

<sup>1</sup> Department of Biomedicine - Experimental Biology Unit, Faculty of Medicine of the University of Porto, Al. Prof. Hernâni Monteiro, 4200-319 Porto, Portugal and Instituto de Investigação e Inovação em Saúde (I3S) Rua Alfredo Allen, 208, 4200-135 Porto, Portugal

<sup>2</sup> Department of Urology, Central Hospital of S. João-Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal

<sup>3</sup> Faculty of Medicine, University of Porto, Portugal

<sup>4</sup> Faculty of Nutrition and Food Sciences, University of Porto, Portugal

E-mail address: [andressap16@gmail.com](mailto:andressap16@gmail.com)

(A. Santos Pereira).

**Aim:** This study aims to investigate the signaling pathway AMP-activated protein kinase (AMPK)-Peroxisome proliferator-activated receptor-gamma coactivator (PGC)1 $\alpha$ -sirtuin (SIRT)3 in the human corpus cavernosum (HCC) between healthy individuals and those with cardiovascular disease risk factors (CVDRF).

**Introduction:** SIRT3 is a mitochondrial NAD<sup>+</sup>-dependent protein-deacetylase involved in the regulation of cellular metabolism.<sup>1,2</sup> As a key factor in AMPK and PGC1- $\alpha$  activation in stress, the decrease in SIRT3 expression or activity is associated with diverse pathologies and aging. Actually, SIRT3 expression was found decreased in HCC of aged individuals with CVDRF.<sup>3</sup> CVDRF such as diabetes mellitus (DM), dyslipidemia, hypertension and obesity strongly associate to endothelial dysfunction, which early manifests as erectile dysfunction (ED).<sup>4</sup>

**Methods:** HCC's samples from individuals aged 40-60 years, submitted to programmed urological surgeries at Hospital São João-Porto, were divided in three groups (n=4): (1)-controls without ED or CVDRF; (2)-DM patients; and (3)-patients with three or more CVDRF including DM. Dual immunolabelling of SIRT3 and superoxide dismutase (SOD)2 with alpha-actin was carried out. As well, levels of SIRT1, SIRT3, SOD2, PGC1 $\alpha$ , NADPH oxidase (Nox)1, phospho-AMPK and AMPK were assessed by Western-blotting(WB).

**Results:** We observed SIRT3 and SOD2 expression in  $\alpha$ -actin-labelled fusiform muscle cells in all groups. The semi-quantification by WB demonstrated a significant decrease in SOD2 expression in group 3 relatively to controls, as well as, an increased tendency of Nox1 and PGC1 $\alpha$  and a decreasing trend in phospho-AMPK in groups 2 and 3. No differences in SIRT1 and SIRT3 were observed among groups.

**Conclusion:** This study suggests that CVRF including DM increase oxidative stress in HCC owing to a decrease in SOD2 expression and concomitant increment in Nox1. Further studies with an increased number of HCC samples will be necessary to elucidate the role of the AMPK-PGC1 $\alpha$ -SIRT3 signaling pathway in the response to oxidative damage.<sup>5</sup>

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## PS075

### Examination of antiproliferative effects of the horseradish extracts



L. Đurić<sup>1,\*</sup>, D. Četojević-Simin<sup>2</sup>, M. Milanović<sup>1</sup>

<sup>1</sup> University of Novi Sad, Faculty of Medicine, Department of Pharmacy, Novi Sad, Serbia

<sup>2</sup> University of Novi Sad, Faculty of Medicine, Experimental Oncology Department, Oncology Institute of Vojvodina, Sremska Kamenica, Serbia

E-mail address: [djuriclarisa@gmail.com](mailto:djuriclarisa@gmail.com)

(L. Đurić).

**Aim:** The aim of the study was to investigate in vitro the antiproliferative effects of the horseradish juice and pulp using different solvents for the extraction.

**Introduction:** Horseradish (*Armoracia rusticana*, Brassicaceae) is a perennial herbal plant, which is widely used in human nutrition, as well as in a traditional medicine. Horseradish is a rich source of bioactive compounds such as isothiocyanates, that have proved to be significant antitumor agents.

**Methods:** Samples were prepared by the Kupchak extraction method, and the antiproliferative effects of the horseradish juice and pulp extracts were examined on the human tumor cell line MDA-MB-231 (ER-, human breast adenocarcinoma). Cell growth was determined by measuring the total protein by colorimetric sulforhodamine B assay. The obtained results (expressed as mean  $\pm$  SD) were analyzed by Tukey HSD test and the differences were considered statistically significant at  $p < 0.05$ .

**Results:** According to the IC<sub>50</sub> parameter (the concentration that inhibited the cell growth by 50%), as an important indicator of the antiproliferative effects, the most pronounced antitumor activity was observed for chloroform juice extract (IC<sub>50</sub> = 5.52  $\pm$  1.47  $\mu$ g/ml). In addition, highly potent was chloroform pulp extract (IC<sub>50</sub> = 19.44  $\pm$  3.82  $\mu$ g/ml), as well as the dichloromethane juice (IC<sub>50</sub> = 26.50  $\pm$  4.15  $\mu$ g/ml) and pulp (IC<sub>50</sub> = 26, 01  $\pm$  2.45  $\mu$ g/ml) extracts. On the other hand, significantly lower in vitro antitumor effect was noticed for the butanol pulp extract (IC<sub>50</sub> = 114.52  $\pm$  0.28  $\mu$ g/ml). IC<sub>50</sub> vales for butanol juice extract, as well as water juice and pulp extracts were higher than 500  $\mu$ g/ml.

**Conclusion:** The obtained results suggest that *A. rusticana* is as a significant source of antitumor agents, especially liposoluble isothiocyanates and as such, it should be recommended for further use in a human nutrition and prevention of cancer.

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## PS080

### Contribution of the determination of numeric value of adc map in early detection of prostate cancer



Dj Perovic

Faculty of Medicine, University Novi Sad, Serbia

E-mail address: [djukaperovic@yahoo.com](mailto:djukaperovic@yahoo.com).

**Aim:** To define the range of ADC values for the absence of malignant disease, as well as to determine the threshold of ADC values for suspected prostate cancer.

**Introduction:** Prostate cancer is the second most diagnosed cancer, and the second most common cancer-cause of deaths in men worldwide. The apparent diffusion coefficient (ADC) derived from DWI has been shown to improve the detection of prostate cancer and is the primary imaging method for the differentiation between low to high grade cancers. ADC values show reduction with increasing Gleason's score.

**Methods:** Prospective study included 60 subjects. Male patients were divided into the groups with pathohistology verified benign and malignant lesions (aged, 46–81; average age, 67.7 years) with abnormal PSA values (>4 ng/ml), and into control group (aged, 44–81; average age 65.3) with normal PSA values (0–4 ng/ml). Prostates were first examined on MRI, determining the diffusion values on ADC map, by placing the region of interest (ROI), through the middle of lesions. Later, the TRUS-guided biopsies were performed. Three intersections of the prostate (apex, middle, and base) were observed, and at total of 12 places (4 places per section) the mentioned methods were indicated.

**Results:** Statistically significant difference ( $p < 0.05$ ) between the groups of patients with malignant and benign lesions in relation to the ADC values of the apex, base and middle of prostate. ADC values of malignant lesions at apex were in range 952–1030, at base  $859\text{--}977 \times 10^{-6} \text{ mm}^2/\text{s}$ , while in benign lesion values at apex were in range 1234–1336, and at base  $1096\text{--}1183 \times 10^{-6} \text{ mm}^2/\text{s}$ .

**Conclusion:** Determination of the numerical value of ADC map represents a significant additional diagnostic parameter for prostate cancer. All values in the range of 1179–1229 for base, 1063–1139 for middle, and  $1199\text{--}1379 \times 10^{-6} \text{ mm}^2/\text{s}$  for apex were considered normal. Values between the range of  $857\text{--}1030 \times 10^{-6} \text{ mm}^2/\text{s}$  have been suspected for possible presence of the prostate cancer.

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## PS101

### Deoxycytidine kinase expression in AML blasts and its relationship to leukemia-free and overall survival

L. Ng<sup>1,\*</sup>, C. Chan<sup>2</sup>, T. Au<sup>2</sup>, C.K. Cheng<sup>3</sup>, K.F. Mo<sup>2</sup>, W. Li<sup>2</sup>, K. Lei<sup>2</sup>, T. Mok<sup>2</sup>, M. Ng<sup>3</sup>, R. Raghupathy<sup>2</sup>

<sup>1</sup> Faculty of Medicine, The Chinese University of Hong Kong (CUHK)

<sup>2</sup> Partner State Key Laboratory of Oncology in South China, Sir YK Pao Centre for Cancer, Department of Clinical Oncology, Hong Kong Cancer Institute and Prince of Wales Hospital, CUHK

<sup>3</sup> Blood Cancer Cytogenetics and Genomics Laboratory, Department of Anatomical and Cellular Pathology, Prince of Wales Hospital, CUHK, Hong Kong

E-mail address: [chitat.ng@gmail.com](mailto:chitat.ng@gmail.com)

(L. Ng).

**Aim:** Study the correlation of expression of genes involved in cytarabine metabolism to leukemia-free (LFS) and overall survival (OS) in AML.

**Introduction:** Cytarabine is the backbone of AML therapy. Understanding the roles and polymorphisms of genes involved in cytarabine metabolism and resistance in AML will facilitate development of novel therapeutics.

**Methods:** Adults less than 60 years with non M3 AML were included. Archived diagnostic marrow samples were studied for expression of 10 genes involved in cytarabine metabolism by RT-qPCR; gene expression normalized to GAPDH was compared

using the unpaired t-test with Welch correction. SNP rs4694362 in deoxycytidine kinase (DCK) gene was tested using Taqman assay. Median time to relapse and survival was calculated by Kaplan Meier method.

**Results:** 21 Han Chinese patients (median age: 50) were identified; 15 were male; 16 had intermediate risk cytogenetics; 5 had a blast count of over  $100 \times 10^9/\text{L}$  at diagnosis. 17 patients achieved CR; 12 after first induction. No difference in gene expression was seen between CR ( $n = 12$ ) versus non CR ( $n = 9$ ) with first induction. 17 CR patients were followed for a median duration of 67.5 months; median time to relapse was 15 months. 1 patient who underwent allogeneic transplant in CR1 was excluded. Higher mean DCK expression was seen in patients with LFS longer than ( $n = 7$ ) versus less than 2 years ( $n = 9$ ) ( $1.91 \pm 0.67$ ,  $p = 0.01$ ) and in those with OS longer than ( $n = 8$ ) versus less than 3 years ( $n = 8$ ) ( $2.02 \pm 0.69$ ,  $p = 0.01$ ). DCK rs4694362 TT genotype was less prevalent than CT in patients with >2 year LFS; but not statistically significant. (49% vs 60%,  $p = 0.6$ ).<sup>1,2</sup>

**Conclusion:** DCK phosphorylates cytarabine to its active metabolite. Our work shows that higher DCK expression is correlated to LFS and OS in AML. The role of DCK SNP rs4694362 should be explored further in the Chinese.

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## PS108

### Analysis of MTHFR C677T polymorphism significance in patient preparation for the in vitro fertilization procedure



Amalija Stojanovic\*, Stevan Stojanovic, Suzana Sredic

*Institute for Human Genetics, Faculty of Medicine, University of Belgrade, Serbia*

E-mail address: [amalija.stojanovic@gmail.com](mailto:amalija.stojanovic@gmail.com)

(A. Stojanovic).

**Aim:** The aim of this study is to determine if in relation to general population, there is a statistically significant difference in the frequency of alleles and genotypes of the C677T polymorphism of MTHFR gene, amongst women with unknown cause of infertility, who are undergoing in vitro fertilization preparation.

**Introduction:** Methylene tetrahydrofolate reductase (MTHFR) is an enzyme coded by MTHFR gene. Polymorphism of MTHFR gene C677T leads to decreased function of MTHFR enzyme, which is associated with high level of homocysteine and low concentration of folate, which can undermine female reproductive function and affect the outcome of in vitro fertilization.

**Methods:** The study included the experimental group consisted of 31 women and the control group consisted of 100 women. C677T polymorphism was detected via PCR/RFLPS method. The statistical difference in genotype and allele frequencies was conducted using the Chi-square test.

**Results:** The comparison of genotypes amongst the experimental and control group has not shown a statistically significant difference ( $p > 0.05$ ). Frequency of the MTHFR677 TT genotype is 22.6% in the experimental group, and 12.0% in the control group, while the allele T frequency amongst the experimental group was