PS124

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

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Abstract: To achieve our goal co-cultures of CRC cell lines and human macrophages were set up. Both normoxic and hypoxic conditions were applied. From these conditions, cellular metabolic activity, motility and invasion ability were 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).

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

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Abstract: The aim of this work was to explore the use of phenol-rich extracts from Cymbopogon spp., and on the evaluation of their anti-inflammatory potential in cell and cell-free systems.
Introduction: The ethnopharmacological use of Cymbopogon spp. dates back from ancient times. Traditionally used in tropical and semi-tropical countries for the repellent properties of their essential oil, the consumption of Cymbopogon spp. infusions is growing all over the world. This is not only due to the unique aroma, widely appreciated by the consumers, but also because of the antimicrobial, anti-inflammatory and sedative properties. 

Methods: The chemical characterization of infusions and ethanol:water (50:50, v/v) extracts from Cymbopogon citratus and Cymbopogon schoenanthus was achieved by HPLC-DAD. The anti-inflammatory potential of the extracts was assessed by cell and cell-free assays.

Results: HPLC-DAD analysis allowed the identification of several caffeic acid derivatives and flavonoids in the infusions and in the ethanol:water extracts of both species. The different extracts displayed scavenging activity against superoxide anion and nitric oxide (NO) radicals, and capacity to significantly reduce NO production by LPS-stimulated macrophages (RAW 264.7 cell line). In addition, the extracts were able to prevent hyaluronic acid degradation via inhibition of hyaluronidase, an enzyme recognized to participate in a number of physiological and pathological processes, including inflammation. No toxicity was observed on human gastric adenocarcinoma and hepatocyte carcinoma cell lines, at a maximum concentration of 2.0 mg/lyophilized extract/mL.

Conclusion: This study provided scientific evidence on the ethnopharmacological use of Cymbopogon species on inflammatory conditions, encouraging infusion consumption and future incorporation of Cymbopogon spp. extracts into nutraceuticals.

Acknowledgements: This work received financial support from National Funds (FCT/MEC) through project UID/QUI/50006/2013, co-financed by FEDER through COMPETE, under the Partnership Agreement PT2020, and from NORTE 2020, under the PORTUGAL 2020 Partnership Agreement, through ERDF (NORTE-01-0145-FEDER-000024). MB is indebted to FCT for the grant (SFRH/BD/95861/2013).

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http://dx.doi.org/10.1016/j.pbj.2017.07.099

PS140

Cytotoxic effects of novel synthesized polyoxometalates on human neuroblastoma SH-SY5Y cell line

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Aim: Investigation of cytotoxic effects of newly synthesized and untested polyoxometalates Pd1 and Pd2 on human neuroblastoma cells SH-SY5Y.

Introduction: Polyoxometalates (POMs) are transitional metal complexes, which are important in medicinal chemistry, as potent anticancer, antiviral and antibacterial agents. Inefficiently selective drugs and problems with dosing of usual chemotherapeutics directed the research towards investigation of new agents, such as POM.

Methods: Effects on viability rate of treated cells was tested using acid phosphatase assay. The mechanism of a cell death was examined using flow cytometry. JC-1, dihydroethidium, ApoStat, propidium iodide and acridine orange stainings were conducted in order to elucidate mitochondrial depolarisation, production of superoxide anion, caspase activation, DNA fragmentation and intracellular acidity.

Results: Pd1 and Pd2 have shown dose and time dependent decrease in cell viability rate. Complexes induced mitochondrial depolarisation after 2 h of treatment, which was shown as increase in FL3/FL1 ratio from 1 to 1.3 (Pd1, 6 µM) and from 1 to 1.7 (Pd2, 40 µM). Superoxide anion production was increased after 5 h of treatment using Pd1 and 2 h of treatment using Pd2. Pd1 complex exhibits increase in percentage of cells with fragmented DNA (subG0) and activated caspases after 24 h treatment. Pd2 complex induced decrease in SubG0 and S phase without caspase activation after 24 h treatment. POMs have shown intracellular acidification after 48 h (FL3/FL1 ratio: control 1, Pd1 2.3, Pd2 1.8).

Conclusion: POM complexes indicated cytotoxic effects on examined cell line. The mechanism by which these complexes exert those effects differ from one another. It was shown that both induce oxidative stress and mitochondrial depolarisation, accompanied by activation of caspases and DNA fragmentation in Pd1-treated cells, all indicative of apoptosis. In Pd2-treated group there was no increase in activation of caspases. Complexes have shown increase in intracellular acidification, which may suggest autophagy.

Acknowledgements: This research was a part of bilateral project between University of Belgrade, Belgrade, Serbia and Jacobs University, Bremen, Germany; 451-03-01038/2015-09/16.

http://dx.doi.org/10.1016/j.pbj.2017.07.100

PS153

HuR prevents c-fos mRNA degradation by proteasome-associated ribonuclease in vitro

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Aim: To estimate HuR protective activity against proteasome-associated ribonuclease for c-myc and c-fos mRNAs.

Introduction: Proteasome-associated proteins are attractive targets for multiple myeloma treatment. One of them is HuR protein known to selectively bind ARE-containing mRNAs and protect them from degradation. HuR is supposed to play a role in cancerogenesis since its expression is elevated in many cancer types and it stabilizes a lot of mRNAs encoding proteins involved in oncogenesis. Previously, it was shown that proteasome in addition to its main function – protein degradation – may act as a selective RNase. Moreover, HuR and proteasome have common targets – c-myc and c-fos protooncogene mRNAs.

Methods: HuR-GST fusion protein has been cloned, expressed and purified by affinity chromatography. Fragments of c-myc and c-fos were cloned and mRNAs have been transcribed in vitro. Proteasomes have been isolated from K562 cell line (human proerytrolekemia) and Im-9 cells (human multiple myeloma). mRNAs were treated by proteasomes in presence and absence of HuR. The estimation of mRNA cleavage was held by gel-electrophoresis.

Results: GST-HuR has specifically bound ARE-containing fragments of c-myc and c-fos mRNAs. Proteasomes extracted from Im-9 and K562 cells cleaved target mRNAs in absence of HuR. It was shown that HuR prevents degradation of c-fos mRNA by proteasomal endoribonuclease, whereas c-myc mRNA was cleaved in the...