase. These compounds can be potential candidates for hGST P1 in cancer therapy. The enzyme/drugs activity relationship was investigated by theoretical calculations and molecular modeling methods.

Conclusion: The results indicated that the human pi-class glutathione S-transferase can easily be inhibited by our proposed compounds. Therefore, they represent potential drug leading compounds. Additionaly, molecular docking was performed to better visualize the interactions between the compounds and hGST P1. In the future, synthesis and characterization of the compounds and their effects on cancer cell lines will be studied.

Keywords: Glutathione S-transferase, Oxadiazole derivatives, Quantum chemical parameters, Molecular docking
Interaction of oxadiazole lead-ligand with hGST P1 from docking calculations.

Molecular docking was performed to better visualize the interactions between the compounds and hGST P1.

P-039 - INVESTIGATION OF THE EFFECT OF MELATONIN CELL VIABILITY AND APOPTOSIS IN HNSCC
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Background and Aim: Head and neck squamous cell carcinoma (HNSCC) is one of the most common malignancies which is characterized by aggressiveness, early recurrence and metastasis in humans. Despite significant advances in therapeutic approaches, little improvement has been achieved in overall survival rates for HNSCC. Melatonin has a wide range of biological effects due to an antioxidant, anti-inflammatory and anti-tumor activity. Melatonin has the ability of affecting cell survival, proliferation, and apoptosis associated with signal transduction pathways. The aim of this study is to determine the effect of melatonin on head and neck squamous cell carcinoma cell line (SCC1-MT1).

Materials ve Methods: MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium) analysis was used to investigate the cytotoxic effects of melatonin. Melatonin was applied interval of 0 and 200 μL/mL concentrations and incubated 24 h, 48 h and 72 h respectively in the parenteral SCCL-MT1 cell line. After MTT analysis, the number of apoptotic cells was measured by Annexin V/PI (propidium iodide) analysis. SCCL-MT1 was incubated at the concentration of 200 μL/mL for 24 h and treated with Annexin-V antibody and propidium iodide. Consequently, apoptosis was evaluated by using flow cytometry.

Results: According to MTT results, dose of 200 μL/mL and 24 h treatment in SCCL-MT1 cell line was selected to apoptosis by flow cytometry. Treatment with 200 μL/mL melatonin for 24 h increased apoptosis in SCCL-MT1 (P=0.0061) cell line.

Conclusion: The results of the study, melatonin may decrease cell viability in head and neck squamous cell carcinoma cell line possibly through activation of apoptotic pathway.

Keywords: Head and Neck Cancer, Melatonin, Cell Viability, Apoptosis
6. MULTİDİSİPLİNER KANSER ARAŞTIRMA KONGRESİ

P-040 - IN VITRO EFFECTS OF RESVERATROL ON EXPRESSION OF CYTOCHROME P450 ENZYMES AND PROLIFERATION OF PROSTATE CANCER CELLS

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Introduction: Trans-resveratrol, existing in many different plants especially grape, is one of the important natural phytoalexins and their anticarcinogenic effects have been also demonstrated against various cancers. The main aim of this study is to investigate the in vitro effects of resveratrol on the xenobiotic metabolizing cytochrome P450 enzyme expression as well as proliferation, invasion and apoptosis of androgen non-dependent cancer cell line LnCAP.

Materials and Methods: LnCAP cells were grown in RPMI-1640 medium supplemented with 10% fetal bovine serum and 2 mM glutamine. Cytotoxicity of Resveratrol on LnCAP cell lines was determined with Alamar blue and IC50 value was calculated. The effects of Resveratrol on invasive potential of LnCAP cells were analyzed by matrigel chamber assays, respectively. Effects of Resveratrol on CYP3A4 and CYP2B6 protein and mRNA expression of LnCAP cells were determined using western blotting and qRT-PCR techniques.

Results: By investigating the effect of resveratrol on cell growth, it was found that IC50 value, half-maximal inhibitory concentration, is 76.2 for LnCAP cell line and 156.4 for PNT1A(healthy prostate cell line). Matrigel invasion assay showed that resveratrol significantly inhibit invasion of LnCAP cells as 43%. Western blot studies showed that both CYP3A4 and CYP2B6 protein expression were decreased 80% and 17%, respectively. On the other hand, qRT-PCR results demonstrated that mRNA expression of CYP3A4 interestingly increased as 2.2-fold while CYP2B6 mRNA expression was decreased 90%.

Conclusion: As a result of this study, we demonstrated that resveratrol possesses anticarcinogenic effects on androgen independent LnCAP cells while there is less toxic effect on healthy prostate cell line PNT1-A in the same concentrations. Beside this, modulation of cytochrome P450 protein and gene expressions by resveratrol makes it one of the new drug candidate which may regulates theophapeutic drug in prostate cancer.

Keywords: Cytochrome P450, LnCAP, Proliferation, Prostate cancer, Resveratrol

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P-041 - REAL-TIME MONITORING OF THE EFFECTS OF TAMOXIFEN AND VITAMIN D ON PROLIFERATION OF BREAST CANCER CELL LINE

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Objectives: Breast cancer is the most common malign disorder seen in women. Tamoxifen is the most widely used endocrine agent in this disease. Tamoxifen, upon binding of ER changes receptor conformation and inhibits transcription. Vitamin D refers to a group of sterols which regulates cell proliferation and differentiation pathways. Vitamin D receptors (VDR) has been identified in many tumorigenic cell lines and it has an anti-proliferative effect on cancer. Current study was designed to investigate the anticarcinogenic effect of varying concentrations of tamoxifen and vitamin D on breast cancer cell line.

Methods: In our study we used xCELLigence RTCA system which measures cell proliferation and drug-mediated cytoxicity in a time-dependent manner. 90 µL of the 3x104 cells/mL media mixture were added to the wells of E-plates. After 24 hrs, 10 µl of tamoxifen (60, 40, 20, 10, 1 µM) or vitamin D (125, 100, 75, 50, 10 nM) were added to each well. Cell proliferations were evaluated for 96 hrs.

Results: Tamoxifen treatment at 1 and 10 µM concentrations was found to be proliferative for 80 hrs and afterthat decrease in cell proliferation was observed. 20 µM of tamoxifen was found to be antiproliferative for approximately 50% of the population from 56th hrs. There was seen a significant decrease in the proliferation of almost all cells treated with 40 and 60 µM of tamoxifen. IC50 value of tamoxifen was calculated as 22 µM. For vitamin D, 10 and 50 nM concentrations were found to be proliferative whereas 75, 100 and 125 nM concentrations to be anti-proliferative.

Discussion: Our study showed that low concentrations of tamoxifen and vitamin D increased cancer cell proliferation. Vitamin D alone did not significantly decrease cell proliferation. Taken together, we propose to investigate the role of separate or combined therapy of these agents on tumor growth.

Keywords: Breast cell, xCELLigence, Tamoxifen, Vitamin D
xCELLigence RTCA system which measures cell proliferation for TAM and Vitamin D

P-042 - ANTICARCINOGENIC AND ANTIOXIDANT PROPERTIES OF RESVERATROL ON PROSTATE CANCER PC-3 CELLS

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Introduction: Resveratrol that mainly found in berries and grapes has antioxidant, anticarcinogenic, anti-inflammatory effects and generally synthesized in response to pathogenic and abiotic stress. In this study, we aimed to investigate in vitro effects of resveratrol on antioxidant enzyme activities as well as protein and gene expressions in androgen independent prostate cancer cell line; PC-3. Beside these, the effects of resveratrol on proliferation, invasion and colony formation properties of PC-3 cells were also examined.

Materials and Methods: PC-3 cells were grown in Ham’s F-12 and PNT1A cells RPMI 1640 medium supplemented with 10% fetal bovine serum and 2 mM glutamine. Cytotoxicity studies were performed using Alamar Blue test spectrophotometrically. GST and NQO1 enzyme activities were determined spectrophotometrically and protein and gene expression were determined by western blot analysis and qRT-PCR. Matrigel invasion, migration and colony formation assays were performed to see the effects of resveratrol on the characteristic properties of PC-3 cells.

Results: IC50 value of resveratrol on PC-3 cells were calculated as 76.2 µM while it is 156.4 µM for healthy prostate cells; PNT1A. Resveratrol significantly decreased migration, invasion and colony formation properties of PC-3 cells as 92.7%, 81% and 87.4%, respectively. GST and NQO1 enzyme activities were determined as 120.3±8 nmol/min/mg and 659.9±9 nmol/min/mg in resveratrol treated group while it was 286.6±4 nmol/min/mg and 1980±5 nmol/min/mg in control group. Moreover, resveratrol also significantly altered protein and gene expression of GST and NQO1.

Conclusion: Consequently, resveratrol was modulate expressions of antioxidant enzymes; GST and NQO1 which leads it one of the good candidates against aggressive prostate cancer.

Keywords: GST, NQO1, PC-3, prostate cancer, Resveratrol

This work was supported by a grant from TUBITAK, Project No: 113Z488
**P-043 - THE CYTOTOXIC EFFECT OF ASTEMIZOLE AND IMIPRAMINE ON PROSTATE CANCER CELL LINE**

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**Introduction and Purpose:** Cancer in the world and our country is a common disease and a high mortality rate. The prostate cancer is the common cancer in men. Studies have shown that the an antidepressant, imipramine and an antihistamine, astemizole affect some oncogenic proteins. Because of these features, the determination studies has gained importance whether these drugs are used in cancer therapy. In this study, it were aimed to investigate the cytotoxic effect of different doses of imipramine and astemizole on DU-145, prostate cancer cell lines.

**Materials and Methods:** In this study, prostate cell line, DU-145 was used. This study was performed using the xCELLigence device to determine the effect of different doses of astemizole and imipramine on cell proliferation. n=4 were repeated for real time cell analysis in our study. RTCA (real time cell analysis) programme was used. For blind, 100 µL media is added e-plate system. Later, to be 5x10³ cells in 90 µL of medium was cultured in e-plate system. After 24 hours, the concentration of astemizole (1 nM, 10 nM, 100 nM, 1 µM, 10 µM) and imipramine (10 nM, 100nM, 1 µM, 10 µM, 30 µM ) in 10 µL and for 96 hour cell proliferation was evaluated.

**Results:** The conclusion of the statistical analysis in term of cell index were observed significant differences between control and dose groups (p<0.05). In the cell index values, all astemizole and imipramine group was significantly decreased compared to controls 96th hour.

**Conclusion:** The result of study, by significantly reducing proliferation of prostate cancer cells, clues were obtained about an antidepressant imipramine and an antihistamine astemizole can be used for prostate cancer therapy. But, the subject is need further studies using different doses.

**Keywords:** Prostate cancer, Astemizole, Ìmipramine

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**P-044 - GEN EXPRESSION PROFILING OF MTOR PATHWAY IN RECTUM CANCER**

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**Background:** In this study, gene expression profile of mTOR pathway will be examined in advanced stage rectal cancer patients. mTOR is a member of serine/threonine protein kinase considering its role in the integration of extracellular and intracellular signals to regulate cell growth, proliferation and apoptosis. Many member as Deptor, REDD1 block the mTOR pathway is a substract of SCF3 ligase. The expression levels of the catalytic unit of SCF3 Ligase, SAG, and related genes NOXA, BNIP, p53 are compared in normal and tumor tissues of advanced stage rectal cancer patients.

**Material and Methods:** Tissue samples were collected from 32 patients who had histologically advanced cell carcinoma of rectum at Radiation Oncology Department of Kartal Education and Research Hospital. Total RNA was extracted from biopsies, total RNA was reverse transcribed using Transcriptor High Fidelity cDNA Synthesis kit and used as a template in real-time PCR reactions.

**Results:** The comparing expression levels of normal and cancer tissues, we observed SAG gene has 56% increasing, NOXA gene, 18%, BNIP gene 28% and p53 gene 47% increasing levels. SAG and NOXA has a reverse regulation in 68% patients. The catalytic component of the mTOR protein complex, SAG is an antiapoptotic proteins which are effective in cancer development and radiation response. NOXA is an apoptotic protein which is accumulated in the cells with a reduced level of SAG. BNIP is known as antiapoptotic protein and p53, tumor supressor genes.

**Conclusion:** This study showed that there is an inverse correlation between SAG and NOXA expression, also a high increase (upregulation) as expected in the p53 gene. After all the mTOR pathway protein expression determined, relationship with the prognosis of these levels will be determined.

**Keywords:** Apoptosis, mTOR Pathway, Noxa, Rectum Cancer, SAG
P-045 - THE CROSSTALK BETWEEN P38 AND AKT SIGNALING PATHWAYS ORCHESTRATES EMT IN NSCLC CELLS VIA REGULATION OF SATB2 EXPRESSION

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Epithelial-mesenchymal transition (EMT) is a significant event for metastasis, and could be mediated by several pathways such as PI3K/Akt, MAP kinases and many epigenetic regulators. SATB2 is an epigenetic regulator involved in EMT and osteoblastic differentiation. Since preliminary results indicate that there is a crosstalk between p38 and Akt pathways in NSCLC cells, we aimed to determine whether this crosstalk has a regulatory effects on EMT and SATB2 expression in NSCLC cells. We used A549 and H1650 cells as a model to evaluate the effects of the crosstalk between p38 and Akt on EMT of NSCLC cells. Therefore, cell culture, inhibition of p38 activation via SB203580, transient expression assay for (CA-Akt), Western blot analysis, siRNA transfection for SATB2, wound healing and invasion assay were performed in this study. Firstly, the expression statues of E-cadherin, SATB2, p-p38, p38, p-Akt and Akt was examined in A549 and H1650 cells by Western blot analysis. We observed that E-cadherin and SATB2 are downregulated in A549 cells (highly active p38, lowly active Akt) compared to H1650 cells (lowly active p38, highly active Akt), suggesting that E-cadherin and SATB2 are associated with the crosstalk between p38 and Akt pathways. Our results demonstrated that p38 inhibition in A549 cells leads to decreased PTEN expression and subsequently increased Akt activation. Then, we found that p38 inhibition upregulated SATB2 expression, and reversed EMT in A549 cells. Furthermore, alone SATB2 knockdown is sufficient to induce EMT; and prevented the effects of p38 inhibition on EMT. All these results strongly indicate that the crosstalk between p38 and Akt pathways could regulate EMT of NSCLC cells by controlling SATB2 expression.

Keywords: EMT, NSCLC, p38, Akt, SATB2

This work was supported by TUBITAK (114S007, 215Z283).

P-046 - EFFECT OF P53 ON DICLOFENAC AND IBUPROFEN-INDUCED ROS GENERATION-DEPENDENT APOPTOSIS IN PC3 PROSTATE CANCER CELLS

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Prostate cancer is the most frequent type of cancer prevail among men. Collected evidence reveals that p53, in other words, “Guardian of genome” plays a critical role in cancer. Also, in vivo and in vitro studies suggest that regular use of nonsteroidal anti-inflammatory drugs (NSAIDs) may reduce prostate cancer risk. In this study, we examined the effects of p53 on regulation of programmed cell death mechanisms that are triggered by ibuprofen and diclofenac in PC3 and PC3 p53−/− prostate cancer cells.

To investigate the role of p53, PC3 cells were transfected with p53 plasmid and correlated with immunoblotting. We observed that ibuprofen (1 mM) reduced cell viability by 25% in PC3 and 40% in PC3 p53+/+ cells. Diclofenac (250 µM) decreased cell viability by 60% in PC3 and 50% in PC3 p53+/− cells. Diclofenac induced apoptosis via caspase-8-dependent cleavage of RIP, which is a pro-apoptotic pathway regulator. Ibuprofen activated intrinsic pathway of apoptosis through upregulation of fas and cleavage of caspase 2, which are related with BID cleavage in PC3 cells. However, transfection of p53 downregulated SAPK/JNK signal axis which has an important role on activation of caspase cleavage and mitochondrial membrane potential loss. To better understand the effect of ibuprofen and diclofenac on ROS generation DCFDA staining was performed and flow cytometric analysis showed that diclofenac induced ROS generation.

In conclusion, the mechanism of transition between extrinsic and intrinsic pathways differ depending on presence of p53. Ibuprofen caused fas upregulation in PC3 cells, which was withdrawn by transfection of P53. Presence of p53 affects upregulation of Bax and Bak which are critical for mitochondrial apoptosis pathway. On the other hand, upon diclofenac treatment, activation of RIP was prevented through BID cleavage which is related with p53-dependent fas activation. Also diclofenac-caused ROS generation was observed, which is independent from p53.

Keywords: Apoptosis, NSAID, p53, Prostate Cancer, ROS
Novel cyclin-dependent kinase (CDK) inhibitors, roscovitine and purvalanol, induce apoptosis by triggering cell cycle arrest in cancer cells. Although the molecular targets of CDK inhibitors are well established, there are still unknown targets, which may affect their therapeutic potential. Endoplasmic reticulum (ER) stress is a mechanism that can be induced by elevated levels of oxidative stress, accumulation of unfolded proteins or starvation. Thereby prolonged ER stress in the cells activates unfolded protein response (UPR) and leads to apoptotic cell death. Our recent studies showed that purvalanol induced ER stress, which triggered apoptosis and autophagy in colon cancer cells [1]. In this study, our aim is to investigate the potential role of roscovitine and purvalanol on ER stress and apoptosis in HeLa cells.

Our findings indicated that both CDK inhibitors induced apoptosis as a late response, which was established by cleavage of PARP and activation of caspases. In contrary, drugs initiated ER stress as an early response via upregulating PERK, IRE-1α and ATF-6 levels in HeLa cells. In addition, pm-Cherry-tagged CHOP plasmid transfection studies showed the ER stress-related efficiency of drugs. The increased CHOP expression due to CDK inhibitors may underlie the potential effect of ER stress on apoptosis decision. This early response as ER stress induction may be related to the activation of oxidative stress. In order to evaluate the potential effect of CDK inhibitors, we showed ROS generation by DCFH-DA staining after time-dependent drug treatment. In further studies, the investigation of CHOP-related apoptotic decision is required to evaluate the potential molecular machinery of CDK inhibitors in HeLa cervical cancer cells.

**Keywords:** apoptosis, CDK inhibitors, ER stress, HeLa cells

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**P-047 - CDK INHIBITORS ROSCOVITINE AND PURVALANOL INDUCED ER STRESS-MEDIATED APOPTOSIS IN HELA CERVICAL CANCER CELLS RELATED WITH INDUCTION OF OXIDATIVE STRESS**

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**P-048 - THE EFFECTS OF ELECTROMAGNETIC FIELD EXPOSURE AT 900 MHZ FREQUENCY EMITTED FROM MOBILE PHONES ON COCHLEAR CELLS**

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**Aim:** Technological developments encountered radiofrequency field from mobile phones in our lives. Possible side effects of electromagnetic field (EMF) need to be investigated. The aim of this study is to evaluate cytotoxic, apoptotic and DNA damage effects of 900 MHz EMF emitted by mobile phones on House Ear Institute-Organ of Corti 1 (HEI-OC1 cell line) cochlear cells.

**Methods:** Cochlear cells were cultured in 6 well plates at 33oC, 10%CO2 in humidified conditions. They were exposed to 900 Mhz EMF in conditions of 5 minutes and 15 minutes, directly and 10 cm away from EMF. EMF was applied by a 3G cell phone and measured by Arduino EMF detector. Cell viability and apoptosis were evaluated after 24 and 48 hours by trypan blue and Annexin V methods. DNA damage related gene expressions was evaluated by real time PCR.

**Results:** Cell death was more prominent in cells 5 minutes of EMF at 48 h. The apoptosis ratio in cells situated 10 cm away from EMF were similar to cells that were directly exposed to 5 minutes of EMF. It was observed that DNA damage related gene expressions were increased in cells after EMF exposure in 48 hours. The expression levels are nearly same in cells that were 10 cm away from EMF. The DNA damage related gene expressions was evaluated by real time PCR.

**Conclusion:** EMF at high dose for 5 minutes caused cell death via apoptosis in HEI-OC1 cell line in vitro. This result was supported by apoptosis detection and DNA damage related gene expressions. Apoptosis was prominent in 5 minutes and similar for both direct and close distance exposure. Further in vivo and in vitro studies with different doses and distances are needed.

**Keywords:** electromagnetic field, cochlear cells, mobile cell phones
P-049 - THE EFFECT OF VITAMIN D ON MCF–7 BREAST CANCER CELL METABOLISM

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Background and Aim: It has long been known that vitamin D is important for calcium absorption and bone health. However, recent studies have revealed that Vitamin D modulates breast cancer cell growth and epidemiologic studies have increasingly suggested that vitamin D may be associated with reduced breast cancer risk. The primary objective of this study was to highlight the effect of Vitamin D on MCF-7 breast cancer cell.

Materials and Methods: Changes in the number of cells and the particular cell culture in wells containing micro-electrodes, duration of the experiment were continuously monitored every 15 minutes. With the use of this data IC50 dose was calculated as 145nM. This pre-defined IC50 dose was subsequently applied to cells which were obtained. These samples were used to determine the apoptosis, levels analysis at cancer cells.

Results: We observed that the anti-proliferative effect of Vitamin D on MCF-7 breast cancer cell similar to the literature through real time cell electronic detection system. The apoptosis level which was 18% at the 24th hour vitamin D groups were respectively 28% at the 48th hour and 38.5% at the 72th hour.

Conclusion: Vitamin D was reduced cancer cell proliferation dose and time depending manner. This findings support the application of vitamin D in breast cancer prevention and treatment.

Keywords: Vitamin D, Breast cancer, Proliferasyon, Apoptosis

Table 1

<table>
<thead>
<tr>
<th></th>
<th>7-ADD Negative, Annexin V (%)</th>
<th>7-ADD Negative, Annexin V Positive (%)</th>
<th>7-ADD Positive, Annexin V (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control 24h</td>
<td>87.8</td>
<td>1.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Vit.D 24h</td>
<td>74.1</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>Control 48h</td>
<td>90.6</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Vit.D 48h</td>
<td>67.4</td>
<td>28.6</td>
<td>2.8</td>
</tr>
<tr>
<td>Control 72h</td>
<td>82.4</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Vit. D 72h</td>
<td>52.3</td>
<td>38.5</td>
<td>6.5</td>
</tr>
</tbody>
</table>

The dose dependent inhibitory effects of vitamin D on the growth of MCF-7 cell. 1-DMEM, 2-CONTROL, 3-ETANOL, 4-10 nM vit. D, 5-100 nM vit. D, 250 nM vit. D, 500 nM vit. D, 1000 nM vit. D

The distribution of different phases of MCF-7 cell cycle after 1α,25(OH)2D3 treatment determined by flow cytometry with Annexin V-FITC and 7-AAD staining
Introduction: Tumor size is an important parameter used to determine the prognosis and stage of breast cancer. We investigated a probable correlation between AgNOR area and tumor size to examine whether the AgNOR parameters can also be tools of detecting the stage and predicting of the prognosis.

Materials and Methods: The diameter of the tumor, AgNOR number and AgNOR area of 18 cases were obtained as a retrospectively from archive records. The mean AgNOR number and area were calculated via measurement of 50 cells from each case. Patients were divided into groups according to the TNM classification system by tumor size. Thus, patients with a tumor diameter between 5-10 mm are included in T1b (n = 8), between 10-20 mm are included in T1c (n = 5), and between 20-50 mm are included in T2 (n = 5) groups. Obtained biopsy material was taken into methanol, spread on clean slides and air dried. After silver staining, 50 cells from each patient were evaluated by counting AgNOR spots and measuring AgNOR areas.

Results: Both mean AgNOR area and mean AgNOR count values had strong correlation with tumor diameters of patients (Respectively r=0.740,P<0.000, r=0.771;P<0.000). When the statistical evaluations were done using the “Tumor Nod Metastasis class of the tumor instead of tumor diameter, correlation appeared milder (r=0.709;P<0.001, r=0.646;P<0.004 with same respect). Three groups of tumor diameter had significantly different for mean AgNOR area and mean AgNOR count values (Respectively;P=0.013;P=0.029).

Conclusion: Presence of positive correlations between tumor diameter and AgNOR parameters indicates that AgNOR parameters have potential to be useful tools for staging of breast cancer and predicting the prognosis of the condition. Because of the correlation with tumor diameter is more strong than TNM classification methods, we thought that the directly using of tumor diameter is more useful.

Keywords: AgNOR, Prognosis, Tumor diameter

P-051 - EVALUATION OF THE AGNOR PARAMETERS FOR THE EARLY DETECTION OF URINARY BLADDER CANCER VIA CELLS OBTAINED FROM URINE SAMPLES

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Background and Aim: Urinary bladder carcinoma is one of the most generally diagnosed cancers after prostate, lung and colon cancers. The rates of bladder cancer is as the fourth most common cancer in men in the world. The detection, treatment, and staging of urinary bladder cancer have traditionally been based on an endoscopic examination - cystoscopy.

Therefore additional methods for the early detection of the bladder cancer are important. Our goal was to determine the usage of cut-off values of some AgNOR parameters of exfoliated cells in urine samples from urinary bladder patients and healthy individuals for distinguishing malignant and benign lesions by a non-invasive method.

Materials and Methods: Exfoliative urinary bladder cells obtained from the urine samples of 11 healthy volunteers and 24 bladder cancer patients were spread onto the slides, fixed and air dried, and silver-stained. Images of the cells transferred into computer, and mean values of different AgNOR parameters were counted, measured and calculated.

Results: The mean age of patient group were 63.417±12.237 and of control group 55.546±15.572 and mean ages of these two groups were not statistically different (p=0.115)

The differences between control and patient groups were statistically significant for AgNOR numbers (respectively 1.950±0.535 and 3.06±0.863) and for TAA/NA (4.373±2.740 and 11.450±1.185 with the same respect) (p=0.000). According to the Baysian Statistic, the cutoff and AUC values were >2.73 ve 0.858 for AgNOR number(p<0.0001), and >6.4 and 0.939 for TAA/NA values (p<0.0001). Cancer subgroups were significantly different for both AgNOR parameters (p<0.05).

Discussion and Conclusion: We showed the usefulness of AgNOR parameters in cells from urine, for detection of the presence of the tumor in suspected cases, with

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P-052 - GROWTH INHIBITION AND INDUCTION OF PROGRAMMED CELL DEATH IN HUMAN LUNG ADENOCARCINOMA CANCER CELLS BY SHORT TERM CARMOFUR TREATMENT

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Background: Lung cancer is one of the type common cases in the World. In Turkey, the incidence of lung cancer in mans is abot %62 and %5 in womans. There is a fact that resistance to widely used chemotherapeutics occurs frequently. Thus novel agents for cancer treatment are required. Carmofur or HCFU (1-hexilcarbamoyl-5-flourouracil) is a pirimidine analogue used as antineoplastic agent in treatment of breast and colorectal cancer types.

Objective: In the present study we evaluated the cell the cytotoxic and apoptotic effects of carmofur in human lung adenocarcinoma cells (A549).

Material and Methods: For cytotoxicity we used MTT(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) colorimetric assay. Stock solution of carmofur was prepared in dimethyl sulfoxide (DMSO) and further diluted in fresh culture medium. A549 cells were exposed to concentrations of carmofur ranging from 5 to 110 microM for 24 hours. The percentages of viability and IC50 concentration of carmofur for 24 hours were determined. The morphological alterations on A549 cells caused by IC50 concentration of carmofur for 24 hours were investigated on confocal microscope. For confocal microscopy, A549 cells were double stained with acridine orange and phalloidine.

Results: Consequently carmofur inhibited the proliferation of A549 cells leading to morphological changes on the cells indicating apoptosis. On our micrographs we detected shrunked cells with damaged cytoskeleton, condensed and fragmented nuclei. In our results we demonstrated that carmofur was highly cytotoxic in low doses in A549 cells and caused damages in the cell structure.

Discussion: Furthermore, with the support of the results presented in this study, it may be concluded that carmofur holds potential to act as a beneficial agent in the...
treatment of cancer. In this research we showed the apoptotic effects of new agent as a carmofur may be using on human breast cancer cells.

**Keywords:** Carmofur, A549, Cytotoxicity, Confocal Microscopy

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**P-053 - SCREENING THE ANTI-PROLIFERATIVE EFFECTS OF SOME HYPERICUM SPECIES IN MCF-7 HUMAN BREAST CANCER CELLS BY USING REAL TIME CELLULAR IMPEDENCE TECHNOLOGY**

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**Introduction:** Breast cancer is the most common type of cancer among women. Anti-neoplastic agents emerge as one of the major class of cancer therapeutics. Anti-cancer effects of *Hypericum* species, which is a natural agent, has been reported in different cancer types. In this study, we aimed to analyze the anti-proliferative effects of four previously unstudied *Hypericum* species (*H. salsugineum*, *H. olympicum*, *H. scabrum* and *H. pruinatum*) on MCF-7 cell line.

**Materials and Methods:** iCELLigence real time and label-free cell analysis system was used during the study. In order to investigate time and dose dependent effects; MCF-7 cells were seeded in E-plate L-8 and treated with various concentrations (2000, 1000, 500, 250, 125, 62.5 µg and DMSO-only control) of methanolic extracts of *Hypericum* species for 72h. Data analysis was performed by RTCA iCELLigence software. IC50 values were calculated based on the final readings taken at the 72 hour time point.

**Results:** After screening the anti-proliferative activity of four different *Hypericum* species, *H. salsugineum* was the most effective on cell proliferation which was followed by *H. olympicum, H. scabrum* and *H. pruinatum*, respectively. Our results demonstrated that *H. salsugineum* significantly decreased the proliferation of MCF-7 cells compared to control ones and other *Hypericum* species included in the study in all biological repeats.

**Discussion:** *H. salsugineum* displayed significant anti-proliferative effects on MCF-7 cells. These promising results will be the basis for further comprehensive functional assays on breast cancer by using *H. salsugineum*.

**Keywords:** Breast cancer, iCELLigence, *Hypericum*, Anti-proliferative, MCF-7
**P-054 - INVESTIGATION OF SOME PLANT EXTRACTS AND CANCER DRUGS OF THE CYTOTOXIC EFFECT OF BCL 2-BAX GENE EXPRESSION AND EFFECT ON CELL CULTURE**

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**Objective:** Cancer is a term used to describe the uncontrolled growth and abnormal spread of cells. It is made for the treatment of cancer with chemotherapy and radiotherapy made by using cancer drugs that destroy cancer cells, to inhibit the growth and proliferation. In our study, it aimed to investigate of cytotoxic effects of different doses of various plant extracts and cancer drugs and effect on BAX and BCL2 gene-expression in cell culture.

**Materials and Methods:** Extraction was performed from plant tissue to be used in this study. Cancer drugs were selected to specific types of cancer to be studied. Plant extract (0.005 mg/ml, 0.01 mg/ml, 0.015 mg/ml and 0.02 mg/ml) and cancer drug (0.5 µg/ml, 1.00 µg/ml, 2.00 µg/ml, 4.00 µg/ml) at different doses was applied to the cell cultures. Article of the MTT assay was performed at intervals of 24, 48, 72 hours. Cells were plated on a 6 well plate in place a certain amount. RNA isolation was performed after administration of plant extract and cancer drugs at certain dose. Real Time PCR was performed by performing cDNA synthesis.

**Results:** Showed increased of toxic effects on the cells both hours and increasing doses of cancer drugs at the of the study. The highest dose used in the experiment, 72 h, that was found killed of medications and plant extracts more than 50% of cancer cells. Observed of increasing doses of the plant extract to decrease the BCL-2 gene expression, to increase the BAX gene expression according to Real Time PCR results.

**Conclusion:** As compared to the comparative plant extracts used cancer drug was found to lead to apoptosis in cancer cells.

**Keywords:** Bcl-2-BAX, plant extract, anticancer drugs, cell culture, cytotoxicity, gene expression, Real Time PCR

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**P-055 - INVESTIGATION OF CYTOTOXIC AND ANTI-METASTATIC EFFECTS OF RHEUM RIBES METHANOL EXTRACT ON MCF-7 BREAST CANCER CELLS**

Çınar İlknur, Eroğlu Canan, Avci Ebru, Çetinkaya Sümayra, Dursun Hatice Gül, Kurar Ercan

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**Rheum ribes** species that one of the herbs commonly used in pharmacological researches, are grown in most Iran, Lebanon, and Eastern Turkey. This plant is determined to contain powerful active compounds. Studies about this plant are generally intended to assess the impact of antioxidant. Our aim in this study is to assess cytotoxic and metastatic changes by way of implementing methanol extract of the *R. ribes* (root) to the MCF-7 breast cancer cells.

Cytotoxic effect of *Rheum ribes* extract was evaluated by using the XTT (2,3-Bis(2-metoksi-4-nitro-5-sulfofenil)-2H-tetrazolyum) test. In order to determine the dose of IC50, plant extracts were applied as time and dose dependent in the range of 10-500ug. In 72nd hour, the IC50 value is determined as 400ug. To examine the anti-metastatic effects of the extract, total RNAs were isolated from dose group and the control cells firstly, then cDNAs were synthesized. Expression profile of the target genes (MMP-2, MMP-9, TIMP-1, TIMP-2, CDH1, CDH2) are determined by qPCR.

According to the results, when the control group compared with the cells, it was determined that 1.6 and 2.07-fold respectively decrease in the gene expressions of MMP-2 and CDH2 of dose group cells. No significant difference was observed in the other genes examined.

Epithelial-mesenchymal transition (EMT) is a critical step for the initiation of cancer metastasis. N-cadherin and MMPs is known as significant mesenchymal marker. Our data suggested, the methanol extracts of *R. ribes* may not only have cytotoxic effect but also anti-metastatic effect on MCF-7 cells.

**Keywords:** Anti-metastatic, Cancer, Cytotoxic, *Rheum ribes*
P-056 - IMPACT OF nanopARTICLES OF C60 FULLERENE ON GFAP AND NF-KB IN THE U373 GLIOMA CELL LINE

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Background: Gliomas are the most common type of primary tumor in brain and are characterized by high resistance to programmed cell death and invasion pattern. Water soluble nanoparticles C60 fullerene have been reported to be effective antioxidant and nontoxic antiproliferative agent. The malignant progression of astrocytic tumors is accompanied by a decrease in the proportion of cells expressing cytoskeleton protein glial fibrillary acidic protein (GFAP) as well as by a reduction in the GFAP content. The goal of this study was to elucidate antiproliferative effects of C60 and the role of cytoskeleton changes in astrocytic tumors on malignant glioma U373 cells.

Material and Methods: Glioma U373 cells were treated with fullerene C60, (1 µM) and H2O2 (500 mM) for 24h, and cells were treated with vehicle as control. Viability of cells were determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) analysis. GFAP and NF-kB proteins expression were checked by Western blot.

Results: MTT analysis showed that fullerene C60 inhibited cell proliferation in glioma U373 cells. There are observed an inhibition of proliferation in normal control on 16%. Contrary, in stressed cells C60 induced a rising of cell viability on 25%, that can be relate to power antioxidant ability of C60. Western blot revealed that GFAP and NF-kB expression decreased in U373 cell line with treated H2O2 on 48% and 64% respectively. The preincubation U373 with C60 fullerene 1 hour before H2O2 treatment ameliorates the reduction of glial intermediate filament and NF-kB expression almost to control level.

Conclusion: These results illustrate that water soluble C60 fullerene provides protection against the system disturbance induced oxidative stress, especially ameliorates expression of cytoskeleton marker GFAP and key regulator for main pathways of cell response NF-kB. Along with antioxidant effect this nanoparticles C60 fullerene have an ability to inhibit the proliferation of malignant glioma U373 cells.

Keywords: C60 Fullerene, GFAP, NF-kB, Glioma

P-057 - Evaluation of cytotoxic and anti-angiogenic effects of methanolic extract of Lawsonia inermis

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Introduction and Aim: Lawsonia inermis, commonly named as henna, belongs to the Lythraceae family and its widely used for medicinal and cosmetic purposes. We aimed to determine the effects of methanolic extract of L. inermis on the human breast cancer cells (MCF-7), human colorectal adenocarcinoma cells (Caco-2), human neuroblastoma cells (SH-SY5Y) and human umbilical vein endothelial cells (HUVECs) and also whether use of the extract would be an alternative for current anti-cancer agents.

Material and Methods: The cytotoxic effects of the methanolic extract were determined by in vitro MTT and Neutral Red Uptake (NRU) assays. The anti-angiogenic effect of L. inermis extract on HUVECs differentiation was assessed by examining in vitro tube formation assay.

Results: The data of present study demonstrated that methanolic extract of L. inermis induced cytotoxicity and significantly inhibited cell viability at concentrations above 25 µg/ml in time and dose dependent manner in all cells. According to MTT and NRU assays results, the most cytotoxic effect was observed on MCF-7 cells and IC50 values ranged 35.53 µg/ml to 40.87 µg/ml. The less cytotoxic effect was evaluated on Caco-2 cells and IC50 values ranged 69.55 µg/ml to 110.24 µg/ml. HUVECs and SH-SY5Y cells IC50 values of the extract of L. inermis ranged 69.55 µg/ml to 80.75 µg/ml and 45.72 µg/ml to 48.63 µg/ml respectively. At low cytotoxic concentration on HUVECs, the methanolic extract was destroy the branching and the tube formation.

Conclusions: Our preliminary data indicates that the methanolic extract of Lawsonia inermis may be potential therapeutic agent for cancer therapy.

Keywords: Lawsonia inermis, Cytotoxicity, Tube Formation, Anti-Angiogenic
**P-058 - THE DISTRIBUTION AND EFFECTS OF CDKN2 P16 540 C>G AND 580 C>T, AND MDM2 SNP309 T>G POLYMORPHISMS ON LARYNGEAL CANCER**

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**Background:** Laryngeal squamous cell carcinoma (LSCC) is a multifaceted and genomically complex disease and cellular and preclinical studies have done wide ranging molecular mechanisms which underpin its development and progression. p16INK4, an important member of cell-cycle which inhibits cyclin D1–cyclin dependent kinase (CDK). It is known that changes in functions of p16 and MDM2 are related to tumor pathogenesis by enhancing cell proliferation and malign development. Recent reports have shown that p16 540C>G (rs11515), p16 580 C>T (rs3088440) and MDM2 SNP309 T>G polymorphisms were related to cancer development, prognosis and tumor aggressiveness.

**Materials and Methods:** Using PCR-RFLP technique, we determined SNPs in 79 patients with laryngeal tumors and 73 healthy volunteers without malignancy.

**Results:** We found no significant association for the distributions of CDKN2 p16 580 C>T and p16 580 C>T variants between cases and controls. However, the frequency of TT genotype for MDM2 SNP309 T>G was significantly 2.5 times higher (p<0.001) and possessing G allele had decreased risk (p<0.001) in laryngeal cancer than control group. We also found that the late tumor stage-laryngeal patients having TT genotype had higher 1.8 fold risk (p=0.017) than those with early tumor stage cancer.

**Conclusion:** These findings show that CDKN2 p16 540 C>G, CDKN2 p16 580 C>T and MDM2 SNP309 T>G variants may be risk factors for the development of laryngeal tumors.

**Keywords:** Laryngeal squamous cell carcinoma, p16, MDM2, Polymorphism

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**P-059 - SYNTHESIS AND CYTOTOXIC EVALUATION OF NEW 1,3,4-THIADIAZOLE DERIVATIVES AS POTENTIAL ANTICANCER AGENTS**

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**Introduction and Aim:** The biological properties of 1,3,4-thiadiazole and its derivatives have been studied for more than one hundred years. They are widely known as compounds with various kinds of biological activities showing antitumor against human cancers. New 1,3,4 thiadiazole derivatives were synthesized via the reaction of 5-(4-fluoro/chlorophenylamino) 1,3,4-thiadiazole-2(3H)-thione with N-thiazol/benzothiazol-2-yl)-2-chlorooacetamide derivatives. We aimed to determine the effects of new 1,3,4-thiadiazole derivatives on human neuroblastoma cells (SH-SY5Y), human hepatocellular carcinoma cells (HEPG2), human umbilical vein endothelial cells (HUVECs).

**Methods:** MTT assay was carried out to determine the cytotoxic effects of the compounds on SH-SY5Y, HEPG2, HUVEC cell lines.

**Results:** Our results demonstrated that new 1,3,4 thiadiazole derivatives significantly reduced cell viability in all cell lines in time and dose dependent manner compared to untreated control cells. Cytotoxic effects of N-(Benzothiazol-2-yl)-2-((5-(4-chlorophenyl)amino)-1,3,4-thiadiazole-2-yl)thio)acetamide (I) on SH-SY5Y, HEPG2 and HUVEC cells IC50 values 400 µM. According to N-(6-Methylbenzothiazol-2-yl)-2-((5-(4-chlorophenyl)amino)-1,3,4-thiadiazole-2-yl)thio)acetamide (II) MTT assay results, the most cytotoxic effect was observed on SH-SY5Y cells and HEPG2 cells. The less cytotoxic effect was evaluated on HUVEC cells. Cytotoxic effects of N-(6-Methylbenzothiazol-2-yl)-2-((5-(4-chlorophenyl)amino)-1,3,4-thiadiazole-2-yl)thio)acetamide (III) on SH-SY5Y and HEPG2 cells IC50 values 200 µM and 50 µM respectively and HUVEC cells IC50 value 400 µM.

**Conclusions:** The significant cytotoxic activity observed for I and III derivatives suggest that these derivatives may be potential anticancer agents.

**Keywords:** Cytotoxicity, Synthesis, Thiadiazole Derivatives, Anticancer
P-060 - BAG-1 INDUCES CELL SURVIVAL IN MDA-MB-231 BREAST CANCER CELL LINES

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BAG-1 (Bcl-2 associated athanogene) is a multifunctional protein that interacts with diverse array of cellular targets and modulates a wide range of cellular processes, including proliferation, cell survival, transcription, apoptosis, metastasis and motility. In human cells BAG-1 exists as three major isoforms (BAG-1S, BAG-1M and BAG-1L) derived by alternative translation initiation from a single mRNA, which allows interactions with various molecular targets such as Hsp70/Hsc70 molecular chaperones, components of the ubiquitylation/proteasome machinery, Bcl-2, Raf-1 kinase, nuclear hormone receptors and DNA. Our work aims to investigate how altered Bag-1 expression levels affect cell survival in MDA-MB-231(ER,PR and HER2/Neu negative) breast cancer cell lines. We first cloned Bag-1L gene to a cloning vector, later we transfected MDA-MB-231 cells for overexpression of Bag-1. We also used Bag-1 siRNA to silence Bag-1 gene. Western blot analysis was applied to demonstrate relative expression levels of Bag-1, its interacting partners and certain proteins which are important for apoptosis pathway. We performed XTT cell viability assay for Bag-1 overexpressed cells to check Bag-1’s impact on cell survival, and observed enhanced survival rates on cells compared to that of the untreated cells with Bag-1 overexpression. In addition, our study revealed that once Bag-1 forms a complex with C-Raf/B-Raf/Hsp70/Akt/Bcl-2, modulation of cell survival was observed. We believe that once the exact localization and involved molecular mechanisms of Bag-1 and its isoforms are found, the role of each Bag-1 isofrom in cell survival can be understood better. This can further provide routes to study tumor development.

Keywords: Breast cancer, Bag-1, cell survival

P-061 - ANTI-PROLIFERATIVE AND APOPTOSIS INDUCING EFFECT OF FLUBENDAZOLE ON NEUROBLASTOMA CELLS

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Aim: Neuroblastoma (NB) is the most common extracranial solid tumor of childhood. Flubendazole (Flu) inhibits microtubule activity that is responsible of proliferation and migration of eucaryotic cells as in metastasis and migration of tumor cells. Anti-proliferative effect of Flu has been shown in leukemia and multiple myeloma in the literature. According to this knowledge, the aim of the study was to evaluate the effect of Flu on the viability and characteristics of NB cells.

Method: After C1300 NB cells were cultured in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, 1% L-glutamine, cells were incubated at various concentrations of Flu (100 nM, 200 nM, 400 nM, 500 nM, 1000 nM). Anti-proliferative effect was evaluated after 24, 48, and 72 hours of exposure by WST-1 test. Apoptotic cell levels were tested at 24 hours of exposure to the same concentrations by annexin V flow cytometric analysis. The migration of cells at LD50 dose for 72 hours was monitored and saved by wound healing and metastatic in vitro assays.

Results: Flu significantly inhibited cell proliferation in a concentration-dependent and time-dependent manner. LD50 doses of Flu were found to be 500 nM at 24-h treatment and 400 nM at 48-h. After 24-h treatment, WST-1 and the apoptosis test results were correlated. Apoptosis inducing effect of Flu was 2 to 6 times more than control. Flu decreased cell migration due to in vitro models.

Conclusion: According to this study, Flu is suggested as an anti-proliferative and apoptosis inducing agent on NB cells. At the 24-h treatment, WST-1 and apoptosis test results were correlated. In following studies the anti-tumoral effect and mechanisms of Flu is thought to be evaluated on the in-vivo animal models.

Keywords: Flubendazole, Neuroblastoma, Apoptosis, Anti-proliferation
P-062 - IN VITRO EFFECT OF BORATE DERIVATIVES ON NEUROBLASTOMA CELLS

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Aim: Neuroblastoma (NB) which is the most common extracranial solid tumor of childhood, carries on to have a dismal prognosis for children diagnosed with advanced stage or relapsed disease. Boric acid (BA) which is an antiseptic, insecticide and precursor to other chemical compounds, has been shown to decrease cell growth in prostate cancer, osteosarcoma and malignant melanoma in previous studies but not in NB yet. Hence, we aimed to evaluate the effect of borat derivatives, BA and disodium pentaborate (DSP) of NB cell behaviour.

Method: Kelly (N-myc amplification positive) NB cells were cultured in RPMI 1640 supplemented with 10% FBS, 1% penicillin/streptomycin, 1% L-glutamine and incubated with BA (100 uM) and DSP (5 mM). Anti-proliferative effects were evaluated after 24 hours by WST-1 test. Apoptotic cell levels were detected by annexin V flow cytometric analysis. The migration of cells was monitored and saved by wound healing and metastatic in vitro assays in subgroups of control, BA and DSP treated cells.

Results: Both of the borate derivatives increased late apoptosis three times while total cell deaths were two times more than the control group. When evaluated seperately, BA was found to be inducing apoptosis 5 times more than DSP. According to in vitro migration models, cells migrated more in BA treated cells. Control cells and DSP treated cells showed similar disposition of migration.

Conclusion: The borate derivatives BA and DSP induced apoptosis on NB cells. However they did not show significant anti-metastatic effects. Particularly, the systemic effect of higher concentrations of BA is suggested to be evaluated on experimental NB animal models in future studies.

Keywords: Neuroblastoma, Boric Acid, Disodium Pentaborate
P-063 - INVESTIGATION OF EFFECTS OF OLIVE (OLEA EUROPAEA L.) LEAVES ON LIVER CELL LINES

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Introduction: Olive (Olea europaea L.) is commonly consumed fruit. In addition to dietary consumption olive used for medical purposes for hundreds of years. Olive cultivated nearly everywhere around the world. However highest cultivation quantity concentrated around Mediterranean countries such as Spain, Italy, Greece Turkey, Syria. Main constituent of olive is fixed oil of the fruit and olive oil commonly used for dietary and medical purposes. Flavanoids, iridoids, secoiridoid glycosides, triterpenes, biophenols and many others are the secondary metabolites of olive. Oleuropein is the responsible compound for antioxidant, anti-inflammatory, anticancer, protection on Low Density Lipoproteins (LDL), antihypertensive effects of olive.

Material and Methods: In this study anti proliferative effects of Olea europaea L. leaves extract on liver cancer cell lines has been investigated. Olive leaves has collected around Iznik province of Turkey and these medical herbs extracted by maceration. Cytotoxic and genotoxic effects of extracts of Olea europaea L. Leaves extract have been performed with cell culture techniques. Hepatoma and liver normal cell lines were used due to stability and suitability for our work. Apoptosis induction of extracts has been performed with acridine orange assay. Used method for cytotoxicity determination was ATP assay. Intracellular Reactive Oxygen Species (ROS) has been detected according to Rajesh et al. (2010). Examination of genotoxicity made by comet assay.

Discussion: According to our results olive leave extract were more cytotoxic, genotoxic and apoptotic when compared with hepatoma cell lines.

Keywords: Olive, Olea europaea L., Oleuropein, Hepatoma, Anti cancer

P-064 - ISOLATION AND PURIFICATION OF ANTICANCER AGENTS CUCURBITACIN D AND I BY HPLC FROM EC BALLIUM ELATERIUM (L.) A. RICH

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Introduction and Objective: Ecballium elaterium (L.) A. Rich. (EE) fruit juice is used for the treatment of sinusitis and for several illnesses in Turkish folk medicine. EE also may be a great natural source for the development of new drugs and may provide a cost effective mean of treating cancers and other diseases in the developing world. It has been used against cancer in the last years. This effect arises from cucurbitacins contained in the plant. This study was designed to isolate and purify Cucurbitacin D and I from EE fruit juice by HPLC.

Material and Methods: EE fruits were collected from Adana, Turkey. The fruits were washed, pressed and the collected juice strained. The juice was extracted with chloroform. The aqueous phase in the fractions was removed and the process continued with the organic phase. The chloroform extract was reduced to 10 mL volume and then fractionated with flash chromatography. The residue obtained by removing the organic phase was dissolved in ethyl alcohol and then was fractionated by an analytical column HPLC. The optimised chromatographic conditions were acetonitrile-water [2:8, solvent A and 45:55, solvent B] with gradient elution analysis. Detection was achieved by a DAD detector at 229 nm wavelength. The Cucurbitacin D and I fractions were collected in the fraction collector. The fractions were lyophilized and Cucurbitacin D and I crystals were obtained.

Results: The isolated Cucurbitacin D and I were checked against standards (Extrasynthese, France). The concentrations of the isolated Cucurbitacin D and I were determined as 418 µM and 296 µM, respectively. The isolated Cucurbitacin D and I had the same purity as the standards.

Keywords: Ecballium elaterium (L.) A. Rich., Cancer, Cucurbitacin D and I, HPLC
P-065 - THE BLOOD NEUTROPHIL/LYMPHOCYTE RATIO IN BREAST CANCER

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Background: While the behavior of cancer involves numerous molecular cascades and processes, it is recognized that inflammation plays a major role in cancer biology. It is suggested that there is the stability of neutrophil/lymphocyte ratio (NLR) compared with the absolute leukocyte subtype counts that could be altered by various physiological, pathological, and physical factors. Moreover, NLR may represent the two opposing inflammatory and immune pathways that exist together in cancer patients. We aimed to investigate NLR in breast cancer, in our population.

Methods: Using data retrieved from the medical records, 66 women diagnosed primary breast cancer met our study inclusion criteria as they had a complete blood count with leukocyte differential performed before any anti-cancer therapy. And 44 women with benign mammary neoplasm/disease, followed up in the out-patient clinics of mammary disease and confirmed with sonographical/histopathological examination, made up our controls. Exclusion criteria included laboratory evidence of white blood cells count (WBC) > 10.5 × 10^9/L. Differential leukocyte counts were obtained by BC 6800 (Mindray Medical International Ltd., China), we examined WBC, neutrophil, lymphocyte, platelet counts, and hematocrite, NLR, mean platelet volume values.

Results: Although there is lack of evaluation of tumor-associated neutrophils and lymphocytes, higher NLR median values and lower lymphocyte mean counts (lymphopenia) were found in women with breast cancer (p<0.0001) as shown in Table 1 and Figure 1. There was a weak negative correlation in breast cancer between NLR values and platelet counts (rs = -0.274; p = 0.026).

Conclusion: Studying complete blood count and indices has advantages of short turn-around time, requiring no sample preparation and being cost-effective. The performance of NLR on assessing the risk of breast cancer should be investigated in pre and post-menopausal women in further studies at the molecular level including clinical outcomes and demographical/histopathological data.

Keywords: Blood, Breast, Cancer, Lymphocyte, Neutrophil
P-066 - INVESTIGATION OF THE EXPRESSION STATUS OF TIMP3 IN LARYNX CANCER

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Background: Larynx cancer (LC) is the most common cancer type in the head and neck region. The incidence of LC is around 2.4% among all cancer types in the world. Despite of advances in medicine in recent years, there is not a significant reduce in the mortality ratio of the LC. Although environmental and genetic factors play a role in the development of LC the underlying mechanism is away from being clear. TIMP3, encoded by gene on chromosome 22, and also known as tissue inhibitor of metalloproteinases 3, is a member of the inhibitors of the matrix metalloproteinases which play a role in degradation of the extracellular matrix. Loss of TIMP3 expression was recorded in various types of cancer such as pancreatic ductal and gastrect adenocarcinoma, non-small cell lung, endometrial and renal cell carcinoma. In our study, we investigated the association of differentially expressed level of TIMP3 with LC.

Methods: The expression status of TIMP3 was analyzed in tumor and matched-normal tissue samples of 44 patients with LC by the quantitative real-time polymerase chain reaction method (QRT-PCR).

Results: The TIMP3 and the reference gene expression status were analyzed by calculating the threshold cycle numbers (Ct) as fold changes using the 2-ΔΔCt method, after performing QRT-PCR method. After evaluation of the expression levels obtained from tissue samples, we selected the ratio of >=2 as the threshold for differentially expressed TIMP3. The decreased expression ratio of TIMP3 was observed as 65.9% (29/44) in LC patients. The results will be compared with the clinopathological data.

Conclusions: It is estimated that the differentially expressed levels of TIMP3 was associated with the larynx carcinogenesis. Our study is still in progress to include a larger cohort of patients.

Keywords: TIMP3, Cancer, Investigation

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P-067 - CHEMOMETRIC DISCRIMINATION OF BREAST CANCER USING SPECTRAL HISTOPATHOLOGY

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Like cancer, most disease states are associated with chemical composition changes. Therefore, biochemical changes in a tissue can provide important molecular clues for diagnosis of cancer. These molecular clues can be considered as biological markers of disease state and have a complex chemical nature. Spectroscopic tools can be employed to sense molecular level changes between the healthy and tumor tissues, providing a means of spectral histopathology if they are validated by a standard clinical test.

This presentation covers determination of biochemical markers of breast cancer using Fourier transform Infrared spectroscopy at the attenuated total reflection mode at the molecular level and compare with the results of standard histopathological assessments to validate discrimination of tumor cells from healthy ones. The study involved seventeen breast cancer diagnosed cases from which twelve cases include samples from healthy and tumor cells. Spectral fingerprints suggest that breast cancer creates molecular changes in the amide I and II vibrational bands of protein along with DNA/RNA groups at 1240 and 1082 cm⁻¹. Chemometric discrimination models were developed based on molecular information of amide, and DNA groups and tumor tissue was discriminated successfully from the healthy tissue. DNA modes were found to be the affected peaks so that class models based on DNA could provide the most accurate discrimination of tumor up to 90 percent. The poster will illustrate the methodology of the our spectral histopathology approach, spectral biological markers of breast cancer, use of a canonical variate analysis as a tool of tumor tissue discrimination. In addition to discrimination, spectral histopathology can also offer understanding of mechanistic cancer development by investigating lipid ratio in tumors and grading of cancer from spectral responses of DNA/RNA. The most striking advantage of spectral histopathology is based on rapid discrimination of tumor cells without the help of a priori information.

Keywords: Breast Cancer, Spectral Histopathology, Molecular markers of Cancer
Cancer Tissue and Spectra

The attached figure shows picture of three breast cancer tissues and their FTIR spectra. Spectra was used for histopathological assessment of breast cancer.

P-068 - THE EFFECTS OF SOME ARTIFICIAL SWEETENERS ON MTDNA

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Objective: The aim of the current study, detection of the effects of some artificial sweeteners on mtDNA damage and copy number in Drosophila melanogaster. Artificial sweeteners are added to a wide variety of food, drinks, drugs and hygiene products. A cancer-inducing activity of one of these substances would mean a health risk to an entire population. DNA mutations generated by some artificial sweeteners have been investigated by some researchers but there is no information in the literature about the effects of these substances on mtDNA. mtDNA damage could potentially be more important than deletions in nDNA because the entire mitochondrial genome codes for genes are expressed. Also, somatic mtDNA mutations have been increasingly observed in primary human cancers.

Material and Methods: In this research, the QPCR method was used to measure mtDNA damage. The lesion present in the DNA blocked the progression of any thermostable polymerase on the template, so a decrease in DNA amplification was observed in damaged templates. We used Drosophila melanogaster as a model organism for our research.

Results: Aspartame created statistically significant mtDNA damage. There was no mtDNA damage in Saccharine+Cyclamate, Saccharine, Aceulfam K and Sucralose application groups.

Conclusion: Over half a century ago, Warburg initiated research on mitochondrial alterations in cancer. These alterations include changes in mtDNA content and mtDNA mutations. In recent years, many mtDNA mutations have been identified in various types of human cancer. Aspartame created mtDNA damage in Drosophila according to our research. These results indicate that the effects of aspartame in human should carefully detect.

Keywords: Artificial sweeteners, mtDNA damage, mtDNA copy number
P-069 - EFFECTS AND MECHANISMS OF VARIOUS CALORIE RESTRICTION METHODS ON EXPERIMENTAL CARCINOMA OF SPRAGUE DAWLEY RATS

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Background: This study aimed to investigate the effects of different calorie restrictions, starting after tumor growth progressed to a certain degree, on tumor growth in induced breast cancer models.

Materials and Methods: A total of 34, 21-days old female Sprague Dawley rats were used. Breast cancer was induced via NMU (50 mg/kg/i.p.) on 29 rats. When tumors grow to 10-12 mm, rats were divided into 3 groups paired according to tumor size: animals in one group were given chow diet ad libitum and served as cancer controls (CC), while others undergo 50% calorie restriction (CR-50%) or fed alternate days (ADF) for following 12 weeks. Other 5 animals were given physiological saline and served as ad libitum-fed healthy controls.

Body weights and, palpable, tumor diameters were recorded weekly. At the end of experiment tumors excised, weighed and their diameters measured. In sera prepared from blood samples, concentrations of the insulin, IGF-I, corticosterone, leptin and adiponectin were determined via ELISA.

Results: Rats undergoing CR-50% lost 26-38% of their initial live weights within 6 weeks, and experiment terminated for this group after 6 weeks. Other 5 animals were given physiological saline and served as ad libitum-fed healthy controls.

Conclusions: ADF protocol did not delay tumor growth to rats undergoing this type of CR. CR remains a viable option as a dietary intervention in breast carcinoma.

Keywords: Alternate Day Fasting, Breast Cancer, Calorie Restriction, Sprague Dawley Rats, NMU

P-070 - SCREENING ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF POLYPORUS SQUAMOSUS AND CANTHARELLUS CIBARIUS EXTRACTS AND THEIR EFFECTS ON GLUTATHIONE-S-TRANSFERASE ACTIVITY

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Medicinal mushrooms show an ideal food nature due to their low sugar and oil content, nutritional value and especially because of being good diet products. Nowadays there is a growing interest in new drugs against secondary metabolites derived from fungi and for the discovery of precursor compounds. These bioactive components are becoming popular sources of natural antioxidant, antitumor, antiviral, antimicrobial and immunomodulatory agents. In this study, the ethanol extracts of Polyporus squamosus and Cantharellus cibarius species were analyzed for the polyphenolic contents by using spectrophotometrically methods. The free radical scavenging and antimicrobial activities of extracts were evaluated by DPPH and disc diffusion assays. Besides, the mushroom extract effects were examined on the glutathione-S-transferase (GST) enzyme activity by kinetic assay. According to the results, ethanol extract of P. squamosus has been shown the highest total of phenolic and flavonoid contents with 25.65 ± 0.37 mg GAE/g and 12.36 ± 0.04 QE/g values, respectively. The highest DPPH radical scavenging were observed for ethanol extract of P. squamosus with 0.329641 ± 0.002275 mg/mL IC50 value. Also, the best activity profile for GST was observed with the crude ethanol extract of P. squamosus. In this study we additionally screened antimicrobial activity of mushroom extracts on the Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa strains. However, ethanol extract of P. squamosus had shown moderate effects on the bacteria strains inhibition.

Keywords: Polyporus squamosus, Cantharellus cibarius, Antioxidant, Antimicrobial, Glutathione-S-transferase
P-071 - EFFECTS OF CHRONIC AND INTERMITTENT CALORIE RESTRICTION ON ADROPIN LEVELS IN MMTV-TGFα BREAST CANCER MOUSE MODEL

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Aim: Adropin, a recently identified peptide, has been implicated in insulin resistance and might be one of the potential growth regulator metabolic hormones. The aim of this study is to determine the effect of calorie restriction on circulating levels of adropin in MMTV-TGFα breast cancer mouse model and investigate the effects of adropin peptide on viability of MCF7 breast cancer cells in culture.

Methods: Mice were fed different dietary regimes ad libitum-fed (AL), chronic calorie restricted (CCR), and intermittent calorie restricted (ICR) from ten weeks of age up to 50 weeks. Serum adropin concentrations were evaluated using an enzyme-linked immunosorbent assay. In addition, cells were treated with 5, 10, 25, 50ng/mL adropin for 24 hours. Cell viability was measured with WST-1 assay and cell cycle analysis was examined by flow cytometry.

Results: There was an inverse correlation between serum adropin levels and mouse age that was attenuated by calorie restriction. In the AL group, the level of adropin was significantly lower at week 50 (3.6±0.2ng/mL) compared to levels at week 10 (4.3±0.2ng/mL). However, among the calorie restricted groups, serum levels of adropin remained high at week 50 (CCR 4.3±0.2ng/mL; ICR 4.7±0.2ng/mL). Incubation of MCF7 cells with 50ng/mL adropin for 24h resulted in a statistically significant decrease in cellular viability (p<0.001), while 24h treatment of 10 and 25ng/mL adropin did not show any significant effect. Flow cytometry analysis showed that MCF7 cells entered the early phase of apoptosis after treatment with 50ng/mL.

Conclusions: Our finding that calorie restriction can offset an age-dependent decrease in circulating adropin levels in a breast cancer mouse model suggests that adropin may be involved in the protective effects that calorie restriction has on breast cancer risk. Moreover the preliminary results of adropin on breast cancer cell viability provide a possible mechanism and indicate that additional studies are warranted.

Keywords: Breast Cancer, Calorie Restriction, Adropin, MMTV-TGFα Mice
P-072 - THE INFLUENCE OF DIFFERENT CALORIE RESTRICTION PROTOCOLS ON SERUM PRO-INFLAMMATORY CYTOKINES, ADIPOKINES AND IGF-I LEVELS IN FEMALE C57BL/6 MICE: SHORT TERM AND LONG TERM DIET EFFECTS

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Calorie restriction (CR) is an effective intervention to prevent chronic diseases in particular cancer. Although many factors including sex hormones, IGF-I and mTOR have been studied in response to CR, the molecular mechanisms of CR remain to be determined. Our objective was to determine the short and long-term effects of different CR protocols on pro-inflammatory cytokines. Our hypothesis was that Intermittent CR (ICR) will result in greater inhibition of pro-inflammatory serum cytokines compared to Chronic CR (CCR) as we previously found ICR to be more protective in prevention of mammary tumor development. From ten week old female C57BL6 mice were maintained on either ad libitum (AL) fed, ICR or CCR protocols for up to 74 weeks of age. Blood samples were collected for measurements of serum interleukin-6 (IL-6), tumor necrosis factor–alpha (TNF-alfa), adiponectin, leptin, IGF-I and insulin levels at specified ages and for ICR mice samples were collected by following restriction (ICR-R) and after refeeding (ICR-RF). In general, both modes of CR reduced serum IL-6, TNF-alfa, IGF-I and leptin levels significantly compared to AL feeding with IL-6 levels 24 and 3.5 fold and TNF-alfa levels about 11 and 1.5 folds lower in ICR and CCR groups, respectively at study termination. There was a trend for adiponectin and insulin to be highest in ICR-RF mice. Body weights were positively correlated with IL-6, TNF-alfa, insulin and leptin but negatively correlated with adiponectin-to-leptin ratio. Moreover, there was a positive correlation between IL-6 and TNF-alfa. Beneficial effects of ICR may function through pro-inflammatory cytokine pathways.

Keywords: Cytokines, calorie restriction, IGF-I, adipokines, inflammation

P-073 - CYTOTOXIC AND APOPTOTIC FUNCTIONS OF TOLFENOMIC ACID ON HUMAN PROSTATE CANCER CELLS

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Prostate cancer is the common adenocarcinoma and second common cause of cancer death in men. The progression of prostate cancer is a multistep process. COX-2 is highly expressed in a number of human prostate cancers and has been identified as an important second messenger that play important roles in various aspects of cancer biology including inducing apoptosis and inhibiting proliferation of cells. We questioned whether tolfenamic acid (COX inhibitor) affects the survival and/or promotes apoptosis of prostate cancer cells (LnCaP) in vitro. After growing the cells in culture, we determined viability with MTT, apoptosis with flow cytometry and activity of COX-2 enzyme with real time PCR. Comparing to the control 1, 1, 5, 10, 25, 50 and 100 µM tolfenamic acid reduced the number of LnCaP cells to 91, 83, 82, 76, 61 and 49 % in 24 hr and 90, 83, 82, 78, 52 and 47 % in 48 hr, respectively. Early apoptotic rate of LnCaP cells were with were 2 and 46 % for 24 hr and 9 and 94 % for 48 hr, respectively. Tolfenomic acid (50 µM) increased the level of caspase-9 up to 4 fold comparing to the control. Moreover, 25, 50 and 100 µM tolfenamic acid reduced the level of COX-2 enzyme down to 1, 0.3 and 0.1 fold. Tolfenomic acid posseses a strong dose and time dependent antiproliferative effect on prostate cancer cell line, LnCaP by blocking COX-2 pathway of arachidonic acid metabolism and by activating caspase-9.

Keywords: Prostate cancer, tolfenomic acid, COX-2
P-074 - THE CYTOTOXIC ACTIVITIES OF NOVEL TEREPTHALATO COMPLEXES

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Object: In this study, we examined cytotoxic activities of six new terephthalato complexes on two commonly used human prostate cancer cell lines (LNCaP and DU145).

Material and Method: Cytotoxic activities of all complexes (1 to 500 µM) were performed prostate carcinoma cells and by MTT assay for 24 and 48 h. Cisplatin was used as a positive control. Enzyme-linked immunosorbent assay is used to identify apoptotic and necrotic cells. All statistical analyses were performed using one-way analysis of variance (ANOVA) and followed up by Tukey’s multiple comparison tests.

Results: Complex of 6 was the most potent growth inhibitory effect against these cell lines. The IC50 values of complex 6 was determined as 43, 22 µM for LNCaP and 45, 18 µM for DU145 for 24 and 48 h, respectively. Prostate cancer cell lines were treated with various concentrations (10, 25, and 50 µM) of 6 complex for 24 h. This complex increased the apoptotic and necrotic cell death in both cell lines in a concentration dependent manner.

Conclusion: We evaluated growth inhibitory effect of six new terephthalato complexes on two different human prostatic carcinoma cells. Among these compounds complex 6 had decreased significantly the cell viability time and dose dependently these cell lines. Especially after 24 h treatment, 6 complex increased the apoptotic and necrotic cell death in both cell lines in a concentration dependent manner.

Keywords: Cytotoxicity, MTT, Prostate Carcinoma, Terephthalate Complexes

P-075 - THE EFFECTS OF [Ca+2]I MOBILISATION ON BINDING OF SKOV–3 CELLS TO FIBRONECTIN

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Introduction and Aim: Intracellular calcium, [Ca+2]i increasing via endoplasmic reticulum (ER) stress is known to be involved in several cellular processes, such as adhesion, apoptosis and anoikis. Extracellular proteins such as fibronectin and vitronectin are essential compounds for microenvironment of high metastatic ovarian cancer cells. Particularly, fibronectin trigger the formation of spheroid structure, attachment and disaggregation of ovarian cancer cells.

Therefore, the aim of this study is an investigation of the effects of [Ca+2]i via stimulation of ER stress on binding capacity of SKOV–3 to fibronectin.

Materials and Methods: In the presence or absence of 12-24 µM of ER stress inducer, tunicamycin, the alteration of [Ca+2]i after binding of cells to 50 µg/ml fibronectin was investigated by calcium indicator dye, Fluo–3. The excitation wavelength used for Fluo–3 was 490 nm and the emission was detected at 510 nm. The rate of binding was measured by using RTCA after treatment of tunicamycin for 50 h. The rate of cell adhesion was calculated according to NCI formula.

Results: The amount of [Ca+2]i in SKOV–3 cells was increased after the treatment of 24 µM tunicamycin upon time–dependent manner. In the presence of 24 µM tunicamycin, the cell attachment to fibronectin was reduced to 0.09 NCI value as compared to control NCI value (1.03). This decline in binding was continued for 48 h. Light microscope images also supported these results.

Discussion and Conclusion: ER stress induced by tunicamycin affected on [Ca+2]i mobilisation in SKOV–3 cells, suggesting that increased cytoplasmic calcium might be replaced into mitochondria and therefore the apoptotic process could be triggered. On the other hand, ER stress also reduced the adhesion rate of SKOV–3 cells, suggesting anoikis could be induced as well. This issue still remains to be addressed. This study is supported by Anadolu University Scientific Research Projects (Project No: 1308S303)

Keywords: Ovarium Cancer, ER Stress, Calcium, Adhesion
P-076 - RAB25 CONFRS RESISTANCE TO CHEMOTHERAPY-INDUCED CELL DEATH IN OVARIAN CANCER CELLS BY INHIBITING MITOCHONDRIAL APOTOTIC PATHWAY

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Background: In spite of the increase in patient survival rates promoted by increased screening and prevention efforts, much faster tumor genome sequencing and developed smart targeted-therapies, de novo or acquired chemoresistance still remains to be a significant factor for treatment failure in cancer therapeutics. Conventional chemotherapy and radiotherapy constitute the main two approaches in addition to surgery in cancer treatment. BCL-2 protein family members regulate chemotherapy-induced mitochondrial cell death pathway and the release of cytochrome c into the cytosol, which is the point of no return for cell death. Rab GTPases play important roles in critical cellular processes such as intracellular vesicular trafficking and cellular viability and their contribution to carcinogenesis and cancer progression becomes increasingly clear. In particular, altered expression of Rab25 has been shown in ovarian, breast and hepatocellular cancers.

Objective: Here we explored how Rab25 regulates proliferation, cell death response and the expression of BCL-2 protein family members in ovarian cancer cells. Methods: Confocal immunofluorescence microscopy was used to determine the intracellular localization of Rab25. The expression status of Rab25 and BCL-2 protein family members were detected by means of immunoblot analysis. CellTiter-Glo, colony formation assay and Annexin V/PI staining were used to evaluate cell death response. CyQUANT and MTT assays were used to assess cell proliferation.

Results: Rab25 was expressed only in two of eight ovarian cancer cells, in which immunofluorescence analysis revealed mainly cytoplasmic localization. Moreover, we demonstrated that Rab25 protected against carboplatin-, etoposide- and paclitaxel-induced cell death in ovarian cancer cells by blocking mitochondrial apoptotic pathway. Hence, siRNA-mediated knockdown of Rab25 in OVCAR3 and OVCAR4 cells resulted in decreased proliferation and increased sensitivity to chemotherapy.

Conclusion: Our results indicate a prominent prosurvival role for Rab25 in ovarian cancer cells, which is mediated through inhibition of mitochondrial apoptotic pathway.

Keywords: Ovarian cancer, Rab25, BCL-2, Apoptosis, Proliferation
P-077 - DETERMINATION OF APOPTOTIC EFFECT OF DEGUELIN ON GLIOMA CELL LINES

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Aim: Glioblastoma multiforme is the most common and very aggressive type of brain tumors. Isolated from several plant species deguelin is a natural plant rotenoid. Several studies have shown that it is a promising cancer-preventive and therapeutic agent. Since the function of deguelin on glioma cells has not been defined yet, we decided to test its possible apoptotic effect on rat (C6) and human (T98G) glioma cell lines.

Methods: Alterations in the morphology and chromatin structure of glioma cells which underwent apoptosis, were examined by fluorescence dye 4′,6-diamidino-2-phenylindole (DAPI) staining. Cells were seeded in a 24 well plate (3x10^4) and cultured for 24 h and were treated with or without 25 and 100 µM deguelin. At the end of 24 h, cells were collected and fixed in 0.5 % paraformaldehyde. Slides were stained with 1 µg/mL DAPI in the dark and were rinsed with PBS. At least 300 cells per condition were subjected to examination using a digital fluorescence microscope.

Results: Although untreated cells displayed a normal nuclear size, menadione treated and stained cells showed typical morphological features of apoptotic cells, with condensed and fragmented nuclei. Apoptotic changes have started at 25 µM dose and increased parallel with the dose increase to 100 µM both glioma cell lines.

Conclusions: In this study a new anticancer agent, deguelin, was examined for the first time in rat and human glioma cells and obtained some preliminary results about the apoptotic effects of this agent. The results indicated that deguelin induced apoptosis in a dose dependent manner on glioma cells.

Keywords: Apoptosis, Deguelin, DAPI, Glioma

P-078 - SKOV-3 CELLS BINDING TO FIBRONECTIN INDUCE MITOCHONDRIAL APOPTOTIC PATHWAY AFTER STIMULATION OF ENDOPLASMIC RETICULUM STRESS

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Introduction and Aim: [Ca2+]i increased by endoplasmic reticulum (ER) stress is transferred into mitochondria in order to reduce the [Ca2+]i to normal levels, This transition of [Ca2+]i is known to trigger the mitochondrial pathway of apoptosis. In vitro studies with ovarian cancer indicated that the mitochondrial membrane depolarization (MMP) can be occurred by [Ca2+]i mobilization and consequently, cytochrome c is released via Bax/Bak formation. The integrin activation by fibronectin is also induced the [Ca2+]i, however the role of SKOV-3 cells-fibronectin interaction in apoptosis has not investigated yet.

The aims of this study are the investigation of MMP and localisation of cytochrome c and Endo G releases from mitochondria in SKOV-3 cells attached to fibronectin after stimulation of ER stress.

Materials and Methods: MMP was investigated using flow cytometry after the cells were plated onto 50 µg/ml fibronectin coated wells. After 2 or 12 h, the cells were treated with either 18 µM tunicamycin or 10 µM dinitrofenol as a positive control. The localization of cytochrome c and Endo G was demonstrated using fluorescence labeling method.

Results: The adhesion of SKOV-3 cells to fibronectin for 2 or 12 h stimulated an increase in MMP (61,9±15,78 and 91,35±2,38, respectively) as compared to control (37,95±5,2). The fluorescence images showed that the accumulation of cytochrome c into the cytosol increased after adhesion of cells to fibronectin as compared to control at 120 min.

Discussion and Conclusion: SKOV-3 cell binding to fibronectin caused both mitochondrial membrane deposition and the releases of cytochrome c and Endo G from mitochondria after stimulation of ER stress. Induction of MMP, followed by cytochrome c and Endo G releases from mitochondria is a critical step of the
P-079 - INHIBITION OF 5-HT1B RECEPTOR SIGNALING INDUCES CASPASE-INDEPENDENT APOPTOSIS IN PANCREATIC DUCTAL ADENOCARCINOMA CELLS

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Pancreatic cancer (PaCa) is the fourth leading cause of cancer deaths with 100% mortality rate in Western countries. PaCa is intrinsically resistant to apoptosis and poorly responds to existing therapeutics. To be able to overcome drug resistance and improve PaCa therapy, novel molecularly targeted therapies have to be developed. Serotonin (5-hydroxytryptamin, 5-HT) was known to acts as mitogenic growth factor for several types tumor cells, including pancreatic carcinoïd cells, breast and colorectal carcinomas. Among 5-HT receptors, we previously shown that 5-HT1B and 5-HT1D subtypes promotes pancreatic ductal adenocarcinoma (PDAC) cell proliferation and invasion (Gurbuz& Ozpolat, PlusONE 2012). Thus in this study, we aimed to investigate the effects of targeting 5-HT1B receptor using highly specific antagonist, SB216641, in PDAC cells. Inhibition of 5-HT1B receptor by SB216641 at 5 μM and 10 μM doses induced significant cell death of Panc-1 and MiaPaCa-2 cells by about 50% and 70%, respectively. The apoptotic protein expressions, inducing factor (AIF), the major mediator of caspase-independent apoptosis, and Cytochrome C (Cyt-C), the major mediator of mitochondrial apoptotic death, were evaluated both in mitochondrial and cytosolic fractions of PDAC cells treated SB216641. We found a marked induction and release of both of AIF and Cyt-C from mitochondria to cytosol compared to non-treated control cells. In conclusion, our data suggest that inhibition of 5-HT1B receptor-signaling induces mitochondrial dependent apoptosis. Considering the intrinsic apoptotic resistance, 5-HT1B receptor antagonists might be used to treat PDAC and further in vivo studies are warranted.

Keywords: Serotonin, 5-HT, AIF, Apoptosis, Pancreatic Cancer
P-080 - ANTI-TUMOR EFFECTS OF BEMIPARIN IN HEPG2 AND MIA PACA-2 CELLS

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Purpose: In this study, we examine the effects of bemiparin on apoptosis and cell cycle related gene expression, viability, colony formation and migration/invasion of the cultured MIA PaCa-2 pancreatic cancer cell line and HepG2 hepatocellular carcinoma cells.

Material and Methods: Effects of bemiparin on cell viability and detecting of IC50 dose in MIA PaCa-2 and HepG2 cells were performed by using XTT assay. Total RNA was isolated from the cells exposed to IC50 doses of bemiparin using Trizol Reagent. Effects of the bemiparin on apoptosis and cell cycle related genes were determined via RT-PCR. Potential effects of the bemiparin on cell invasion, colony formation and cell migration were detected by matrigel-chamber, colony formation assay and wound-healing assay, respectively. The comparison of the control and dose groups has been analysed by “RT² Profiles PCR Array Data Analysis” through “Student t-test”.

Results: IC50 dose of bemiparin was found to be 200 IU/mL in the 48th hour in the MIA PaCa-2 cell line and 50 IU/mL in the 48th hour in the HepG2 cell line. In HepG2 dose group, CCND1 expression was reduced and p53, caspase-3, p21, caspase-8 expressions were increased in the dose group cells when compared with the control group cells. In MIA PaCa-2 dose group, CCND1, CDK4 and CDK6 expressions were reduced and p53 expression was increased. Cell invasion and migration was significantly inhibited and colony formation was significantly decreased through bemiparin treatment in both cell lines.

Conclusion: In present study, bemiparin inhibits cell proliferation by inducing cell cycle arrest, apoptosis and also decreases invasion, migration and colony formation in HepG2 and MIA PaCa-2 cells. Bemiparin may be a novel agent for treatment of hepatocellular and pancreatic cancers as a single agent or in combination with other agents.

Keywords: Apoptosis, Bemiparin, Cell cycle, HepG2, Mia Paca-2
P-081 - AN IN VITRO AND IN VIVO EVALUATION OF THE EFFECTS OF DOXORUBICIN AND CHLOROQUINE COMBINATION ON MICE EHRlich ASCITES CARCINOMA (EAC)

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Background and Aim: Cancer still remains the most horrifying disease due to treatment failure, cause development of resistance to chemotherapeutics. Autophagy is regarded as one of the key mechanism for drug resistance and chloroquine (CQ) is an inhibitor of autophagy. Therefore, the combination of Doxorubicin (DXR) and CQ may be a realistic strategy for a new treatment modality on Ehrlich Ascites Carcinoma (EAC) cells in vivo and in vitro.

Materials and Methods: EAC cells were treated with DXR (1µM) alone or in combination with CQ (8µM). ATP assay were perfomed to determine cytotoxicity after 48h treatment. Apoptosis and autophagy related proteins were also determined by Western blot analysis. Changes in survival pathway was detect by Luminex xMAP. For in vivo evaluation, 88 Balb-c mice with EAC were divided into control (n=8) and 8 experimental groups (n=10). DXR and CQ have been investigated following intraperitoneal administration of doses of 1.5 and 3 mg/kg X 3 and 25 and 50 mg/kg X 3 respectively. Tumor volume determined in vivo by caliper and relationship between apoptosis, proliferation and autophagy was evaluated by immunohistochemistry.

Results: According to ATP viability assay, combination of DXR and CQ caused a significant decrease in cell viability compared doxorubicin alone. Also 24h treatment of CQ and DXR combination showed increased autophagy protein levels. Cleavage of PARP1 and caspase-3, besides increase in expression of PTEN and FAS protein indicating the enhanced apoptosis. Moreover proteins related to survival pathway were found to decrease by using Luminex xMAP. In vivo results showed the combination of CQ and DXR reduced tumor volume and increased apoptosis by suppressing the autophagy and proliferation.

Conclusion: The combination of DXR and CQ enhances apoptosis, possibly via the inhibition of autophagy and this might be a promising therapeutic strategy in EAC cells.

Keywords: Chloroquine, In vivo, Doxorubicin, Autophagy, Apoptosis

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**P-082 - MIR376B IN BREAST CANCER**

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Autophagy, is one of the most well known catabolic processes whose activation can degrade accumulated proteins as well as damaged organelles for maintaining cellular homeostasis. Beside this, autophagy was found to be associated with cancer. In addition, miRNAs have been implicated in several fundamental biological processes. Moreover, evidence also suggests that miRNAs play a role in cellular transformation and carcinogenesis. Thus, understanding the regulation of autophagic mechanisms through miRNAs might have tremendous importance in the field of cancer.

Overexpression of MIR376B in MCF-7 cells has been utilized and several mono clone cells picked and cultured under selection condition. For further analysis, mono clones were evaluated by their autophagic capacity via LC3 shift, p62 accumulation and MIR376B target protein status. After the characterization of clones, several growth analyses were performed either short or long term assays in vitro. On the other hand, Gamma-H2AX foci analysis and ROS measurement by DCFDA was carried out to identify the DNA damage and oxidative stress, respectively. We also observed; in colony formation assay, those cells formed more and bigger colonies.

As a consequence of autophagy deregulation, accumulation of p62 was observed in MIR376B stable cells. Intriguingly, intracellular ROS level was also increased and accumulation of ROS localized around the mitochondria. In addition to susceptibility of oxidative stress, loss of autophagy makes cells more prone to DNA damage. Although in short term assays, growth attenuation of MIR376B stable cells was observed; in colony formation assay, those cells formed more and bigger colonies. In addition, we also figured out that MIR376B clones have a capacity to establish a bigger tumor in comparison to control clones in vivo.

We identified for the first time that MIR376B as a key miRNA which might has a role in tumorigenesis in breast cancer.

**Keywords:** MicroRNA, MIR376 Family, MIR376B, Cancer, Autophagy

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**P-083 - MIR-548A-3P, MIR-548AS-3P AND MIR-8078 ARE RESPONSIBLE FOR TNF-a MEDIATED NF-KB INDUCTION OF NSCLC INVASION**

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**Introduction:** Lung Cancer is the leading cause of cancer related deaths in the world and approximately 90% patients with lung cancer ultimately die from metastatic disease. Metastasis is the most dangerous step of cancer. In our recently published work showed that Akt/NF-kb pathway is continuously active and induces cellular invasion and PTEN suppresses cellular invasion via inhibition of Akt/NF-kb pathway. In this study we aimed to show NF-kb mediated induction of miRNA expression can responsible for inducing NSCLC invasion. Materials-Methods: We used Chromatin Immunoprecipitation (ChIP) Assay Kit for detection of TNF-a induced NF-kb mediated miRNAs. Therefore, H1299 and PC14 cells treated by TNF-a (30ng/ml) for ChIP assay. Chromatin regions, reading with ChIP-Seq, were analyzed using bioinformatics tools. We also performed additional bioinformatics search to find NF-kb related miRNAs which potentially take a role in NSCLC invasion. We investigated the effects of miRNA which determined at the bioinformatics analysis results on invasion using invasion chamber method.

**Results:** We found 16 miRNAs which potentially induced by NF-kb and related with NSCLC invasion. Our invasion results indicate that miR-548a-3p, miR-548as-3p, miR-8078, miR-1915, miR-6814-3p, miR-548q mimics can induce cellular invasion on H1299, miR- 548v, miR-548h-5p, miR-138-5p, miR-548a-3p, miR-548as-3p and miR-8078 mimics can induce cellular invasion on PC14. We also verified our results by qRT-PCR, because we want to sure that miRNAs which can induce invasion, can also transcriptionally regulated by NF-kb or not.

**Discussion and Conclusion:** We found that miR-548q, miR-548a-3p, miR-584as-3p, miR- 1915 and miR-8078 in H1299, miR-138-5p, miR-548a-3p, miR-548as-3p and miR-8078 in PC14 can induce cellular invasion by NF-kb. As a conclusion, Our investigation indicate that NF-kb can induce NSCLC invasion via miR-548a-3p, miR-548as-3p and miR- 8078.

**Keywords:** NSCLC, microRNA, Invasion, NF-kb Pathway

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P-084 - EXPRESSION ANALYSIS OF MIR-181C, MIR-34A AND MIR-375 IN GASTRIC CANCER

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Background: Gastric cancer (GC) has high incidence and casualty rate in different countries is still a standout amongst the most continuous and fatal diseases. MicroRNAs (miRNAs) initiate translational controlling and play a critical role in developmental timing. Presently, miRNAs have been determined to play an important roles in several pathological and physiological conditions, specially miRNA deregulation in different types of cancer. In the current study, we aimed to examine the contribution of miR-181c, miR-34a and miR-375 expressions to unravel their role in gastric cancer tissue, the miRNAs to the risk of gastric cancer.

Methods: MiRNAs of 38 paired tumor and normal tissue samples that were grouped according to the types of gastric cancer and clinical characteristics of patients, including gender and average age were determined by quantitative real time polymerase chain reaction (qRT-PCR) technique.

Results: The expression level of miR-34a and miR-375 were significantly down-regulated while the expression level of miR-181c was significantly over-expressed in gastric cancer tissues according to gastric normal tissues.

Conclusion: Our results shows decreased expression in miR-34a and miR-375 might be a risk factor for gastric cancer. Further analysis is required to identify the responsible miRNAs rather than miR-181c, miR-34a and miR-375 in gastric cancer tissues.

Keywords: Gastric cancer, microRNA, Expression analysis, qRT-PCR

P-085 - EXPRESSION EVALUATION OF MIR-132-5P, MIR-184 AND MIR-34C-5P IN BREAST CANCER TISSUE

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Background: Breast cancer is the most common malignancy among women in the worldwide, the incidence rates are highest in the Western world. non-coding RNAs that regulate the expression of target genes, microRNAs (miRNAs) belong non-coding RNA, play a significant role in the post-transcriptional regulation of gene expression by mRNA degradation or translational repression. The target of this study was to examine the expression level of miR-132-5p, miR-184 and miR-34c-5p in breast cancer tissues.

Methods: Expression determining of miR-132-5p, miR-184 and miR-34c-5p in 40 pairs of tumor and normal samples of breast cancer patients were performed by quantitative real time polymerase chain reaction (qRT-PCR).

Results: The expression level of miR-132-5p and miR-34c-5p in breast cancer tissue were significantly decreased (down- regulated) according to normal tissue samples. However, The expression level of miR-184 in breast cancer tissue was significantly increased according to normal tissue samples (over-expressed).

Conclusion: Decreased expression level of miR-132-5p and miR-34c-5p and increased expression level of miR-184 might be risk factors for breast cancer development, and suggesting these miRNAs expression alteration modify individual susceptibility to breast cancer. In order to understand molecular mechanism of breast cancer further analysis warranted.

Keywords: Breast cancer, microRNAs, Reverse transcription, qRT-PCR
**P-086 - INVESTIGATION OF THE PROMOTER METHYLATION PROFILES OF THE MISMATCH REPAIR GENES IN LARYNX CANCER**

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**Background:** The incidence of larynx cancer (LC) is around 2% among all cancer types. Although environmental and genetic factors play a role in the development of LC, the underlying mechanism is far from clear. Recent studies indicate that silencing of DNA repair genes by promoter hypermethylation may play a significant role in LC. The mismatch repair system (MMR) plays a crucial role in the maintenance of genomic stability. The primary function of the MMR genes is to remove mismatch base errors, insertions and deletions. The MGMT protein removes alkyl-adducts at the O6-position of guanine and prevents mutagenic effects. Cells with MMR deficiency may accumulate mutations and can progress to cancer. In this study we investigated methylation levels of six MMR genes (MLH1, MSH2, MSH6, MSH3, MLH3, PMS2) and the MGMT gene in LC patients.

**Material and Methods:** The promoter methylation status of the MMR genes were analyzed by using Methylation-Specific Multiplex Ligation Dependent Probe Amplification (MS-MLPA) in the DNA obtained from tumor tissues from 73 LC patients. PCR products were analysed in a ABI 310 genetic analyzer. The peak areas of the signals were normalized by dividing to the areas of reference probes. A difference higher than 20% was considered positive.

**Results:** In 37 (50.6%) patients, more than one gene was methylated while only one methylated gene was observed in 24 (34.8%) patients. Methylation of two genes was shown in 6 (16.2%) patients. MGMT was the most frequently methylated gene and its methylation rate was 45.2% (33/73). There were statistically significant associations between stage (p=0.02), metastasis (p=0.0006) and the presence of methylation.

**Conclusions:** Our results indicate that methylation of the repair genes is a frequent event in LC and may play a role in the development of the disease.

**Keywords:** Methylation, Epigenetics, Larynx Cancer, Mismatch Repair Genes, MGMT

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**P-087 - A COMPARATIVE STUDY OF MCF-7S AND MDA-MB-435S FOR RELATIVE DATA NORMALIZATION TO ENDOGENOUS REFERENCE GENES**

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**Purpose:** microRNAs (miRNAs) play essential roles both physiological conditions and human malignancies. Additionally, obtain from miRNAs produced by cells shows their normal function state and these molecules have been examined as pathological biomarkers. A lot of methodologies have been adjusted to characterize quantitatively the expression patterns of miRNAs, one of them quantitative real-time PCR (qRT-PCR). In qRT-PCR evaluation of gene expression, normalization of data against RNA variant by using appropriate references gene is essential. The present study aimed to investigate the alterations in breast cancer cells MCF-7s and MDA-MB-435s of endogenous reference genes from 2 to 48 hours.

**Methods:** In order to determine the quantitative expression profiles of 84 miRNAs and 6 candidate housekeeping genes (SNORD68, SNORD72, SNORD95, SNORD96A, SNORD61, RNU6-2) in MCF and MDA-MB-435 cell lines were investigated by Fluidigm Microfluidic Dynamic Array. The software program NormFinder was used for selection of candidate housekeeping genes. Total RNAs including miRNAs were isolated from breast cancer cells at 2nd, 4th, 6th, 12th, 24th and 48th hours. Determinations of relative gene expression values were carried out by using the 2-ΔΔCt method (normalized threshold cycle (Ct) value of sample minus normalized Ct value of control).

**Result:** Our results demonstrated that statistically differences 6 housekeeping genes were detected in MCF-7 cells and MDA-MB-435 cells groups at different time period (4th, 6th, 12th, 24th, 48th hours) comparing with control groups (2nd hour). We found fluctuations in other housekeeping genes except for SNORD61. Thus we showed that, notably, SNORD61 is suitable and can be used for MCF-7 and MDA-MB-435 miRNA's normalization (foldchange<2, p<0.05).

**Conclusion:** This study provides for the time-dependent a comprehensive list of suitable housekeeping genes for experimental conditions of breast cancer cells. Thus, our result promote miRNA-based studies on MCF-7 and MDA-MB-435 cells.

**Keywords:** microRNA, Breast Cancer, Reference Gene, RT-PCR
**P-088 - TIME-DEPENDENT EFFECT OF VITAMIN-D ON EXPRESSION OF MIR-548C-3P IN BREAST CANCER**

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**Purpose:** Vitamin D is an important regulator via cellular differentiation and proliferation in a lot of cancer including breast cancer. Enormous evidence that microRNAs (miRNAs), which endogenous small RNAs, are known essential player in carcinogenesis. In this report, we overview detailed information on the vitamin D effect on level of miR-548c-3p from 2 to 6 hours in MCF-7 breast cancer cell.

**Methods:** Total RNA including miRNAs were isolated from 140 µM D vitamin treated (from 2 to 6 hours) with MCF-7s and the expression of miR-548c-3p was examined by high-throughput real-time quantitative polymerase chain reaction (qPCR). All statistical analyses were performed using the Biogazelle’s qbase PLUS 2.0 software.

**Result:** We observed significantly up-regulation (nearly sixty-fold regulation higher) of miR-548c-3p both 4th hour and 6th hour in 140 µM D vitamin treated MCF-7 cell compared with D vitamin non-treated MCF-7 cells (control group) (p<0.05). Besides miR-548c-3p target genes were related with pathways such as ErbB signaling pathway (hsa04012), Pathways in cancer (hsa05200), PI3 kinase/AKT (hsa04151).

**Conclusion:** miR-548c-3p may act as a tumor suppressor-miRNA in breast cancer. Additionally, vitamin D increased expression of miR-548c-3p in MCF-7 cell. Thus, these data support that vitamin D may be an effective agent for breast cancer therapy.

**Keywords:** microRNA, vitamin D, MCF-7, Realtime-PCR

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**P-089 - MIR193B INHIBITS ERK ACTIVATION IN PANCREATIC DUCTAL ADENOCARCINOMA CELLS**

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miRNAs have been known to play key roles in various cellular mechanisms, including cell proliferation, both in nontumorigenic and tumorigenic cells. Many miRNAs were shown to be involved in the progression of pancreatic ductal adenocarcinoma (PDAC) cells. Considering the strong regulatory effects, miRNAs might be used as a novel therapeutic approach for the prevention of PDAC, which has the highest mortality rate of all major cancers. For this purpose, we aimed to investigate the potential role of miR193b in cellular proliferation of PDAC cells, Panc-1 and MiaPaCa-2. To be able to evaluate the change in cell proliferation, ERK activation was detected both in cell lines transfected with miR193b or control miRNA. We clearly obtained that miR193b inhibits ERK activation compared to total ERK protein expression in Panc-1 and MiaPaCa-2 cells. When the inhibitory effect of miR193b on the ERK activation was compared with gemcitabine, which is currently used as a clinical drug for pancreatic cancer patients, we determined that miR193b was much more effective than gemcitabine. Having tumor suppressive properties, miR193b might be served as a novel gene therapeutic approach for pancreatic cancer patients and further in vivo studies are needed.

**Keywords:** miRNA, miR193b, Cell proliferation, MAPK, Pancreatic cancer
P-090 - DIFFERENTIAL EXPRESSION LEVELS OF DICER1 ANTISENSE RNA 1 (DICER1-AS1) AND DICER1 IN DIFFERENT CANCER CELLS

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Introduction and Aim: As we all know, miRNAs are the most abundant member of the small RNAs that suppress gene expression at the post-transcriptional level. DICER1 is a type III ribonuclease involved microRNA biogenesis by producing active miRNAs. Additionally, long non-coding (Lnc) RNAs are a newer class of regulatory RNA molecules with a size longer than 200 nucleotides. A mass of indication suggest that Lnc RNAs are involved in the variety of cellular processes such as regulation of gene expression. Accordingly, the aim of the present study was to investigate interrelation between DICER1 and DICER1-AS1, a tail-to-tail natural antisense transcript.

Materials and Methods: HUVEC, and HEL299 normal cells and, MCF-7, DU-145, HeLa, and A549 cancer cells were included in the present study. For the analysis of gene expression levels RT-PCR method was used. Results were analyzed via Image J software.

Results: As a result, expression levels of DICER1 were found to be elevated in all cell lines as compared to DICER1-AS1 levels. In addition, DICER1 expression was found to be not much altered among cancer cell lines. Furthermore, DICER1-AS1 was shown to be differentially expressed in cancer cells. While its expression was up-regulated in MCF-7 and A549 cells, it was reduced in HeLa cervix cancer cells and not altered in DU-145 cells.

Discussion and Conclusions: DICER1-AS1 seems to have important regulatory function in cancers and further studies more functional studies are needed to understand its comprehensive regulatory role. Also, it is of great interest to determine the interrelation between DICER1 and DICER1-AS1.

Keywords: Cancer, DICER1, DICER1-AS1, NAT

P-091 - GENE EXPRESSION PATTERN OF MTUS1, A NEWLY IDENTIFIED TUMOR SUPPRESSOR, IN VARIOUS TYPES OF CANCERS

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Introduction and Aim: MTUS1 (Microtubule Associated Tumor Suppressor 1 gene or Mitochondrial Tumor Suppressor Gene 1) is a newly identified tumor suppressor gene located at chromosome 8p21.3-22. This tumor suppressor gene is known to encode five different protein isoforms (known as ATIPs; ATIP1, ATIP2, ATIP3a, ATIP3b and ATIP4) as a result of alternative exon usage. Furthermore, accumulating evidence suggest that MTUS1 is downregulated at various types of malignancies including prostate, bladder, liver, colon, head and neck, ovarian, and breast cancers. Thus, the aim of the present study was to evaluate differential expression of MTUS1 gene in various cancer and normal cell lines.

Materials and Methods: HUVEC, BEAS2B, and hfOB1.19 normal cells and U2OS, A549, DU-145, PC3, HGC27, BCPAP, and PANC1 cancer cells were included in the study. For the expression analysis of MTUS1 gene, Real-Time PCR method was used.

Results: As a result, expression level of MTUS1 gene were found to be highly reduced in BCPAP, PANC1, HGC27 and U2OS cancer cells and lost in DU-145 cancer cells. Surprisingly, MTUS1 expression was also found to be lost in HUVEC and hfOB1.19 normal cells. In contrast, expression levels of MTUS1 were found to be highly elevated in A549 and PC3 cancer cells. MTUS1 levels were also found to be reduced in BEAS2B bronchial epithelial cells compared to A549 cells.

Discussion and Conclusions: MTUS1 gene seems to be an important tumor suppressor regulating vital cellular process. In the present work, total MTUS1 expression levels were demonstrated. MTUS1 gene may have other cellular functions in different cancers. In the future studies, studying the role of MTUS1 gene variants can more informative in understanding its various functions.

Keywords: Cancer, MTUS1, Tumor Suppressor
P-092 - ACTIVATION OF IMMUNE RESPONSES TO COLORECTAL CANCER BY USING A COMBINATION OF RADIATION AND PROTEASOME INHIBITOR

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Colorectal cancer (CRC) is the third most common cancer worldwide and the 5-year survival rate is lower than 60%. As CRC progress, tumor cells become less susceptible to the induction of apoptosis. Radiotherapy has been extensively used for cancer therapies including colorectal cancer. Sub-lethal doses of radiation can modulate gene expression, making tumor cells more susceptible to T-cell-mediated immune attack. However, radiation treatment alone isn’t sufficient to generate strong immune responses. Inhibition of the ubiquitin-proteasome system causes inhibition of cell proliferation in all cells, including cancer cells. As inhibition of the ubiquitin-proteasome system is a promising strategy of cancer therapy, a combination treatment of proteasome inhibition and irradiation may further induce activation of tumor-specific immune responses. The goal of this study is to investigate the effects of the 26S proteasome inhibitor, bortezomib, alone or in combination with radiotherapy, on the expression of immunogenic genes in colorectal carcinoma cells. We examined two colorectal carcinoma cell lines (HCT116 and SW620) for changes in expression of multiple co-stimulatory molecules (OX40L and 41BBL) and death receptors (DR4, DR5 and CD95). Our results indicate a combination of 26S proteasome inhibition and sub-lethal radiation significantly increases the sensitivity of carcinoma cells to apoptosis. Combination treatment upregulates cell surface expression of death receptors and co-stimulatory molecules by increasing transcriptional activation of each gene. Thus, the combination treatment enhanced sensitivity to killing through FAS and TRAIL receptors by CD8+ T cells. Our studies show for the first time that combining radiotherapy and proteasome inhibition may simultaneously enhance tumor immunogenicity and the induction of antitumor immunity by enhancing tumor-specific T-cell survival and activation.

Keywords: Colorectal cancer, Bortezomib, HCT116, SW620

P-093 - ENHANCING SENSITIVITY OF BREAST CANCER CELLS TO ANTI-TUMOR IMMUNE RESPONSES BY RADIATION AND 26S PROTEASOME INHIBITION

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Irradiation leads to DNA damage by inducing cellular stress responses, which result in activation of apoptotic pathways. Radiation treatment is an effective breast tumor therapy, however, its usage is limited by dose and toxicity. Sub-lethal doses of radiation can modulate tumor gene expression, making tumor cells more susceptible to immune responses, but radiotherapy alone is insufficient to generate strong tumor-specific immune responses. The proteasome presents a novel target for combination therapies in cancer: it plays a key role in cancer cell proliferation, inhibition of radiation-induced apoptosis and development of drug resistance. The objective of our study is to investigate the effects of the 26S proteasome inhibitor, Bortezomib, alone or in combination with radiotherapy, on the expression of immunogenic genes in breast cancer cells. Here, we examined MDA231 breast cancer cells for changes in expression of multiple immuno-stimulatory molecules and death receptors. Our preliminary data indicates a combination of bortezomib and low-dose radiation significantly increases the sensitivity of MDA231 cells to apoptosis. Furthermore, this novel combination treatment upregulates cell surface expression of multiple death receptors (TNFRSF10A, TNFRSF10B and Fas/APO-1) and co-stimulatory molecules (TNFSF4 and TNFRSF9) by increasing their transcriptional activation. These data will guide experiments to determine how this combination therapy can best enhance anti-tumor immune responses.

Keywords: Bortezomib, MDA231, 26S Proteasome
P-094 - COMPARISON OF HEMATOLOGICAL PARAMETERS IN PATIENTS WITH ENDOMETRIAL CANCER

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Introduction and Aim: Endometrial cancer is the most common gynecologic cancer after breast, lung and colon cancer especially in the elderly and postmenopausal women with the disease. The aim of our study was to compare levels of neutrophil, lymphocyte, mean platelet volume (MPV) and neutrophil/lymphocyte ratio (NLR) in patients with endometrial cancer and healthy control groups.

Materials and Method: Full blood samples were collected from 38 healthy control and 199 patients with endometrial cancer. The mean age for controls and patients were 63,15 ± 10,68 and 61,70 ± 8,56 respectively. Neutrophil, lymphocyte, MPV levels and NLR measured with Abbott Cell-Dyn 3700 Hematology Analyzer. Statistical analysis was performed with IBM SPSS 20 by using Mann-Whitney U and Wilcoxon T test.

Results: Neutrophil, lymphocyte, MPV levels and NLR parameters showed statistically significant difference between patients and healthy control groups (p<0,01).

Discussion and Conclusion: In our study, the healthy and endometrial cancer groups were compared with hematological parameters, it was observed that neutrophil levels and NLR as peripheral markers of systemic inflammatory response were decreased, lymphocyte and MPV levels were increased. As it has been shown in some studies that when NLRs decreasing MPV levels increase in advanced stage endometrial malignancies and can be used as a prognostic factor. Significant increase of MPV values which observed in our study support these studies.

Keywords: Endometrium, Mean Platelet Volume, Neutrophil/Lymphocyte Ratio, Cancer

P-095 - MPV VALUES IN PATIENTS WITH PROSTATE CARCINOMA

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Introduction and Aim: In this study, our aim was to compare the level of Mean Platelet Volume (MPV) levels in Prostate Carcinoma patients and healthy control groups.

Materials and Method: Serum samples were collected from 36 healthy control and 23 patients with prostate carcinoma. The mean age for controls and patients were 70,47±7,60 and 69,43±9,26 respectively. MPV levels measured with Abbott Cell-Dyn 3700 Hematology Analyzer. Statistical analysis was performed with SPSS v21 by using independent sample t test.

Results: The MPV values in patients with prostate carcinoma (7,702±1,40) were higher from control group (6,97±0,83) but this was not statistically relevant (p=0,85).

Discussion and Conclusion: Although MPV is a inflammation marker, our analyses showed that MPV cannot be used for a inflammatory marker in patients with prostate carcinoma.

Keywords: Prostat, Mean Platelet Volume, Cancer
P-096 - PEPPERS’ DNA PROTECTIVE ACTIVITY PROPERTIES

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Peppers, vegetables of the Solanaceae family, is a species in the genus Capsicum. Pepper and pepper products have strong antioxidant capacity. The origin of chili with antioxidants, especially to determine the mechanism of UV protection is extremely important. In this study, the Latin name Capsicum Annuum L. pepper is grown in Gaziantep. Type of color cayenne pepper green and red chili peppers and red sweet pepper varieties are dried in the shade. These three kinds of conditions suitable optimization pepper hexane, dichloromethane and methanol and water extracts were obtained. Different concentrations of each peppers were exposed to H₂O₂ and UV-C in four different solvents. Then the effect on DNA damage were investigated using plasmid pBR322 DNA. Compatible with the controls of the pepper extract DNA bands were displayed. When the results were analyzed, all examples of red sweet pepper, red chili pepper and green chilies have demonstrated a protective effect against DNA, Hydrogen peroxide and UV-C, While water extract of red pepper 10 mg concentration showed a protective effect, 40 mg concentration showed a protective effect in other extracts. But, all of the green pepper extract concentration of 10 mg was observed that the protectiveness effect. Compared with other types of green peppers DNA hydrogen peroxide and UV-C said to be more protective against the rich potential of the compound.

Keywords: pBR322, H₂O₂, UV-C, DNA, Capsicum Annuum L.

P-097 - TRPV1 EXPRESSION DECREASES IN SPLEENS OF MICE BEARING METASTATIC BREAST CARCINOMA

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Introduction and Aim: Breast cancer is the leading cause of cancer-related deaths among women. Tumor induced inflammatory response is believed to be involved in metastasis of breast cancer. Transient Receptor Potential (TRP) channel 1 (TRPV1) is activated by warm temperatures and capsaicin and mainly expressed in sensory nerve endings. Recently it was also shown that TRPV1 is expressed in immune cells and might be involved in anticancer immunity.

Material and Method: We previously isolated liver (4TLM), heart (4THM) metastatic cells of 4T1 (metastatic breast tumor cell line) cells. 4TLM and 4THM cells (100,000 cell/mouse) were inoculated into the right upper mammary pad of 8-10 weeks old female Balb-c mice. Necropsies were performed 25-27 days after injection. TRPV1 immunoreactivities were examined in primary tumors and spleens by using immunohistochemistry. Tissues from control animals (no-tumor) were also used.

Results: We observed that TRPV1 expression was mostly confined to red-pulp and approximately 20% of monocyctic cells were strongly positive and stained both cytoplasmic and membranous. Staining pattern was markedly altered in spleens of animals injected with 4TLM cells such that diffuse staining all though the red-pulp was observed. Strongly positive cells found in control spleens were lacking in spleens of 4TLM injected mice. TRPV1 positive cells were significantly decreased in spleens of 4THM injected mice. Occasional TRPV1+ immune cells were also observed in primary tumors.

Discussion: These results demonstrated that for the first time that TRPV1+ cells decreases in spleens of highly metastatic breast carcinoma cells. Further studies are required to understand significance of these findings in metastasis.

Keywords: TRPV1, metastatic breast cancer, immunohistochemistry

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Neurodegenerative diseases are group of pathologies characterized by the progressive loss of structure or functions of neurons. Amyotrophic Lateral Sclerosis (ALS) is a rapidly progressive and fatal neurodegenerative disease affecting all ethnic groups. Both sporadic and familial cases of the disorder have been reported, where sporadic ALS (SALS) accounts for 5-10% of the total ALS cases. The pathogenic mechanism of the ALS is not yet clear, this pathology could result from complex interaction between various cellular mechanisms, including genetic and environmental factors, alteration in RNA metabolism, oxidative stress, etc. Recent studies have been shown that several genes were mutated in AML. The first one reported was SOD1. Recently, TAR DNA-binding protein-43 (TDP-43) emerged as a key protein involved in the pathogenesis of ALS. TDP-43 is a highly conserved and ubiquitously expressed nuclear protein reported to be involved in pre-mRNA splicing, transcription, mRNA stability and mRNA transport. Recent studies documented role of TDP-43 as the main protein component of the intracellular inclusions observed in Amyotrophic Lateral Sclerosis (ALS) patient brain.

In the present study, we investigated the role of TDP-43 analogue in the model organism Drosophila melanogaster. We have used different transgenic flies expressing TDP-43 analogue TBPH and checked whether the expression of TBPH is causing the neurodegeneration as compared to the control groups. Expression of TBPH caused development of black spot (necrosis) in the eyes of the flies clearly suggested neurodegeneration in the eye. Moreover, 2D gel analysis on TBPH mutated flies showed reduced fat body proteins 1 (FBP1) levels in the eyes of mutated flies as compared to wild type, suggesting the role of TBPH in the protein aggregation. In conclusion our date showed TBPH is involved in the neurodegenerative process by enhancing the production of FBP1.

**Keywords:** Neurodegenerative Disease, Amyotrophic Lateral Sclerosis, TDP-43

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Breast cancer is resulting in death is a serious global problem that especially a significant increase among women in recent years. Calcium ion accumulation into the cancer cells plays an important role in the survival and cell death. Accumulating evidences indicated that increase of intracellular free calcium concentration is the negative effect on the proliferation and metastasis of cancer cells. Transient receptor potential vanilloid1 (TRPV1) cation channels are non-selective calcium channels. In vivo studies in animals have shown that TRPV1 channel overstimulation of the sensory neurons selectively destroys the sensitivity of capsaicin. In a recent study, we observed modulator role of selenium (Se) on TRPV1 channel and oxidative stress in cancer cell and the similar effect of selenium may occur on oxaliplatin-induced oxidative stress and TRPV1 channel activity in MCF-7 breast cancer cells. In this study, aim of the study was investigate protective effect of selenium on TRPV1-mediated oxidative stress caused by an important chemotherapeutic oxaliplatin.

**Keywords:** Apoptosis, Oxaliplatin, Oxidative stress, TRPV1, Selenium
P-100 - EFFECT OF MELATHONIN ON PON LEVEL IN RATS GENERATED SEPSIS MODEL

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Aim: There are two different methods that were used to create melatonin deprivation experimentally in rats. The exposure of rats to light for 24 h continuously, or the removal of the pineal gland operation. These methods cause to be done many researches related to biological rhythms as well as having the melatonin secretion destroying property of pineal gland. These methods also have to destroy property as well as the melatonin secretion of the pineal gland lot of research on biological rhythms are reasons to be made. The aim of this study was to determine the effect on the PON of change in the level of melatonin and to investigate therapeutic effect of melatonin.

Materials and Methods: In this study, 54 piece 4-5 month old adult male rats were used. First-third groups were hosted without food and water restriction under 12 h light-12 h dark at 21-22 °C. The rats in second group were hosted without food and water restriction under 24 h lights at 21-22 °C. All rats were hosted in this way for 10 days. Sepsis model was created by applying the cecum mount-drilling method (CLP). Rats in Group 2 to ensure the lack of melatonin were hosted under lights daily for 24 hours throughout hosted days. The level of PON in blood samples of rats was detected.

Findings: The levels of PON in bloods which were taken from two groups with and without sepsis after 24 hours were compared. The level of PON in group which was uncreatet melatonin deprivation was found lower than that of created melatonin deprivation.

Results: The results obtained from this study suggest that low PON values in rats applied to pinealectomy can be due to the effect on PON values of melatonin. This case reveals that the level of melatonin may be the effect on the immune system.

Keywords: Pinealectomy, PON, Sepsis