same conditions. GST protein didn't bind with target mRNAs and didn't affect proteasome cleavage activity.

Conclusion: HuR protects c-fos mRNA from proteasome ribonuclease cleavage in vitro, but can't prevent c-myc mRNA degradation. HuR and proteasome compete with each other for manifestation of their opposite activities. Thus, a new mechanism of regulation of proto-oncogenes expression was observed. However, the functional role of this process in vivo should be evaluated in further studies.[1–4]

References

1. Myer VE, Fan XC, Steitz JA. Identification of HuR as a protein implicated in AUUUA-mediated mRNA decay. EMBO J. 1997;16:2130–9.
2. Srikantan S, Gorospe M. HuR function in disease. Front Biosci. 2012;17:189–205.
3. Pouch MN, Petit F, Buri J, Briand Y, Schmid HP. Identification and initial characterization of a specific proteasome (prosome) associated RNase activity. J Biol Chem. 1995;270:22023–8.
4. Savant-Bhonsale S, Cleveland DW. Evidence for instability of mRNAs containing AUUUA motifs mediated through translation-dependent assembly of a > 20S degradation complex. Genes Dev. 1992;6:1927–39.

PS156

Metformin interferes with glucose cellular uptake by both estrogen and progesterone receptor-positive (MCF-7) and triple-negative (MDA-MB-231) breast cancer cell lines

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Aim: Transport experiments with 3H-DG, culture growth and proliferation rate assays were performed.This work aimed to investigate the possible interference of metformin with glucose uptake by MCF-7 and MDA-MB-231 human breast adenocarcinoma cell lines as a mechanism contributing to its anticarcinogenic effect.

Introduction: Breast cancer, the most common cancer among women, remains one of the leading causes of mortality among women worldwide.[1] Metformin has been widely used as a treatment for type 2 diabetes for over 40 years.[2] The first report of a reduced risk of developing cancer for diabetic patients treated with metformin was published in 2005.[3] Several mechanisms of action of metformin appear to be implicated in this effect.[2,4]

Methods: Transport experiments with 3H-DG, culture growth and proliferation rate assays were performed.

Results: Acute (26 min) exposure of MCF-7 cells to metformin significantly inhibited uptake of 3H-deoxy-D-glucose (3H-DG) (maximal inhibition found with metformin 0.5 mM: $27 \pm 2\%$ reduction). Chronically (24 h), metformin induced a concentration-dependent increase in 3H-DG uptake (maximal increase observed with metformin 1 mM: $81 \pm 15\%$ increase). Acute (26 min) exposure of MDA-MB-231 cells to metformin slightly inhibited uptake of 3H-DG (maximal inhibition found with metformin 1 mM: $10 \pm 3\%$ reduction). Chronic (24 h) exposure to metformin significantly increased 3H-DG uptake by MDA-MB-231 cells (maximal increase observed with metformin 1 mM: $30 \pm 8\%$ increase).

Chronic (24 h) exposure of both cell lines to metformin (1 mM) decreased culture growth/cell mass; in contrast, it increased cell proliferation rates. Combination of metformin (1 mM) with the facilitative glucose transporter (GLUT) inhibitor kaempferol (30 μM) did not result in a more marked effect on culture growth and cell proliferation rates.

Conclusion: Summarizing, chronic exposure of MCF-7 and MDA-MB-231 cells to metformin induces a marked increase in glucose uptake, associated with an anticarcinogenic effect of the drug. We suggest that the increase in glucose uptake is a compensatory mechanism to cellular energy depletion induced by metformin.

References

1. DeSantis, et al. CA Cancer J Clin. 2014;64:52–62.
2. Jara L-M. Pharmacol Res. 2015;101:102–8.
3. Evans, et al. BMJ. 2005;330:1304–5.
4. Daugan, et al. Pharmacol Res. 2016;113:675–85.

PS162

Endocannabinoids induce placental trophoblast reticulum stress

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Aim: We aim to investigate in cytotrophoblasts whether these effects on cell viability loss are due to endoplasmic reticulum (ER) stress mediated apoptosis.

Introduction: Placental development relies on a balance between proliferation, differentiation and apoptosis of trophoblasts, a process tightly regulated by growth factors, cytokines and hormones. Endocannabinoids (eCB), such as 2-arachidonoylglycerol (2-AG) and anandamide (AEA) may play a role in these processes. We previously demonstrated that both eCB induced trophoblast cell death.[1,2] Here we investigated in cytotrophoblasts whether these effects on cell viability loss are due to endoplasmic reticulum (ER) stress mediated apoptosis.

ER stress is caused by the accumulation of unfolded proteins leading to an unfold protein response (UPR) triggered by transmembrane ER signaling proteins including: pancreatic ER kinase (PKR)-like ER kinase (PERK), inositol-requiring enzyme 1 (Ire1) and Activating transcription factor 6 (ATF6). The dissociation of Grp78 (BiP) from these sensors triggers a series of mechanisms that can restore homeostasis or lead to apoptosis. Placental stress has been implicated in the pathophysiology of pregnancy complications, including growth restriction and pre-eclampsia.

Methods: BeWo cells (ATCC, USA), an accepted model of cytotrophoblast stem cells were treated with AEA or 2-AG (10 micromolar) for 24 h. Through quantitative real time polymerase chain reaction (qPCR), we evaluated mRNA levels of ER stress markers: CHOP, Grp78, ATF4 and spliced mXBP1. Protein expression of CHOP was evaluated by western-blot.

Results: After 24 h of treatment with both eCB, we found an increase in mRNA levels of ER stress markers: CHOP, Grp78, ATF4 and spliced mXBP1. Protein expression of CHOP also increased in both cases.

Conclusion: These results suggest that cell viability loss promoted by 2-AG and AEA was associated with ER-stress since both PERK and IRE1 arms of UPR are activated. Prolonged ER-stress, contributes to the expression of pro-apoptotic proteins, such as CHOP.

These findings shed light to the impact of endocannabinoids induced-ER stress which may negatively affect trophoblast cell turnover and pregnancy outcomes.

References

1. Costa MA, Fonseca BM, Keating E, Teixeira NA, Correia-da-Silva G. 2-Arachidonoylglycerol effects in cytotrophoblasts: metabolic enzymes expression and apoptosis in BeWo cells. Reproduction. 2014;147:301–11.
2. Costa MA, Fonseca BM, Teixeira NA, Correia-da-Silva G. The endocannabinoid anandamide induces apoptosis in cytotrophoblast cells: involvement of both mitochondrial and death receptor pathways. Placenta. 2015;36:69–76.

PS163

Analysis of imaging characteristics, incidence, and prognosis of brain metastases from thyroid cancer

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Aim: The main objectives of this study were to evaluate the incidence, imaging characteristics, and prognosis of parenchymal brain metastases originating in thyroid cancer.

Introduction: While thyroid cancer is a relatively common type of cancer, it is usually highly curable.[1] Brain metastases from thyroid cancer are rare and their imaging appearance has not been well defined.[2]

Methods: Review of case records of thyroid cancer patients within the IPO Porto data base from 2005 to 2015 was conducted in order to identify the patients with thyroid cancer and evidence of brain metastases.

Results: We identified 3175 patients with thyroid cancer, with only five having evidence of brain metastases (two from papillary thyroid cancer, two from follicular thyroid cancer and one from poorly differentiated thyroid cancer). At the time of brain metastases detection, 100% of the patients had concurrent lymph node metastases, 80% lung metastases and 60% osseous metastases. Of those brain metastases, 60% were multifocal and 40% presented as partially cystic/necrotic. Of the two cases in which the patients died, the median overall survival after brain metastasis detection was less than one year.

Conclusion: Brain metastasis from thyroid cancer remains a rare phenomenon that most frequently occurs in the setting of widely disseminated lymph node disease. The imaging appearance is highly variable and the prognosis is poor.

References

1. Hjiyiannakis P, Jefferies S, Harmer CL. Brain metastases in patients with differentiated thyroid carcinoma. Clin Oncol. 1996;8:327–30.
2. Tsuda K, Tsurushima H, Takano S, Tsuboi K, Matsumura A. Brain metastasis from papillary thyroid carcinomas. Molec Clin Oncol. 2013;1:817–9.

PS166

The association of GSTP1 genotype with the risk and survival in ccRCC patients with advanced tumor stage

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Aim: The aim of this study was to evaluate specific role of glutathione S-transferase P1 (GSTP1) gene variants as determinants of ccRCC risk in patients with advanced tumor stage (pT3 and pT4). Furthermore, we evaluated the effect of GSTP1 gene variants on postoperative prognosis in these patients.

Introduction: Renal cell carcinoma (RCC) accounts for up to 90% of malignant kidney tumors with clear renal cell carcinoma (ccRCC) being the most frequent and the most aggressive subtype of sporadic RCC in adults. Unfortunately, most RCCs are asymptomatic in early stages, whereas symptomatic RCC correlates with aggressive histology and advanced disease. Aside from known risk factors for RCC, evidence suggest that the development of RCC can be partially explained by genetic variations among the populations. Highly polymorphic cytosolic glutathione S-transferases are known to be involved in both the development and the progression of renal cell carcinoma.

Methods: GSTP1 genotype was determined in 99 ccRCC patients and 326 matched-controls by qPRC method, using TaqMan® SNP Genotyping Assay. The risk for disease was computed by odds ratios (OR) and 95% confidence intervals (CI) using logistic regression analysis Furthermore, overall survival was analyzed as well by Kaplan-Meier method and Cox proportional hazard regression model.

Results: GSTP1-variant genotype was associated with 5-fold increased risk for ccRCC in comparison with GSTP1-wild type genotype ($p < 0.001$). Moreover, survival analysis clearly indicated shorter overall survival in ccRCC patients with GSTP1-variant genotype, however without reaching statistical significance ($p = 0.166$). Additionally, ccRCC patients with GSTP1-variant genotype had a 7-fold higher hazard ratio ($p = 0.177$), compared to the carriers of GSTP1-wild type genotype.

Conclusion: GSTP1-variant genotype contributed independently towards the risk of ccRCC in our patients. Moreover, GSTP1-variant genotype is associated with poor postoperative prognosis in ccRCC.