

41.9%, and the frequency of the aforementioned allele amongst the control group was 34.5%.

**Conclusion:** The results of this study show that there is no statistically significant correlation between MTHFR C677T polymorphism in women with infertility of unknown cause, who are undergoing in vitro fertilization preparation, but also underline the need for further research.

**Acknowledgements:** Mentor: Professor Dr. Ivana Novaković.

<http://dx.doi.org/10.1016/j.pbj.2017.07.094>

#### PS114

### Assessing the oxidative modification of proteins in inflamed placenta combined with iron deficiency anemia in the pregnant through histochemical method with bromophenol blue based on Mikel Calvo



Y. Karliichuk\*, V. Ilika

Department of Pathological Anatomy and Morphology, USA

E-mail address: [j.m.karliichuk@gmail.com](mailto:j.m.karliichuk@gmail.com)  
(Y. Karliichuk).

**Aim:** To set features of OMB in the cytoplasm of decidua cells in basal plate of the placenta at chorioamnionitis with iron deficiency anemia in pregnant women by means of histochemical methods combined with computer microspectrophotometry.

**Introduction:** Decidua cells are important cells to the placenta, playing a significant role both in the physiology of pregnancy and during inflammation. The processes of oxidative modification of proteins (OMB) in inflammation are associated with increased levels of oxygen free radicals, which alter the properties of these macromolecules while oxidating amino groups of proteins. Anemic condition is accompanied by intensification of free radical processes in the blood and tissues, and iron deficiency additionally significantly modifies these processes.

**Methods:** 125 studied placentas, to compare the studied placental physiology of pregnancy and monitoring iron deficiency anemia without inflammation.

A histochemical reaction of bromophenol blue for “acidic” and “basic” proteins by Mikel Calvo was set in histological sections 5 μm thick.

Delta Optical Evolution 100 and Olympus SP-550UZ were used to obtain a digital copy of the image. Ratio R/B, which is the ratio between the amino and carboxyl groups in proteins, was determined by “ImageJ”.

Unpaired Student’s test calculated arithmetic mean and its error.

**Results:** When assessing visual histochemical preparations decidua cells are clearly stained, that is suitable for quantitative research, cell boundaries are defined through clear cell membrane coloring and contrasting color around decidua cells fibrinoid. Nuclei and nucleoli were visualized fairly well. “Basic” proteins prevailed in nucleoplasm, while “sour” in the nucleolus.

The decidua cells’ cytoplasm specific color has been mostly granular in nature, and spectral characteristics and optical density of color varied greatly.

Factor R/B at physiological pregnancy ( $n = 20$ ) was  $-1.04 \pm 0.008$  and in iron deficiency anemia ( $N = 21$ )  $-1.06 \pm 0.009$   $P > 0.05$ . In acute chorioamnionitis ( $n = 23$ )  $-1.08 \pm 0.009$ , and combined with iron deficiency anemia ( $N = 21$ )  $-1.09 \pm 0.009$   $P > 0.05$ . Regarding chronic chorioamnionitis ( $n = 20$ ) ratio  $-1.24 \pm 0.011$ , and combined with iron deficiency anemia ( $N = 21$ )  $-1.64 \pm 0.016$   $P < 0.001$ .

**Conclusion:** Conclusion. The intensity of OMB increases only in chronic form of chorioamnionitis in the decidua cells cytoplasm,

and combined with iron deficiency anemia significant performance increase has been observed.

<http://dx.doi.org/10.1016/j.pbj.2017.07.095>

#### PS121

### Comparison of Ras/Raf/MAPK signaling pathway in primary tumour and lymph node metastases – A report on an experimental study of two colorectal cancer cell lines (SW480 and SW620) and tissue samples



K. Kałuzińska\*, A. Wach, P. Frączek

Chair of Medical Biochemistry, USA

E-mail address: [ka.kaluzinska@gmail.com](mailto:ka.kaluzinska@gmail.com)  
(K. Kałuzińska).

**Aim:** To compare the presence of mutations in essential genes of CRC pathogenesis pathway between tissues derived from the primary tumour site and lymph node metastases.

**Introduction:** Colorectal cancer (CRC) remains the third most commonly diagnosed malignancy worldwide and a leading cause of cancer - related death. One of the pivotal pathways leading to CRC development is Ras/Raf/MAPK which is regulated by the receptor for the EGF. Mutations in these genes predict lack of response to EGFR-targeting monoclonal antibodies. However it is a common practice to assess only the primary tumour site, while mutations in metastasis may also affect the response to treatment.

**Methods:** The study was conducted on 10 patient-derived tissue samples and two ATCC human CRC cell lines obtained from the same individual: SW480 (primary tumour) and SW620 (lymph node metastasis). Cell lines were cultured according to the protocol. Genomic DNA and RNA were isolated, and PCR and RT-PCR were conducted. Primers for PCR included the following fragments: KRAS (exons 2,3,4), NRAS (exons 2,3,4), BRAF (exon 15); and for RT-PCR: KRAS, NRAS, BRAF and EGFR. Restriction enzymes were used. Proteins were extracted, purified and Western-Blot (RAS, RAF, MAPK) was performed.

**Results:** For SW480 we detected a mutation in exon 3 of NRAS gene, whereas SW620 presented a wild type. The level of Ras protein remained the same. Raf protein expression was abundant in the primary tumour site as compared to the lymph node metastasis, whereas MAPK protein presented the opposite level of expression.

**Conclusion:** The analysis of Ras-Raf-MAPK pathway may suggest that along with the tumour progression, the dominating signal is located at deeper levels of signaling pathway. Due to existing differences in key molecular points between the primary tumour and its metastases, in the era of targeted therapy, pre-treatment assessment of both sites has a potential to become a standard of care.<sup>1,2</sup>

#### References

1. Van Cutsem E, Cervantes A, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. *Ann Oncol.* 2016;27:1386–422.
2. Negru S, Papadopoulou E, Apeessos A, et al. KRAS, NRAS and BRAF mutations in Greek and Romanian patients with colorectal cancer: a cohort study. *BMJ Open.* 2014;4:e004652. <http://dx.doi.org/10.1136/bmjopen-2013-004652>.

<http://dx.doi.org/10.1016/j.pbj.2017.07.096>

#### PS122

### PI3K-Akt and Ras-Raf-MAPK signaling in colorectal cancer – Comparison of activity in primary tumor tissues and primary tumour – Derived human colorectal cancer cell lines



A. Wach\*, K. Kałuzińska, P. Frączek

Chair of Medical Biochemistry, Estonia

E-mail address: [adamwach27@gmail.com](mailto:adamwach27@gmail.com)

(A. Wach).

**Aim:** The study aimed to compare the differences in activity of PI3K-Akt and Ras-Raf-MAPK pathways, and changes in the Ras-Raf-MAPK activity after PI3K-Akt silencing, between different cell lines and tissue samples from primary tumour sites of human CRC.

**Introduction:** Alterations in EGFR-related Ras-Raf-MAPK and PI3K-Akt pathways are involved in the pathogenesis of up to 55% and 15% colorectal cancers (CRC) respectively. The Ras-Raf-MAPK pathway mutations are assessed before introducing a standard anti-EGFR treatment, as they indicate lack of response. However, the autonomic activity of alternative PI3K-Akt pathway may also have an impact on the effectiveness of targeted therapy.

**Methods:** The study was carried out on three ATCC human CRC cell lines derived from primary tumours (COLO320, SW480 and HT29) and ten patient tissue samples. Cell lines were cultured according to the protocol. Genomic DNA and RNA were isolated, PCR and RT-PCR were performed. Restriction enzymes were applied. Primers for the following fragments of genome were used: KRAS (exons 2, 3, 4), NRAS (exons 2, 3, 4), and BRAF exon 15 for PCR; KRAS, NRAS, BRAF, PIK3CA for RT-PCR. Proteins were extracted, purified and Western Blot was conducted. siRNA for Akt and specific PI3K inhibitors were used to silence PI3K-Akt activity.

**Results:** The analyzed material presented variable profiles of pathways activity. Interestingly, high expression of Ras protein was positively correlated with Akt protein level. In case of low level of Ras, Raf protein was dominating whereas Akt expression was significantly decreased.

**Conclusion:** Ras and Akt can simultaneously present a high level of expression. Thus, as PI3K-Akt is an alternative pathway to Ras-Raf-MAPK for EGFR signaling and its autonomic activity may affect the efficacy of anticancer treatment, it has a potential to be taken into consideration while planning a treatment and developing new anticancer agents.<sup>1,2</sup>

## References

1. Muzny DM, Bainbridge MN, et al. Comprehensive molecular characterization of human colon and rectal cancer. *Nature*. 2012;487:330–7.
2. Khwanraj K, Madlah S, et al. Comparative mRNA expression of eEF1A isoforms and a PI3K/Akt/mTOR pathway in a cellular model of parkinson's disease. Hindawi Publishing Corporation; 2016. p. 11. Article ID 8716016.

<http://dx.doi.org/10.1016/j.pbj.2017.07.097>

## PS124

### The role of the hypoxic tumor microenvironment on the macrophage-tumor cell interplay

F. Martins<sup>1,2,3,\*</sup>, F. Castro<sup>2,3,4</sup>, M.L. Pinto<sup>2,3,4</sup>, A.J. Silva<sup>2,3</sup>, B. Sousa<sup>2,5</sup>, M.J. Oliveira<sup>2,3,6</sup>, Â.M. Costa<sup>2,3</sup>

<sup>1</sup> Department of Biology, Faculty of Sciences, UPorto, Porto, Portugal

<sup>2</sup> i3S - Instituto de Investigação e Inovação em Saúde, UPorto, Porto, Portugal

<sup>3</sup> INEB - Institute of Biomedical Engineering, UPorto, Portugal

<sup>4</sup> ICBAS- Instituto de Ciências Biomédicas Abel Salazar, UPorto, Porto, Portugal

<sup>5</sup> IPATIMUP- Institute of Molecular Pathology and Immunology of the University of Porto, Portugal



CrossMark

<sup>6</sup> Department of Pathology and Oncology, Faculty of Medicine, UPorto, Porto, Portugal

E-mail address: [flavia.martins@i3s.up.pt](mailto:flavia.martins@i3s.up.pt)

(F. Martins).

**Aim:** The aim of this work is to unveil the role of the hypoxic microenvironment on macrophage-tumor cell interplay, using colorectal cancer (CRC) as a model.

**Introduction:** Microenvironment, in most cases hypoxic, is composed by cancer cells, extracellular matrix, stromal and immune cells, that cooperate and affect each other activities. Macrophages are one of the most abundant immune cells at the tumor microenvironment, acting as tumor suppressors or promoters. Previous research had shown that both hypoxia and immunosuppressive macrophages are associated with tumor progression. Nevertheless, these studies did not focus on the interplay between hypoxia and macrophage-cancer cell crosstalk.

**Methods:** To achieve our goal co-cultures of CRC cells and human macrophages, both in normoxia and hypoxia, were established. Macrophages were characterized functionally and phenotypically and their potential to induce cancer cell invasion was evaluated.

**Results:** Our results suggest that hypoxia, and the presence of cancer cells, decreases the cell surface expression of an anti-inflammatory marker (CD163), however the mRNA expression was not altered. Nevertheless, hypoxia induced an increase in the mRNA expression of the macrophage pro-inflammatory marker (CCR7).

Macrophages metabolic activity was not altered by hypoxia but decreased when co-cultured with cancer cells. In addition, lactate production decrease in co-culture while glucose consumption increased. Notably, macrophages in normoxia presented a more rounded morphology while in hypoxia are more elongated with evident cellular protrusions, suggesting dynamic alterations at the actin cytoskeleton organization. Interestingly, MMP-2 and MMP-9 activity profiles were not altered by the presence of cancer cells or hypoxia. Nevertheless, cancer cell invasion ability increased in the presence of macrophages, suggesting that other MMPs might be involved.

**Conclusion:** Findings in normoxia regarding macrophage potential to induce cancer cell invasion are consistent with those previously described by our group. Interestingly, we demonstrate now that hypoxia potentiates the invasive behavior of cancer cells and also macrophage pro-invasive ability.

<http://dx.doi.org/10.1016/j.pbj.2017.07.098>

## PS129

### Ethnopharmacological use of *Cymbopogon citratus* (DC.) Stapf and *Cymbopogon schoenanthus* (L.) Spreng.: Anti-inflammatory potential of phenol-rich extracts



CrossMark

Elisabete Gomes\*, João Bernardo, Mariana Barbosa, Paula B. Andrade, Patrícia Valentão, Graciliana Lopes

REQUIMTE/LAQV, Laboratório de Farmacognosia, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira, n° 228, Porto 4050-313, Portugal

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

(E. Gomes).

**Aim:** The aim of this work consisted on expanding the knowledge on the chemical composition of different extracts from *Cymbopogon* spp., and on the evaluation of their anti-inflammatory potential in cell and cell-free systems.