5th Kaunas / Lithuania International
Hematology / Oncology Colloquium

26 June 2020

Online Poster Abstract Book

Cover picture: Kaunas Garrison Officers' Club

www.KIHOC.lt

Editor

Prof. Elona Juozaitytė (Kaunas, Lithuania)

Abstracts' Reviewers

Prof. Arturas Inčiūra (Kaunas, Lithuania)
Assoc. Prof. Rolandas Gerbutavičius (Kaunas, Lithuania)
Assoc. Prof. Sigita Liutkauskienė (Kaunas, Lithuania)

Cover Photo's Author Gintaras Česonis

2020

5th Kaunas / Lithuania International Hematology / Oncology Colloquium

26 June 2020

ONLINE POSTER ABSTRACT BOOK

5th Kaunas / Lithuania International Hematology / Oncology Colloquium

SCIENTIFIC AND ORGANIZING COMMITTEE

SCIENTIFIC COMMITTEE

Chair
Prof. Dietger Niederwieser (Leipzig, Germany)

Members
Prof. Arturas Inčiūra (Kaunas, Lithuania)
Assoc. Prof. Rasa Jančiauskienė (Kaunas, Lithuania)
Dr. Laimonas Jaruševičius (Kaunas, Lithuania)
Prof. Elona Juozaitytė (Kaunas, Lithuania)
Assoc. Prof. Rolandas Gerbutavičius (Kaunas, Lithuania)
Assoc. Prof. Sigita Liutkauskienė (Kaunas, Lithuania)
Domas Vaitiekus (Kaunas, Lithuania)

ORGANIZING COMMITTEE

Members
Assoc. Prof. Rolandas Gerbutavičius (Kaunas, Lithuania)
Prof. Elona Juozaitytė (Kaunas, Lithuania)
Prof. Dietger Niederwieser (Leipzig, Germany)
Domas Vaitiekus (Kaunas, Lithuania)

COLLOQUIUM SECRETARIAT
EVENTAS (PCO&AMC)
Mob.: +370 686 44486
E-mail: info@eventas.lt
www.eventas.lt

POSTER ABSTRACTS

1. Polymorphisms in MDM4 Gene: Effects on Clinicopathological Characteristics in Breast Cancer Patients

Agnė Bartnykaitė[1], Aistė Savukaitytė[1], Rasa Ugenskienė[1], Erika Korobeinikova[2], Jurgita Gudaitienė[2], Elona Juozaitytė[2]

[1]Oncology Research Laboratory, Oncology Institute, Lithuanian University of Health Sciences
[2]Oncology Institute, Lithuanian University of Health Sciences

Background and Objectives
Breast cancer is one of the most common malignancy among women. The main clinical parameters (tumor size, status of estrogen, progesterone, HER2 receptors, lymph node involvement, tumor histological grade, etc.) are indicators routinely used in clinical practice to assess the prognosis of the disease and to select treatment methods. Genetic factors also play a substantial role in breast cancer. The evidence suggests that the MDM family may be related to breast development, function and protection from cancer. The elevated level of the MDM family member MDM4 is associated with various human cancer types (including breast cancer). This suggests that single nucleotide polymorphisms (SNPs) in the MDM4 gene may have functional implications for breast cancer morphology. However, the effect of SNPs in MDM4 gene has not been sufficiently studied yet. The aim of the current study was to evaluate the influence of polymorphisms in MDM4 gene on the clinical and morphological characteristics of breast cancer.

Material and Method
A total of 100 patients (with mean age of 42 years) with breast cancer were enrolled in the study. For SNP analysis genomic DNA was extracted from peripheral blood leukocytes. SNPs (rs1380576 and rs4245739) in MDM4 gene were analyzed by the polymerase chain reaction-restriction fragment polymorphism (PCR-RFLP) assay. All clinical and tumor pathomorphological data of the patients were obtained from the medical records by the oncologists. Informed consent was obtained from every participant. Study data included the age at diagnosis, pathological tumor size (pT), status of pathological lymph node involvement, status of estrogen, progesterone and human epidermal growth factor receptor 2 (HER2), intrinsic subtype (luminal A, luminal B, HER2 or triple negative), tumor grade (G1 and G2, G3), progress, metastasis and death. The associations between SNPs and clinical characteristics of the patients were evaluated. Statistical data analysis was performed using SPSS.

Results
The results of analysis showed some significant associations between rs1380576 and rs4245739 polymorphisms, located in MDM4, and tumor features. Our data indicated that compared to GG genotype, MDM4 rs1380576 CC genotype showed 3.97-fold increased chance for developing estrogen-positive breast cancer (95 % CI 1.016-15.520, p=0.047). In addition, compared with the GG genotype, the CC was significantly associated with increased chances of positive progesterone receptor status (OR=12.150, 95 % CI 1.416-104.252, p=0.023). It was found that carriers of rs1380576 CC genotype had higher probability for developing luminal A subtype (CC versus GG OR=5.635, 95 % CI 1.082-29.337, p=0.040). Moreover, an increased chance for developing estrogen-positive breast cancer was significantly associated with rs4245739 AA genotype, compared to CC (OR=5.739, 95 % CI 1.027-32.065, p=0.047).

Conclusions
The study suggests that there is correlation between MDM4 genetic polymorphisms and breast cancer morphology. SNPs in the MDM4 (rs1380576, rs4245739) may have the potential to operate as markers contributing to the assessment of breast cancer clinical characteristics.

2. The Effect of Ionizing Radiation on MCF-7 Breast Cancer Cell Line | *Best Poster Award Received*

Agnė Bartnykaitė[1], Rasa Ugenskienė[1], Arturas Inčiūra[2], Elona Juozaitytė[2]

[1]Oncology Research Laboratory, Oncology Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Oncology Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania

Background and Objectives
Breast cancer is one of the most common cancers worldwide. Radiotherapy is widely applied for breast cancer treatment, however, cancer cells resistance to ionizing radiation (IR) causes problems for the best treatment outcomes. The evidence suggests that some chemical substances in combination with IR could generate synergistic effect and

increase death of cancerous cells. Mefenamic acid (MEF) is one of non-steroidal anti-inflammatory drugs. Literature sources present the data of MEF potential to improve the radio-sensitization of cancer cells. However, this type of analysis has never been done on breast cancer cells. Lithuanian University of Health Sciences participates in Horizon 2020 project "INfraStructure in Proton International Research" (INSPIRE) in the Framework Programme for Research and Innovation. In INSPIRE project we aimed to analyze the molecular basis of breast cancer cell resistance to ionizing radiation. Consequently, the aim of this study was to determine the radiation-induced effects on MCF-7 breast cancer cells in vitro and to analyze the effect of different MEF concentrations on breast cancer cell proliferation.

Material and Method
Human breast cancer cell line MCF-7 was used for the study. Cells were exposed to 2, 4, 6, 8, 10 Gy of IR from Clinac 2100C/D linear accelerator. Radiation-induced effect was assessed using colony formation, apoptosis and cell-cycle assays. To determine the effect of MEF, MCF-7 cell proliferation was estimated using MTT assay. The powders of MEF were dissolved in dimethyl sulfoxide (DMSO) to achieve a concentration of 178 mM of stock solution. The stock solution was frozen at -20 ˚C in small quantities to prevent freeze-thaw cycles. Working solutions were prepared before each experiment. Final DMSO concentration was 0.28 % in all the samples. Exponentially growing cells were plated into 96-wells plates for 24 hours and then treated with indicated concentrations (0, 10, 25, 50, 100, 250, 500 µM) of MEF. Then cells were grown at 37 °C in a 5 % CO_2-enriched humidified air atmosphere for 24, 48 and 72 hours.

Results
Colony formation assay demonstrated that MCF-7 viability decreased with increasing doses of IR. Results of apoptosis revealed that MCF-7 cell line exhibited a significant number of apoptotic cells at 24 hours after the highest doses of IR (8 and 10 Gy). Changes of cell cycle distribution following IR did not show significant results. Nevertheless, MCF-7 cell number increased in the G0/G1 phases following the exposure to all IR doses. In the following steps we aimed to analyze the effect of MEF on MCF-7 cells proliferation. During the experiments we observed that MEF was not completely dissolved and the selected concentrations produced needle-like crystals which could change biological activity of MEF pharmaceutical compound. Even though we performed proliferation analysis, however, we tend to think that MTT assay provided inaccurate results as they were very diverse in replicate samples. In the subsequence steps we aimed to dissolve MEF in the different solutions (for example, PBS pH 7.4), though, this resulted in even worse solubility. Our data suggests that due to its poor solubility MEF is not suitable for further research in our setting.

Conclusions
Our results are in agreement with the literature and suggest that MCF-7 cells are not highly resistant to IR. MCF-7 cell line could be use as control for comparison to other cell lines response to IR analysis. Furthermore, the study demonstrated that MEF tends to produce crystals which could exist in various polymorphic forms. Therefore, this drug will not be considered for further radiosensitizing analysis in our study. ACKNOWLEDGMENTS: This project has received funding from the European Union's H2020 Research and Innovation Programme, under Grant Agreement No: 730983.

3. Alginate-based 3D Cell Phantom for Radiobiological Experiments | *Best Poster Award Received*
Justė Čepaitė[1], Walter Tinganelli[2], Walter Bonani[3], Elona Juozaitytė[4], Marco Durante[2], Olga Sokol[2]
[1]*Institute of Biology Systems and Genetic Research, Lithuanian University of Health Sciences*
[2]*Departament of Biophysics, GSI Center for Heavy Ion Research*
[3]*BIOtech Research Center and European Institute of Excellence in Tissue Engineering and Regenerative Medicine*
[4]*Institute of Ocology, Lithuanian University of Health Sciences*

Background and Objectives
Cell culture has been one of the most common tools for the discoveries and testing of different cancer treatments since the 1900s. Traditionally, cell monolayers are used for this purpose. Despite the ease of use, there are, however, several drawbacks of using 2D cultures: cells get equal exposure to nutrients, oxygen, or toxins, while tumours in living organisms are forming three-dimensional (3D) structures and thus have a gradient exposure to these factors. Moreover, 2D cultures only demonstrate cell-cell interactions, while cells in living organisms also interact with their environment. Thus, cells in monolayers may respond differently to medications or radiation exposure compared to living tissues. More complex 3D cell cultures are considered to mimic the real tissues better than a monolayer. Numerous studies report different protocols for gel-based 3D cell cultures. However, most of them are either based

on expensive materials, or not applicable for selected types of radiobiological experiments. The work, carried out at GSI, is focused on the development of a relatively inexpensive 3D cell culture phantom out of stable, rigid gels, but without sacrificing the cell proliferation and viability. These gels should be sufficient for the experiments, studying cellular effects after the irradiation with ion beams. In this work, a protocol for cell culture in gels, consisting of alginate, type I collagen, and silk fibroin, was established. Here we report the preliminary results of cell morphology and proliferation analysis.

Material and Method
Gels were prepared using a mixture of alginate (AG), collagen type I (COLI), silk fibroin (SF), and cells suspension in the growth medium. For the preliminary tests, two human cancer cell lines were used: U2OS (bone osteosarcoma) and A549 (lung carcinoma). First, the gelatine molds containing 100 mM $CaCl_2$ for alginate ionic cross-linking were prepared inside a 12-well plate. COLI was neutralized using 1N NaOH and 10x PBS and mixed on ice with AG, SF, cell suspension, and culture medium. The mixture was poured on gelatine molds and incubated for 40 minutes until solid. After the incubation, gels were washed in 10mM $CaCl_2$ solution, PBS with Ca ions, and twice with medium. Final concentrations of COLI, AG and SF were 0.1875%, 0.25% and 0.25% respectively with cell concentration of 1.75×10^6 cells/ml. The medium was changed every 2-3 days. Pictures of the gels were taken using an inverse light microscope for ten days at different time points. Additionally, the cell growth was assessed by dissolving the gel slices using PBS with 0.046% EDTA and estimating the cell numbers and viability using trypan blue dye and Bio-Rad TC20 Automated Cell Counter.

Results
At 0h, both U2OS and A549 cells could be seen under the microscope as round spheres encapsulated in a gel. After 48h, most U2OS cells had elongated phenotype, while most A549 cells still appeared rounded. After 96h, U2OS cells spread out and made connections. For A549 cells, it took additionally approx. 48h for similar changes to occur. For the U20S, dissolving the gels at different time points yielded the following cell numbers (N) and viability (V): N $= 0.57 \times 10^6$, (SD* = 0.13) and V = 91.75 % (SD = 2.9) after 0 hours of incubation. Measurements at further time points show an increased cell number: the final measurement after 240h yielded a cell number of N $= 1.71 \times 10^6$ (SD = 0.34) with a viability V = 64% (SD = 4.1). Preliminary results provide an insight on gel's ability to support cell survival and proliferation.
*SD - standard deviation in absolute values.

Conclusions
Cell elongation seen under a microscope could indicate that cells are interacting with collagen in the gel and acquiring phenotype closer to one seen in living tissues. However, more thorough testing is required to confirm it. Preliminary results showed steadily increasing cell number, although cell viability within the first 48 hours dropped significantly from 92% to 42%. However, after 96 hours of incubation, it started to increase further and reached 64% after 240 hours. This viability drop can be explained by the stressful encapsulation and temperature changes that cells undergo. Gel dissolving might also be damaging to the cells, so it could also contribute to lower viability values. Other counting and viability assessment methods should be considered for evaluating gel's ability to support cell survival and proliferation. One of those methods could be MTT performed directly in the gel.

The gels, produced using the described protocol, are rigid enough for being moved around and assembled into a more complex structure for irradiation. The future experiments include assembling of an elongated gel phantom to study the relative biological effectiveness of a proton beam along the spread-out Bragg peak. Another planned study would investigate the effect of irradiation on a co-culture or mixed culture of normal and cancerous cells in the same gel.

4. Impact of Genetic Variations of Polymerase γ Gene (POLG) on Breast Cancer Patients
Ieva Golubickaitė[1], Rasa Ugenskienė[1,2], Erika Korobeinikova[3], Jurgita Gudaitienė[2,3], Domas Vaitiekus[3], Lina Poškienė[4,5], Elona Juozaitytė[2,3]
[1]Institute of Biology Systems and Genetic Research, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Institute of Oncology, Lithuanian University of Health Sciences, Kaunas, Lithuania
[3]Department of Oncology and Hematology, Hospital of Lithuanian University of Health Sciences, Kaunas, Lithuania
[4]Department of Pathological Anatomy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[5]Department of Pathology, Hospital of Lithuanian University of Health Sciences, Kaunas, Lithuania

Background and Objectives
Breast cancer is highly prevalent in females and is the leading cause of cancer-related deaths among women worldwide. Cancer is commonly considered a genetic disease that involves mutations in nuclear genes. Variations in mitochondrial DNA (mtDNA) and nuclear DNA, coding for mitochondrial proteins or regulatory molecules, are seldom under consideration.

Material and Method
In this study single-nucleotide variants in POLG, which encodes alpha subunit of polymerase gamma, was analyzed. This polymerase functions in mitochondria and is the only polymerase that is involved in mtDNA replication. It also plays an important role in mtDNA repair. The aim of this study was to assess POLG gene variations (rs3087374, rs2307441, rs2072267, rs976072) and associations with tumor phenotype and disease outcome for 234 breast cancer patients. Variations were determined with Real-Time PCR using TaqMan® probes.

Results
We observed that patients with POLG rs2307441 TT and CT genotypes had a lower probability for tumor vascular invasion than those with CC genotype (p=0.001). Also, patients with rs2307441 C allele had a 2.5 higher probability of shorter metastasis-free survival than those without C allele (p=0.026). Patients with POLG rs2072267 AG genotype were found to be predisposed for disease progression when compared with GG genotype (p=0.015).

Conclusions
Our data indicate that nuclear DNA variations in POLG (rs2307441, rs2072267) gene play an important role in breast cancer patients.

5. Cell Processing in Kaunas

Ieva Golubickaitė[1,2], Birutė Šabanienė[1], Inga Valančiūtė[1], Elona Juozaitytė[1,2], Rolandas Gerbutavičius[1,2], Domas Vaitiekus[1,2], Aurija Kalasauskienė[1,2], Diana Remeikienė[1,2], Jonas Šurkus[1,2], Rūta Lekšienė[1,2], Marius Perminas[1,2], Milda Rudžianskienė[1,2], Martyna Beitnerienė[1,2], Vilma Svetickienė[1], Rūta Dambrauskienė[1,2], Vaida Didžiariekienė[1,2], Daiva Urbonienė[1,2], Dietger Niederwieser[1,3]
1Hospital of Lithuanian University of Health Sciences Kauno Klinikos, Lithuania
2Lithuanian University of Health Sciences, Lithuania
3University of Leipzig, Germany

Background and Objectives
Peripheral blood stem cells (PBSCs) are widely used for blood stem cell transplantation around the world. Viable post thaw CD34+ cell count is the important parameter for quality assurance of the cryopreserved cells. The cell processing results from the Hospital of Lithuanian University of Health Sciences Kauno Klinikos are presented in this study.

Material and Method
The study included 128 patients undergoing blood stem cell transplantation from the Hospital of Lithuanian University of Health Sciences Kauno Klinikos from 2015 to 2020 June. It included 288 apheresis, 405 cryopreserved PBSC products and 141 autologous and 1 allogeneic hematopoietic stem cell transplantation. Before autologous transplantation cells were cryopreserved using patient's plasma and DMSO (final concentration of 10%). All PBSC products were initially cryopreserved in a controlled freezer, stored in vapor phase nitrogen. Viability was assessed from a vial no less than 24 hours after cryopreservation, thawed immediately before testing with BD FACS Canto flow cytometer and BD Stem Cell Enumeration Kit. PBSC for transplantation were thawed before use in a water bath at 37°C. Allogeneic cells had a minor incompatibility and were processed by removing donor's plasma. Cells were kept at +2 - +8°C and transplanted in less than 24 hours.

Results
In total 128 patients (69 females, 59 males) with a median age of 59 (range 18–73) years were harvested after G-CSF (10µg/kg) or Cyclo+G-CSF or additional mobilizing agents such as plerixafor (0.24mg/kg, n=4) for auto-SCT. Most patients received one auto-SCT. The first allogeneic stem cell transplantation was performed for a Hodgkin lymphoma patient. Indications for autologous transplantations were multiple myeloma (n=120), non-Hodgkin lymphoma (n=14), germ-cell testicular tumor (n=3), CNS lymphoma (n=1), Ewing's sarcoma (n=1), multiple sclerosis (n=1) and autoimmune encephalitis (n=1). The median CD34+ cells harvested per kilogram of body weight was 4.3, post-thaw CD34+ viability - 78% (range 42-96). Median leukocytes (>1.0 x 109/l) engraftment on two consecutive days was 11 (range 8-13) days, and 15 days for platelets (>50 x 109/l), ranging 9-23 days after SCT.

Statistically significant (p<0.05) negative correlations were observed between CD34+ cell post-thaw viability and CD45+(r=(-0.351)); CD34+ count (r=(-0.275)); volume of leukapheresis product (r=(-0.315)).

Conclusions
These findings revealed that lower amounts of CD45+ and CD34+ cells and lower leukapheresis volume correlated with better post-thaw cell viability. Further studies will be needed to confirm such findings and optimize the process.

6. Cryopreserved Peripheral Blood Stem Cell Products for Multiple Myeloma Patients

Ieva Golubickaitė[1,2], Birutė Šabanienė[1], Inga Valančiūtė[1], Elona Juozaitytė[1,2], Rolandas Gerbutavičius[1,2], Domas Vaitiekus[1,2], Aurija Kalasauskienė[1,2], Diana Remeikienė[1,2], Jonas Šurkus[1,2], Rūta Lekšienė[1,2], Marius Perminas[1,2], Milda Rudžianskienė[1,2], Martyna Beitnerienė[1,2], Vilma Svetickienė[1], Rūta Dambrauskienė[1,2], Vaida Didžiariekienė[1,2], Daiva Urbonienė[1,2], Dietger Niederwieser[1,3]
[1]Hospital of Lithuanian University of Health Sciences, Kaunas Clinics, Lithuania
[2]Lithuanian University of Health Sciences, Lithuania
[3]University of Leipzig, Germany

Background and Objectives
Autologous peripheral blood stem cell (PBSC) transplantation has been associated with improved response and progression-free survival rates. The high-dose therapy followed by PBSC transplantation is the standard treatment for multiple myeloma patients. Tandem or double autologous PBSC transplantation is usually performed for myeloma patients within a time period of no more than six months. It is associated with better outcomes compared to a single transplant. Therefore, it is important to collect enough PBSC to ensure availability to perform tandem transplantation and save some PBSCs in case of relapse.

Material and Method
In this study, we present the results of 110 patients with multiple myeloma treated by autologous blood stem cell transplantation in the Hospital of Lithuanian University of Health Sciences (HLUHS) Kauno Klinikos from 2015 to 2020 June. Patients underwent apheresis, collected PBSCs were cryopreserved using controlled rate freezing and stored in vapor phase nitrogen tanks until transplantation.

Results
The median of 2 aphereses were done for one patient (range from 1 to 4). Only one apheresis was done for 8 patients (7.3%), 2 aphereses for 63 patients (57.3%), 3 aphereses for 34 patients (30.9%), 4 aphereses for 5 patients (4.5%). After one cycle of stimulation the median of 3 bags of cryopreserved cells were prepared per patient, ranging from 1 to 5. Only 1 bag was prepared for 2 patients (1.8%), 2 bags were prepared for 18 patients (16.4%), 3 bags were prepared for 43 patients (39.1%), 4 bags were prepared for 35 patients (31.8%), 5 bags were prepared for 12 patients (10.9%). After one apheresis, the median of 1 bag of cryopreserved PBSC product was prepared per patient (range from 1 to 3) with the median of 3.3 CD34+ cells harvested per kilogram of body weight (range from 0.6 to 38.0). In Kauno Klinikos 70 (63.6%) patients were supplied for both tandem transplantation and stock for relapse, 28 of these patients had one additional and 5 patients had two additional cryopreserved PBSC products available. In HLUHS 20 patients (18.2%) with multiple myeloma had tandem transplantations and 13 (11.8%) patients had the second transplant after relapse.

Conclusions
These data indicate that stem cells collected, cryopreserved and stored in vapor nitrogen at the Hospital of Lithuanian University of Health Sciences, Kaunas Clinics, were sufficient for 70 (63.6%) patients to undergo tandem transplantation and have supplies for future use in case of relapse. If PBSCs were collected to supply 2 transplantations, cells were used for one transplantation and second bag was kept in case of relapse.

7. The Effect of Kaempferol on the Change in Vitality of Breast Cancer Cells in Combination with Ionizing Radiation

Greta Gudoitytė[1], Rasa Ugenskienė[1], Aistė Savukaitytė[1], Agnė Bartnykaitė[1], Antanas Vaitkus[2], Elona Juozaitytė[3]
[1]Oncology Research Laboratory, Institute of Oncology
[2]Institute of Oncology
[3]Department of Radiology

Background and Objectives
Breast cancer is the most common women's oncologic disease and leading cause of morbidity and mortality. The main treatment of breast cancer is radiotherapy, which is based on ionizing radiation (IR) activity. IR causes DNA structure instability and distruption causing activation of DNA repair mechanisms which leads to cell cycle arrest and cell death. Although radiotherapy is widely used and provides substantial benefits it has several limitations. It has been observed, that cancer cells develop radioresistance and are able to repopulate, therefore decreasing effectiveness of the treatment. Consequently, there is a growing interest in compounds which could inhibit cancer cell viability or sensitize them, thus improving treatment efficiency. Phytochemicals have attracted attention of many researchers as potential anti-cancer agent. In addition, phytochemicals might inhance cancer cells sensitivity to IR. Therefore, the aim of this study was to evaluate human breast cancer MCF-7 and MDA-MB-231 cell line response to a single dose of IR in combination with phytochemicals.

Material and Method
MCF-7 and MDA-MB-231 breast cancer cell lines were used to test kaempferol (KMF) effect. KMF was dissolved in dimethyl sulfoxide. Initially, we examined cell viability after the exposure solely to KMF. In order to assess survival, we incubated cells with different KMF (0, 5, 10, 20, 30, 40 µM) concentrations for various incubation times (24, 48, 72 h). For cells survival analysis MTT test was performed. Afterwards, the combination of KMF and IR (0, 2, 10 Gy) on cells survival (MTT method) was analysed.

Results
This study showed that KMF had different effect on chosen breast cancer cells survival. There was a significant decline in MCF-7 cell viability which was KMF concentration-dependent. The same effect was observed following all three incubation periods. However, significant decrease of MDA-MB-231 cell viability was not found. Therefore, only MCF-7 cell line was selected for further analysis. Our study revealed that a combination of 2 Gy and 40 µM KMF caused a significant decrease in cell viability when compared to 2 Gy alone. Furthermore, we found that combination of 10 Gy with KMF at all tested concentrations (10, 20, 30, 40 µM) led to significant decrease in cell viability.

Conclusions
Our study revealed that KMF negatively affected MCF-7 cell viability and sensitized them to IR. However, KMF had no significant impact on MDA-MB-231 cell viability decrease. Therefore, further studies are required to elucidate molecular mechanisms response for sensitising effect of KMF.

8. Associations of p21 Gene Functional Polymorphisms with Early-stage Breast Cancer Clinicopathologic Features and Prognosis

Erika Korobeinikova[1], Rasa Ugenskienė[2,3], Rūta Insodaitė[2], Elona Juozaitytė[1]
[1]Oncology Institute, Lithuanian University of Health Sciences
[2]Biology System and Genetic Research Institute, Lithuanian University of Health Sciences
[3]Oncology Research Laboratory, Oncology Institute, Lithuanian University of Health Sciences

Background and Objectives
p21 plays multiple functions in cell cycle arrest, apoptosis and transcriptional regulation. Higher expression of p21 facilitates breast cancer progression and metastasis. Functional single nucleotide polymorphisms (SNPs) in p21 gene have influence on p21 production and therefore are potential breast cancer prognostic biomarkers. The aim of the tis study was to evaluate the associations between functional SNPs in the p21 gene with the early-stage breast cancer clinicopathologic features, locoregional and distant disease progression.

Material and Method
Genomic DNA and clinical data were collected for 202 adult Lithuanian women with primary I–II stage breast cancer. Genotyping of the SNPs was performed using TaqMan genotyping assays. Two functional polymorphisms in p21 gene (rs1801270, rs1059234) were analysed. The associations of polymorphisms with clinicopathologic variables (age at diagnosis ≤50y/>50y), tumour size (≤2cm/>2-5cm), lymph node status (positive/negative), estrogen receptor (ER) status (positive/negative), progesterone receptor status (positive/negative), HER2 status (positive/negative), differentiation grade (G1+G2/G3) were evaluated by Pearson's chi-square or Fisher's exact test. The survival

endpoints were locoregional recurrence-free survival (LRFS) and metastasis-free survival (MFS). For survival analysis Kaplan-Meier's procedure and Cox regression models were used. Patients were prospectively followed until 30 April 2019.

Results
All studied genotypes were in Hardy–Weinberg equilibrium. Evaluating the associations between studied polymorphisms and clinicopathologic variables it was found, that carrying of CC genotype (CC vs CA+AA) in p21 rs1801270 SNP was associated with larger tumour size (Odds ratio (OR) 2.79; 95% confidence interval (CI) 1.16-6.72; p=0.022), positive lymph node status (OR 2.35; 95%CI 1.04-5.29; p=0.041). p21 rs1059234 polymorphism CC genotype was also associated with larger tumour size (OR 2.66; 95%CI 1.10-6.44; p=0.034). In the mean follow-up time of 67 months (range 28–202) locoregional recurrence was observed for 11 patients, 28 patients developed distant metastases. Univariate Cox survival analysis revealed that p21 rs1801270 CC genotype carriers had worse LRFS (hazard ratio (HR) 3.79; 95%CI 1.02-14.14; p=0.047). Mean LRFS for CC genotype carriers was 137 months versus 193 months for CA+AA genotype carriers (log-rank p=0.032). Multivariate Cox survival analysis, adjusted for clinicopathologic variables, revealed that p21 rs1801270 CC genotype has a borderline statistical significance as an independent negative prognostic marker for LRFS (HR 2.33, 95%CI 0.99-9.60; p=0.05). No other significant associations were observed.

Conclusions
Functional polymorphisms in p21 gene may help to identify patients with more aggressive breast cancer phenotype. Furthermore, p21 rs1801270 SNP might contribute to the identification of early-stage breast cancer patients at higher risk for development of local recurrence. Further investigations with larger sample sizes are needed to confirm our findings.

9. Case of Covid-19 in Patients with T-large Granular Leukemia

O. Y. Mishcheniuk[1], O. M. Kostiukevich[1], S. V. Klymenko[2]
[1]State Institution of Sciences "Research and Practical Center of Preventive and Clinical Medicine", State Administrative Department, Kyiv
[2]State Institution "National Research Center for Radiation Medicine of National Academy of Medical Sciences of Ukraine", Kyiv

Introduction and Aim
SARS-CoV-2 is a new coronavirus, which first appeared at the end of 2019, and associated with a high level of mortality in elderly patients or patients with comorbidity, including oncological diseases, among which death rate reaches 15% (Yang X. et al, 2020; Guan W.J. et al, 2020). The last data show that among patients with cancer, the infection rate is higher, than in the general cohort (0.79% vs 0.37%) (Jing Yu. et al). At this moment, only a few clinical cases and one study with inclusion of patients with hematological pathology have been published. All clinical cases of COVID-19 in hematological patients included subjects under 65 y. with lymphoproliferative diseases and favorable outcome (Liang W. et al, 2020; Jin X.H. et l, 2020; Zhang X. et al, 2020). In the study with 25 hematological patients and COVID-19, also confirmed, that most of them had lymphoproliferative neoplasms and the one-month survival was equaled 60% (Malard F. et al, 2020). In a few studies with COVID-19 patients were found, that one of the markers of an unfavorable prognosis is the high level of ferritin, which is one of the acute-phase proteins (Thirumalaisamy P. et al., 2020). The ferritin level in the non-surviving COVID-19 patients was 1297.6 ng / ml, while, in the cohort with the favorable outcome – 614.0ng / ml (Mehta P., 2020). Currently, there does not exist publications with the analysis of the impact of hyperferritinemia on survival prognosis of COVID-19-positive patients with hematological diseases.

The aim: demonstration of the clinical case of the T-large granular leukemia (T-LGL) patient with COVID-19 and hyperferritinemia.

Case Report
The patient L., 67 y. In the peripheral blood smear (PBS) analysis (12/17/17): leukocytes-6.4 per mcL, erythrocytes-2.15 million mcL, hemoglobin-74 g / l, platelets-384 per mcL, ESR-22, eosinophils-5%, basophils-0%, bound neutrophils-6%, segments-20%, lymphocytes-64%, monocytes-5 %. The morphological examination of the peripheral blood cells revealed an increased number of the large granular lymphocytes, the immunophenotyping detected the clonal mature lymphocytes (CD3+, TCRab+, CD42-, CD5dim, CD81+, CD161+, CD27-, CD28-, CD45R0-). T-large granular leukemia was diagnosed. The patient was transfusion-dependent. On December of 2017,

the ferritin level was 396 ng / ml, the transferrin saturation was 73.7%. In January 2018, cyclosporine 200 mg / day was prescribed for 4 months, the response was not achieved. 05/25/18 cyclosporine was changed to cyclophosphamide 100 mg / day. In the PBS of 10/19/2018: leukocyte-3,1 per mcL, erythrocyte-2,5 million mcL, hemoglobin-68 g / l, thrombus-320 per mcL, ESR-24 mm / hour, eosinophils-3%, basophils-2%, bound neutrophils-5%, segments-59%, lymphocytes-21%, monocytes-9%. The normalization of the blood formula with preserved anemia attracted the attention. Due to the long period of the transfusion dependency, 10/14/18 ferritin concentration was detected – 4168 ng / ml. Chelation with deferoxamine at a dose of 37.5 mg / kg was started. After 6 chelations (12/26/18) the hemoglobin level reached 98 g / l and cyclophosphamide therapy was completed. After 15 chelations (02/12/19) the hemoglobin and ferritin level was 144 g / l and 2410 ng / ml, respectively. At this moment 26 chelations were performed.

On March 10, 2020, the patient consulted a hematologist, it was confirmed clinic-hematological remission of T-LGL, complaints were absent and ferritin level was 1807 ng / ml. 18/03/20 patients felt general weakness, the temperature (37.5°C) and infrequent dry cough was appeared. 23/03/20 temperature was 39.0°C. The patient was prescribed acetaminophen. 24/03/20 lung auscultation: breath was rigid, rales were absent. Chest X-ray was performed: infiltrative or other changes of the lung were not detected. On March 27, 2020, the nasopharyngeal lavage material was examined by PCR for the SARS-CoV-2 virus RNA and a positive result was obtained. The patient was prescribed therapy: azithromycin 500 mg / day per os, hydroxychloroquine 200 mg / 2 times / day, acetaminophen 500 mg / day. 03/31/20 in the bacteriological analysis of sputum - S. aureus 1.2×10^5 CFU / ml, S. pneumoniae 3×10^8 CFU / ml, in the blood C-reactive protein -13.94 mg / l. According to the chest X-ray from 03/31/20: the pulmonary fields are focally infiltrated on the right. It was verified the lower lobe pneumonia on the right. Ceftriaxone 2.0 iv / day was prescribed. 04/07/2020 the chest radiography was repeated: bilateral lower lobe pneumonia. On the background of antibacterial therapy, the next control radiography was performed on 13/04/20: focal-infiltrated changes were not detected. 15/04/20 PCR for the presence of SARS-CoV-2 RNA was performed, the virus RNA was detected. The PCR analysis from 04/22/20 and 04/27/20 - the result is negative. The level of ferritin from 04/29/20 - 1852 ng / ml.

Discussion
Thus, the use of ferritin as an additional marker for assessing the inflammatory intensity due to COVID 19 in hematological patients could be limited on the start of the infection, given its often-high base level. On the other hand, elevated synthesis of pro-inflammatory cytokines associated with the increasing ferritin level. According to the study with hemodialysis patients, the ferritic level before SARS-CoV-2 infection corresponded to 584 ng ppm, and after - 1446 ng / ml (Bataille S. et al, 2020).

Conclusions
Therefore, hyperferritinemia in patients with inadequate hematopoiesis and transfusion dependence cannot serve as a powerful marker of the intensity of the inflammatory process at the beginning of infection, without other markers of "cytokine storm", because of this group subjects have its high level without an inflammation.

10. Aspirin and Salicylic Acid Induce REDD1 Expression and Dephosphorylate 4E-BP1 in Breast Cancer Cells

Aistė Savukaitytė[1], Greta Gudoitytė[1], Agnė Bartnykaitė[1], Rasa Ugenskienė[1], Elona Juozaitytė[2]
[1]Oncology Research Laboratory, Institute of Oncology
[2]Institute of Oncology

Background and Objectives
While the anticancer activity of aspirin is strongly supported by epidemiological data, the underlying molecular mechanism remains uncertain. Inhibition of mTORC1 (mechanistic target of rapamycin complex 1) signaling has been proposed to contribute to the effect. Attenuation of mTORC1 activity by aspirin has been reported to be dependent and independent of AMP-activated protein kinase (AMPK). Although REDD1 (protein regulated in development and DNA damage response 1), encoded by the DDIT4 gene, is known to function as an mTORC1 inhibitor, the involvement of REDD1 in aspirin anticancer action has not been reported in the literature. Since we have previously found that DDIT4 mRNA is elevated upon aspirin treatment in breast cancer cells, here, we seek to determine whether this increase is concomitant with the induction of REDD1 protein. While aspirin has been shown to suppress mTORC1 signaling in PIK3CA-mutant breast cancer cells, we investigate the activity of mTORC1 signaling following aspirin exposure in PTEN-mutant breast cancer cells.

Material and Method
MDA-MB-468 (PTEN-mutant) and MCF-7 (PIK3CA-mutant) breast cancer cell lines were used for the analysis. Cells were treated with aspirin or its metabolite salicylic acid for 24 hours before cell lysates were prepared. Protein expression and phosphorylation was assessed by western blotting. mTORC1 activity was evaluated by phosphorylation levels of 4E-BP1 and S6K1.

Results
We demonstrated that aspirin and salicylic acid increase REDD1 protein levels in MDA-MB-468 and MCF-7 cells. In order to investigate the effect of aspirin on mTORC1 activity in MDA-MB-468 cell line we probed for phosphorylation levels of S6K1 and 4E-BP1 which are the main known targets of mTORC1. However, we found no detectable baseline S6K1 phosphorylation and therefore assessed only phosphorylation of 4E-BP1. We observed that aspirin and salicylic acid dephophorylates 4E-BP1.

Conclusions
Our observations show an increase in REDD1 protein level and decrease in phosphorylation of mTORC1 target 4E-BP1. Further research aimed at unraveling whether the aspirin-mediated dephosphorylation of 4E-BP1 is dependent on REDD1, is warranted.

11. Multidisciplinary Approach to Ewing Sarcoma - Combining Chemotherapy, Radiotherapy and Hematopoetic Stem Cell Transplantation: a Case Report

Lina Simaškaitė1, Domas Vaitiekus1, Elona Juozaitytė1, Rasa Jančiauskienė1, Rolandas Gerbutavičius1, Laimonas Jaruševičius1, Dietger Niederwieser2
1Hospital of Lithuanian University of Health Sciences Kaunas Clinics, Department of Oncology and Hematology
2University of Leipzig, Germany

Introduction and Aim
Ewing sarcoma (ES) is aggressive form of round cell mesenchymal sarcomas commonly appearing in children and young adults. It is very rare disorder with annual incidence of 2,9 - 3 cases in 1 mln. Clinical presentation differs depending on the localization of the tumor. One quarter of patients diagnosed with ES already has a metastatic disease. On the other hand – subclinical metastases are presumed to be present in almost all patients. This is the reason of high relapse rate (80-90 percent) in patients who were treated only with local therapy. Combination of chemotherapy with multiple anticancer drugs and local treatment by surgery or/and radiotherapy (RT) are typical methods with curative intent. In addition to, high dose myeloablative chemotherapy followed by stem cell transplantation is thought to improve outcomes for high risk non- metastatic patients. About 65-75 percent of patients with localized ES who undergone treatment survives for 5 years. If disease is metastatic 5 year OS varies from less than 30 to 50 percent. We are presenting a case of multidisciplinary treatment of patient with localized Ewing sarcoma.

Case Report
In June of 2019, 20 years old patient with progressing paralysis in both legs and dysfunction of urogenital organs was admitted.

Non-homogeneous heterogeneous mass compressing spinal canal of 7,5 x 4,1 cm in size in the sacrum were found in the CT and MRI. In performed CT and PET/CT no metastases were detected.

Biopsy confirmed Ewing sarcoma which was proved by EWSR1 (22q12) translocation. We started treatment with 5 months of neoadjuvant chemotherapy of vincristine, doxorubicin, cyclophosphamide alternating with ifosfamide, etoposide (VDC/IE), overall 9 cycles were realised. After 2 cycles of chemotherapy neurological and urogenital dysfunction started to decrease. After 6 cycles in PET/CT full metabolic response was detected. With intend of HSCT, mobilisation of stem cells was completed successfully after 7 cycles of chemotherapy. In the end of systemic treatment, we performed a biopsy of persisted PET/CT negative masses in the sacrum - necrotic cells were detected and full pathological response was confirmed. Multidisciplinary team decided to chose RT as a local treatment, surgery was not feasible option in this case, RT (60 Gy) to sacrum was completed. In March of 2020 HSCT was performed (high dose melphalan and busulfan conditioning) with no major complications. In the end of this multidisciplinary treatment patient had no neurological deficiency. Three months after completing HSCT there are no signs of diseases recurrence.

Discussion

Even if Ewing sarcoma is localized micro metastases are presented in most of the cases. This is why ES is considered as a systemic disease. It has been known that local treatment alone for ES is not sufficient and systemic treatment is needed. EURO EWING 2012 trial which just ended in 2019, compared VIDE and VDC/IE induction regiments. Compelling results of this trial was revealed in ASCO 2020 annual meeting demonstrating that EFS and OS is better with VDC/IE with no excess toxicity. By treating our patient presented in this case report with the same regime we do expect the best outcomes as well.

In addition to, HSCT appears to be beneficial in improving overall survival in patients with localized Ewing's sarcoma. Although there is not one established treatment algorithm as the standard of care for localized ES and further trials are needed, we believe that bringing the chemotherapy, radiotherapy or surgery and HSCT therapy together could lead to best results in treating patients with this disease.

Conclusions

Combining chemotherapy, radiotherapy and hematopoetic stem cell transplantation in treatment of ES may be the one of the ways to achieve long term recurrence free and overall survival.

12. MMP-2 and MMP-8 Polymorphism Analysis and Their Prognostic Value Assessment in Breast Cancer Patients

Rasa Ugenskienė[1], Bar Lahmi[1], Agne Bartnykaitė[1], Erika Korobeinikova[2], Jurgita Gudaitienė[2], Aistė Savukaitytė[1], Roberta Vadeikienė[1], Elona Juozaitytė[2]
[1]Oncology Research Laboratory, Oncology Institute, Lithuanian University of Health Sciences
[2]Oncology Institute, Lithuanian University of Health Sciences

Background and Objectives

Breast cancer (BC) is the most common women cancer and it accounts for approximately 25% of total cancer cases. It remains the leading cause of cancer mortality among women worldwide. Consequently, there is still an urgent need to improve our knowledge in BC biology what might lead to better treatment options. Matrix metalloproteinases (MMP) are calcium-containing zinc-dependent endopeptidase. Their function is to degrade any type of extracellular matrix, what is important for tissue architecture and remodeling. Previous studies suggested that germline MMP polymorphisms are associated with inherited risk for tumor development and poor outcome in human breast cancer, however the results are inconclusive. This study aimed to analyse the effect of MMP2 rs243665 and MMP8 rs112255395 polymorphisms on tumor pathomorphological characteristics and the cause of the disease.

Material and Method

A retrospective study, involving 100 breast cancer patients, was conducted. The study research protocol was approved by Kaunas Regional Biomedical Research Ethical Committee (protocol number BE-2-10 and BE-2-10/2014). Patient peripheral blood samples, obtained by clinicians in a time period 2014-2016, were used for the genomic DNA extraction. MMP2 -1306 C>T (rs243865) and MMP8 -799 C>T (rs11225395) promoter polymorphisms were analyzed with polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. Patient clinical data were collected from medical records. The statistical analysis was performed using IBM "SPSS".

Results

A group of young (30-40 years – 34%, 41-50 years – 66%) patients (N- 100) was involved in the study. The distribution of tumor pathomorphological parameter was as follows: estrogen positive (57%), progesterone positive (48%), HER2 overexpression - 22% of tumors. Approximately half of the studied BC patients (45%) had positive lymph nodes. The majority (71%) of the tumors were well to moderate differentiated (G1+G2) and most of them were classified as T1 (64%). The distribution of MMP2 rs243865 and MMP8 rs11225395 genotypes was according to the Hardy-Weinberg equilibrium. The MAF of analyzed polymorphisms followed Hap-Map CEU, 1000 Genomes, ALFA and other world-wide conducted studies. In the association analysis it was determined that the carriers of C allele in MMP8 rs11225395 polymorphism were less likely to develop tumors with positive PR when compared to the non-carriers in both univariate ($p=0.011$) and multivariate models ($p=0.033$). In the survival analysis it was demonstrated that the carriers of C allele in MMP2 rs243865 polymorphism had lower probability of shorter PFS that the non-carriers in both univariate ($p=0.042$) and multivariate models ($p=0.033$).

Conclusions

Our data suggest that MMP2 rs243865 and MMP8 rs11225395 polymorphism are important for breast cancer phenotype and prognosis.

13. Development of Cellular Model with CALR Gene Mutations Using Genome Editing Tool CRISPR/Cas9 System

Roberta Vadeikienė[1], Tautvydas Karvelis[2], Baltramiejus Jakštys[3], Rasa Ugenskienė[1], Elona Juozaitytė[4], Saulius Šatkauskas[3], Virginijus Šikšnys[2]

[1]*Institute of Oncology, Oncology Research Laboratory, Lithuanian University of Health Sciences, Kaunas, Lithuania*
[2]*Department of protein-DNA interactions, Vilnius University, Vilnius, Lithuania*
[3]*Biophysical Research Group, Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania*
[4]*Department of Oncology and Hematology, Hospital of Lithuanian University of Health Sciences Kauno klinikos, Kaunas, Lithuania*

Background and Objectives

BCR/ABL negative classic myeloproliferative neoplasms (MPN) include primary myelofibrosis, polycythemia vera and essential thrombocythemia. Mutated calreticulin (CALR) was discovered to be involved in MPN pathogenesis. Although CALR involvement in MPN pathogenesis is an area of active research, there is no commercially available cell line with specific CALR mutations, characteristic to MPN. CRISPR/Cas9 system is one of the newest and the most advanced methods for cell genome editing. We hypothesized that CRISPR/Cas9 system is appropriate tool for CALR 52bp del and 5bp ins mutations initiation in cell culture. The aim of this study was to initiate CALR gene mutations that are specific to MPN pathogenesis and provide electroporation settings to deliver designed constructs into cell culture model.

Material and Method

After selection of potential DNA targets in gDNA, corresponding tabs were cloned into vector (pSpCas9(BB)-2A-Puro (PX459) V2.0, Addgene plasmid #62988) that is optimized for Cas9 and RNA-guided expression in eukaryotic cells. The effectivity of CRISPR/Cas9 system in CALR gene mutations (52bp del and 5bp ins) induction was assessed in HEK293T cells. Designed plasmids were transfected ("TurboFect" Transfection Reagent, Thermo Scientific) into HEK293T and after 48 hours the analysis was done. To assess the effectiveness of double-stranded break induction by Cas9, T7E1 nuclease method was used. This method enables finding the deletions and insertions that occur after NHEJ (non-homologous end joining). Band intensities were quantified by imageJ. Furthermore, we provided electroporation settings to balance highest possible transfection efficiency with haematological UT-7 cell line viability and growth post-electroporation. UT-7 cells were transfected with green fluorescent protein plasmid (pMaxGFP) by electroporation at various conditions using the electroporation system BTX T820. The transfection efficiency was determined by the counting pGFP positive cells 48 hours after post-transfection.

Results

Widely used type II CRISPR-Cas9 system derives from Streptococcus pyogenes. It is known that for DNA cleavage, the corresponding Cas9 protein depends on binding to its cognate 5'-NGG protospacer adjacent motif (PAM). We screened human CALR gene to identify matching target sites that respect the PAM requirements. Four sites (two for each mutation type) with lowest off-target rate effect were found using online tool available at ZHANG LAB website. After selection of potential DNA targets in genomic DNA, corresponding tabs were cloned into vector PX459. To generate the plasmids containing CALR 52bp del and 5bp ins were used standard procedures and some specific modifications were applied.

The selected constructs were used to introduce CALR 52bp del and 5bp ins in relatively easy to transfect HEK293 cell line and after 48 hours the T7EI analysis was done. This method enabled finding CALR 52bp del and 5bp ins mutations that occured after NHEJ in HEK293 cells. T7EI activity was detected only in samples, in which catalytically active Cas9 proteins were expressed, confirming that Cas9 proteins have their DNA-cleavage properties. Based on this, it was possible to choose optimal tabs which effectively guide Cas9 complex to cut gDNA at the selected location. According to our results, only in samples with CALR 52bp del significant T7EI activity was detected and reached 30%. In samples with induced 5bp ins the amount of cleavage was below the 5% detection limit of the assay and was omitted.

Because hematological UT-7 cells are recalcitrant to transfection with standard liposomal or calcium phosphate methods, we have used electroporation as a technique for delivery of plasmid into UT-7 cell line. Pilot experiments was carried out with GFP constructs in order for electroporation settings optimization. The best results, represented by 76.05±1.20% GFP-positive cells, were obtained in the combination of 1.4kV/cm (electric field strength), 100μs (duration) and 3HV (high voltage pulses) regimen. 100 μg/ml of the pMaxGFP was appropriate concentration for carried experiments. Cell viability under these conditions was 22.60 ±0.05%.

Conclusions

CALR gene mutations initiation with CRISPR/Cas9 system was appropriate. Construct with CALR 52bp del was designed properly and can be transfected in cell culture relatively easily. Despite successful construction of plasmid containing CALR 5bp ins, further experiments with corresponding plasmid delivery into cell culture are warranted. Additionally, there is a need of improving UT-7 cell line viability during electrotransfection.

14. Polymorphisms of the Drug Transporters ABCB1 SNP rs8187710 and it's Impact on Subclinical Anthracycline-based Chemotherapy Cardiotoxicity | *Best Poster Award Received*

Domas Vaitiekus[1], Gintarė Muckienė[2], Rūta Inčiūraite[3], Rūta Insodaitė[4], Rasa Ugenskienė[4,5], Renaldas Jurkevičius[2], Elona Juozaitytė[1]

[1]Department of Oncology and Hematology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Department of Cardiology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[3]Institute for Digestive Research, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[4]Biology System and Genetic Research Institute, Faculty of Animal Science, Veterinary academy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[5]Oncology Research Laboratory, Oncology Institute, Faculty of Medicine, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania

Background and Objectives
Background: Oncology progress has allowed to improve outcomes in many breast cancer patients. The core stone of breast cancer chemotherapy is anthracycline-based chemotherapy. With evolution of echocardiography, subclinical damage is identified, and more sensitive evaluation can be performed. This leads to understanding the heart damage beyond cumulative dose in early phase and importance of other risk factors. There are many risk factors for anthracycline-based chemotherapy cardiotoxicity (ABCC). One of possible pathways is intra cellular drug transporters and ABCB2 rs8187710 gene regulated pathway could be one of possible targets for investigation.

Objectives: The main objective of our study was to identify the impact of ABCB2 rs8187710 SNP (single nucleotide polymorphism) on the development of subclinical heart damage during and/or after doxorubicin-based chemotherapy in breast cancer patients.

Material and Method
Prospective study was done. Data of 73 women with breast cancer treated with doxorubicin-based chemotherapy in outpatient clinic were analyzed and SNP RT-PCR tests performed.

Results
Statistically no significant association between ABCB2 rs8187710 and ABCC after completion of chemotherapy was observed (p=0,92).

Conclusions
Consequently, our study demonstrated that ABCB2 rs8187710 SNP has no statistically significant important role in the development of ABCC. Further, larger volume studies are required.

15. The Analysis of Polymorphisms in the TLR4 Gene and Their Associations with the Pathomorphological Characteristics of Cervical Cancer and the Course of the Disease

Eglė Žilienė[1], Arturas Inčiūra[1], Rasa Ugenskienė[2], Agnė Bartnykaitė[2], Rūta Brazaitytė[2], Elona Juozaitytė[1]
[1]Oncology Institute, Lithuanian University of Health Sciences
[2]Oncology Research Laboratory, Oncology Institute, Lithuanian University of Health Sciences

Background and Objectives
The risk of cancer is increased by additional factors that activate the immune system and cause inflammation. Toll-like receptors (TLRs) family plays essential role in the pathway of activating the immune response associated with autoimmune diseases, inflammation, and tumor-associated diseases. The signaling pathway of TLRs begins in the cytoplasmic TIR domain, contributes to the recognition of antigenic molecules such as lipopolysaccharides, nucleic acids, and activates the protein complex (NF-kB, IRFs MAP kinases) via the MyD88-dependent or MyD88-

independent pathway. In this way, it regulates the production of cytokines, chemokines, type I interferon, thus eliminating antigens. Negative regulation of the signal path helps protect the host from inflammatory damage. TLRs can produce the desired antitumor effects by inducing the expression of inflammatory cytokines and the response of cytotoxic T lymphocytes. TLR4 gene polymorphisms and interactions with the characteristics and the influence of various cancers are widely studied worldwide. Changes in TLR4 gene expression are involved in carcinogenesis and tumor process progression through chronic inflammation, forming a tumor microenvironment. However there is very little information on the impact of TLR4 gene polymorphisms on cervical cancer as one of the most common oncological diseases.

We performed a study to determine the distribution of TLR4 gene functional polymorphisms (rs7276633, rs2838342, rs2051407, rs4986791) in a group of patients with cervical cancer. Then we analyzed the correlations between genotypes and alleles with tumor pathomorphological parameters and course of the disease.

Material and Method
Ninety-two patients with cervical cancer were enrolled in the retrospective study. Subjects were recruited from October 2014 to January 2019. Clinical data on patients and peritheral blood sample were collected. Genomic DNA was extracted from leucocytes. Molecular genetic studies were performed using the real time polymerase chain reaction method. The obtained SNPs were used in further statistical analysis in genotype and allelic models. The statistical analysis was performed using SPSS. The associations between the genotypes and alleles with tumor characteristics were assessed using Pearson's Chi-square or Fisher's Exact tests. Univariate and multivariate analysis to present odds ratios with 95% confidence intervals (CIs) and p-values were calculated with logistic regression. Differences in PFS and OS were assessed using hazard ratios (HRs) from univariate and multivariate Cox proportionate hazard models. p-value of <0.05 was considered statistically significant for all analysis.

Results
92 patients (mean (+/- SD age, 56.6 (+/- 11.7), 69.9% ≥50 years) were involved in the study. The majority (93.5%) of patients had squamose cell carcinoma histopatology type, adenocarcinoma accounted for 6.5% of cases. Approximately half of patients had T1-2 tumors (56.5%) and 43.5% were T3-4. 38% of patiens had positive lymph nodes. A large part (68.5%) of tumors were well or moderate differentiated (G1 or G2). Only 5.4% of cases metastasis were detected. The distribution of genotypes was according to the Hardy-Weinberg equilibrium. The distribution of rs7276633 genotypes was as follows: TT-33.7%, TC-48.9%, CC-17.4%. Data showed that rs7276633 CC genotype compared to TT genotype increased chance for T3-4 tumors (OR = 5.455, 95% CI: 1.414-21.035, p = 0.014). T allele was significantly associated with less chance for T3-4 tumors (OR = 0.194, 95% CI: 0.057-0.661, p = 0.009). Borderline significant association detected between CC genotype and stage III-IV (OR = 4.062, 95% CI: 0.963-17.138, p = 0.056). Patients with T allele were less likely to have stage III-IV cancer (OR = 0.197, 95% CI: 0.052-0.748, p = 0.017). Carrying the T allele statistically significantly reduced the chance of having a worse prognosis (T3-4 and G3) for cancer (OR = 0.118, 95% CI: 0.024-0.574, p = 0.008). Rs2838342 genotypes are distributed as follows: AA-33.7%, AG-48.9%, GG-17.4%. GG genotype compared to AA genotype increased chance for T3-4 tumors (OR = 5.455, 95% CI: 1.414-21.035, p = 0.015) and likely chance for a higher stage (III-IV) of cancer (OR = 4.062, 95% CI: 0.963-17.138, p = 0.056). Patients with GG genotype are more likely to have a worse prognosis (T3-4 and G3) cancer (OR = 10.0, 95% CI: 1.558-64.198, p = 0.015). In cox regression analysis of rs2051407 the links between C allele and PFS or OS were detected. The carriers of C allele had a decreased risk for shorter PFS than non-carriers (OR = 0.428, 95% CI: 0.183-1.000, p = 0.042). In a multivariate analysis this association remained significant when the adjustment for tumor differentiation (G) was done (OR = 0.401, 95% CI: 0.169-0.952, p = 0.038). C allele decreased chance to have shorter OS (OR = 0.416, 95% CI: 0.176-0.981, p = 0.045). In case of rs4986791, no significant link between genotypes or alleles and tumor phenotype or patient survival was detected.
Conclusions
The study suggests that SNPs rs7276633, rs2838342 may have the potential to be markers contributing to the assessment of the cervical cancer phenotypes. Rs2051407 may have implications for survival prognosis.

16. MyD88 Gene Polymorphisms and Their Associations with Tumour Pathomorphological Characteristics and Progression of Disease in Patients with Gynaecological Malignancies

Eglė Žilienė[1], Yury Dubinskiy[2], Rasa Ugenskienė[3], Arturas Inčiūra[2], Elona Juozaitytė[2], Agnė Bartnykaitė[3], Rūta Brazaitytė[3]

[1]*Oncology Institute, Lithuanian University of Health Sciences*
[2]*Lithuanian University of Health Sciences*
[3]*Oncology Research Laboratory, Oncology Institute, Lithuanian University of Health Sciences*

Background and Objectives
Myeloid differentiation primary response 88 (MyD88) is a protein that, in humans, is encoded by the MYD88 gene. Signals from outside the cell are received by a group of proteins and the MyD88 functions as a connecting link that transmits the signals further towards proteins within the cell, this includes the signals coming from toll-like receptors (TLRs). TLRs signaling pathways are divided into MyD88-dependent pathways and MyD88-independent pathways. Ultimately, these signaling pathways lead to the expression of different molecules such as cytokines and different inflammatory factors which are essential for the proper immune response enabling natural cellular defences. It has been established that the TLR-MyD88 signaling pathway is involved in the pathogenesis of gynaecologic cancers caused by Human Papillomavirus (HPV) infections.

Different single nucleotide polymorphisms (SNPs) of the MYD88 gene have been identified. Some SNPs have been associated with increased susceptibility to different cancers and other diseases. However, the distribution of the SNPs of the MYD88 gene and their effect, if any, in different gynaecological cancers and in tumor pathomorphological characteristics is poorly understood. Gynaecologic cancer is one of the major causes of global cancer deaths amog women.

Objectives: In this study we aim to investigate the link between MYD88 polymorphisms and tumor pathomorphological characteristics, progression-free survival (PFS) and overall survival (OS) in a group of patients with cervical and uterine cancers in order to further extend our knowledge in processes which may play a crucial role in earlier diagnosis and more selective treatment plans that will improve the overall management of these conditions.

Material and Method
This study included 121 women with cervical and uterine cancer. For SNP analysis genomic DNA was extracted from peripheral blood leukocytes. MyD88 rs6853 and rs7744 SNPs were analyzed by the polymerase chain reaction-restriction fragment polymorphism (PCR‑RFLP) assay. The statistical analysis was performed using SPSS. The associations between the genotypes and alleles with tumor characteristics were assessed using Pearson's Chi-square or Fisher's Exact tests. Univariate and multivariate analysis to present odds ratios with 95% confidence intervals (CIs) and p-values were calculated with logistic regression. Differences in PFS and OS were assessed using hazard ratios (HRs) from univariate and multivariate Cox proportionate hazard models. p-value of <0.05 was considered statistically significant for all analysis.

Results
91 (75.2%) with cervical cancer and 30 (24.8%) with uterine cancer patients were involved in this study (mean (+/-SD age, 62.6 (+/- 12.5). Histopathology types of squamose cell carcinoma majority were in patients with cervical cancer (91.2%), and adenocarcinoma majority were in cases of uterine cancer (90%). The distribution of genotypes was as follows: MYD88 rs6853 AA-72.7%, GA-25.6%, GG-1.7%, 119 (98.3%) patients were A allele carriers and 33 patients (27.3%) were G allele carriers. For MYD88 rs7744 the distribution was AA-61.2% GA-33.9% GG-5%, 115 (95%) patients were A allele carriers, 48 (39.7%) patients were G allele carriers. The distribution of genotypes was according to the Hardy-Weinberg equilibrium. The AA genotype of MYD88 rs6853 was associated with a reduced risk of higher-grade (G3) cancer compared to the GA genotype (OR = 0.400, 95% CI 0.169-0.948, p-value = 0.037) in multivariate analysis following the adjustment for age at diagnosis, cancer type, lymph node status and cancer stage. This study included too few patients with GG genotype to conduct meaningful comparisons. MYD88 rs6853 polymorphism did not show any significant association with the course of the disease. In case of MyD88 rs7744 SNP, no significant link between tumor phenotype and patient PFS or OS was detected.

Conclusions
Our study suggests that MYD88 rs6853 polymorphism has an impact on tumor differentiation grade. For more precise analysis further investigation on larger sample size should be conducted.

CONTENT

5th Kaunas / Lithuania International Hematology / Oncology Colloquium

26 June 2020

Editor

Prof. Elona Juozaitytė (Kaunas, Lithuania)

Abstracts' Reviewers:

Prof. Arturas Inčiūra (Kaunas, Lithuania)
Assoc. Prof. Rolandas Gerbutavičius (Kaunas, Lithuania)
Assoc. Prof. Sigita Liutkauskienė (Kaunas, Lithuania)

9 786099 616704