subsequently deacetylated NF-kB p65 and p53 in WT, however not in NQO1−/− mice thereby attenuating AIC.

Conclusion Dunnione has a cardioprotective effect against ADR-induced cardiomyopathy through NQO1 enzymatic action. Thus, modulation of NAD+/NADH by NQO1 may be a novel therapeutic approach to prevent chemotherapy-associated heart failure, including AIC.

**PO-252** IMPAIRED GLUCOSE METABOLISM IN HUMAN GLIOMA STEM CELLS UPON TREATMENT WITH A CELL-PENETRATING PEPTIDE BASED ON CONNEXIN43

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**Introduction** Connexin43 (Cx43), the main gap junction channel-forming protein in astrocytes, is downregulated in glioma stem cells (GSCs). Restoring Cx43 in GSCs reverses their phenotype through the inhibition of c-Src and consequently reduces GSCs tumorigenicity. We have developed a cell penetrating peptide (TAT-Cx43266–283) containing the region of Cx43 that interacts with c-Src and mimics the effect of Cx43 on GSC phenotype. GSCs reprogram their metabolism to compete for glucose resources through HIF-1-α, which can in turn be regulated by c-Src. Therefore, the aim of this work was to study the effect of TAT-Cx43266–283 on GSC metabolism.

**Material and methods** G166 (human GSCs), Wistar rat organotypic brain slices, neurons and astrocytes from primary culture.

2-NBDG uptake: Cells were incubated with 146 μM 2-NBDG for 1 hour, lysed, and supernatant fluorescence intensity was measured by spectrophotometry and normalised to mg of protein. GSCs-brain organotypic slice co-culture Fluorescently-dyed human GSCs were injected into rat organotypic brain slices and 2-NBDG uptake was analysed by confocal microscopy.

Cell energy analysis were performed on an extracellular flux analyser (Agilent Seahorse XF Technology) using Mito Stress and Glycolysis Stress kits.

**Results and discussions** Because TAT-Cx43266–283 inhibits Src activity in GSCs, we analysed the effect of TAT-Cx43266–283 on the rate of glucose uptake in human GSCs. Our results showed that TAT-Cx43266–283 reduced the uptake of a fluorescent glucose analogue (2-NBDG) into GSCs. Interestingly, TAT-Cx43266–283 did not significantly affect the uptake of glucose in neurons or astrocytes from primary culture, suggesting a specific effect on GSCs. Moreover, experiments using 6-NBDG, a fluorescent glucose analogue that cannot be phosphorylated by HK-2 (whose expression is regulated by HIF-1-α), showed that 6-NBDG uptake does not differ between treated and not treated GSCs. Moreover, we analysed 2-NBDG uptake on a GSCs-brain organotypic slice co-culture. Our results revealed that TAT-Cx43266–283 reduced glucose uptake in tumoral cells when they are within the brain parenchyma. More importantly, data obtained with a cell energy analysis platform (Agilent Seahorse XF Technology) showed impaired metabolism in GSCs treated with TAT-Cx43266–283, but not in neurons or astrocytes.

**Conclusion** In vitro and ex-vivo experiments revealed that TAT-Cx43266–283 reduces the rate of glucose uptake selectively in human glioma stem cells with the subsequent decrease in metabolic activity and survival.

**PO-253** CHARACTERISTICS OF CELLULAR RESPIRATION, GLYCOLYTIC ACTIVITY AND RELATED METABOLIC FEATURES IN WILD TYPE AND IDH1 MUTANT GLIOMA CELLS

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**Introduction** IDH mutations are expressed in 80% of low grade gliomas and secondary glioblastomas with a favourable prognosis comparing to non IDH mutant gliomas [According to WHO (2016)] classification, IDH1 mutation is an important marker to classify gliomas. Our main interest is to study the role of certain metabolic alterations in glioma models, especially mitochondrial functions, glycolytic activity, IDH1 mutations and its regulation.

**Material and methods** U87 MG, U373 MG and U251 MG and U251 MG IDH1 R132H (kind gift of Dr. W. Leenders) were used. We overexpressing cells were used in vitro. Cellular oxygen consumption, glycolytic activity, energy substrates oxidation were measured by Seahorse technique. Furthermore, the amount and the activity of several proteins related mTOR (mammalian target of rapamycin) and other metabolic pathways were studied. The proliferation and apoptosis rates of in vitro cell cultures were also studied. We analysed extra- and intracellular metabolite levels (citrate, succinate, fumarate, alpha-ketoglutarate, malate, 2-hydroxyglutarate – D-2-HG, glutamate) using LC-MS measurements. Addressing the question what are the sources of D-2-HG in IDH1 mutant U251 MG cells, we fed the cells with 13C-labelled energy substrates.

**Results and discussions** The glycolytic activity and oxygen consumption rates of the four cell lines show differences. The wild type U251 MG cell line has higher glycolytic activity and lower basal respiration than its IDH1 mutant variant glioma cell line pair. Addition of D-2-HG (72 hour) to wild type U251 MG cells increased the basal respiration and decreased the glycolytic activity of U251 MG cells. Using various bioenergetic substrates, it has been shown that U251 MG cells can oxidise glutamine, glutamate and malate at significantly higher level than IDH1 mutant U251 MG cells. This may be due to the alternative use of glutamine, we found that glutamine is the main source of D-2-HG in IDH1 mutant U251 MG cells, we fed the cells with 13C-labelled energy substrates.

**Conclusion** Based on the results of the examined glioma cells, IDH1 mutant cells showed a lower glycolytic capacity, a higher respiration and altered glutamine metabolism, which could be therapeutic targets in the future.

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**PO-254** G PROTEIN-COUPLED OESTROGEN RECEPTOR ACTIVATION DECREASES PROSTATE CANCER CELLS VIABILITY CONCOMITANTLY WITH ALTERED PROLIFERATION, APOPTOSIS, AND METABOLIC PROFILE

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Introduction A panoply of studies have been indicating that estrogens are protective agents in prostate carcinogenesis. However, the physiological effects of estrogens in PCa mainly have been associated with the differential activation of the nuclear estrogen receptors (ER), with much less knowledge existing on the membrane ER. The G protein-coupled ER (GPER), known to be involved in the rapid nongenomic responses, has been linked to antiproliferative and proapoptotic effects and is a likely candidate mediating the ‘anti-carcinogenic’ actions of estrogens. This work aims to characterise the GPER role controlling proliferation, apoptosis and metabolism of PCa cells.

Material and methods The nonneoplastic PNT1A cell line and neoplastic LNCaP DU145 and PC3 cells were maintained in culture in RPMI 1640 medium. GPER expression pattern was characterised by Western blot. Fluorescent immunocytochemistry allowed determining the subcellular localization of GPER by colocolization with wheat germ agglutinin, calnexin, and hoescht. PNT1A, LNCaP, DU145 and PC3 cells were treated with the GPER specific agonist G1 (1 μM) for 24 hour. Cell viability was assessed by the MTT assay. The effect of GPER activation on cell proliferation, apoptosis and metabolism was assessed by analysing the expression of key proteins in each process. Also, the enzymatic activity of caspase-3 and LHD was measured. Furthermore, G1-stimulated cells displayed augmented caspase-3 activity comparatively to the control group. G1 treatment also modulated PCa cell metabolism with altered glucose consumption and lactate production were determined using commercial kits.

Results and discussions GPER was differentially expressed in PCa cell line models depending on their aggressiveness and disease status. GPER expression was highest in the androgen-sensitive and less aggressive LNCaP cells and decreased in the more aggressive castration-resistant cell line models (DU145 and PC3). GPER was located at the cell membrane, endoplasmic reticulum, and also in the nucleus. The activation of GPER by G1 decreased PCa cells viability, concomitantly with altered expression of key regulators of proliferation and apoptosis. Furthermore, G1-stimulated cells displayed augmented caspase-3 activity comparatively to the control group. G1 treatment also modulated PCa cell metabolism with altered glucose consumption and lactate production.

Conclusion GPER activation decreased viability of PCa cells whereas enhancing apoptosis. Also, PCa metabolic profile was altered in response to G1. These findings stimulate further research to ascertain the role of GPER as a therapeutic target.