were analysed by MTS assay and colony formation were assessed by clonogenic assay; Proteins expression were detected by westernblotting. Targeted metabolomics were done using LC-MS with stable isotopes labelled intermediates to identify potential metabolic flux changes in GLUL knockdown cells. 

Results and discussions In vitro analyses of cell viability assay showed that GLUL knockdown in A549 cell line mediates resistance towards the treatment of chemotherapeutic drugs including Pazopanib, Imatinib, Dasatinib and Docetaxel. While, westernblotting analyses revealed that reduction in apoptotic marker PARP cleavage in knockdown cells in response to chemo drugs with respect to controls. Further, we also observed that GLUL knockdown activates pro-survival signaling mechanism i.e., PI3K-AKT pathway via increased phosphorylation of AKTser473 and S6 ribosomal proteinser235/236 in mediating resistance. Additionally, in clonogenic assay, cells were grown in the presence of different chemotherapeutic drugs, evidently GLUL knock down cells were able to form more colonies than control cells. Since GLUL activity potentially can be linked to central energy metabolism via glutaminolysis we probed the metabolic fluxes in glycolysis and TCA pathways by means of 13C labelled Glutamine, Glutamate and Glucose respectively and interestingly we didn’t observe any significant changes in metabolic flux linking the resistant phenotype to alterations in energy utilisation by the cells. 

Conclusion Our study shows that GLUL Knockdown could induce chemo-resistance, through modulating apoptotic and pro-survival signalling. Thus, GLUL may serve as a potential target for NSCLC tumour progression with therapeutic significance. 

PO-251 MODULATION OF NAD+-LEVELS BY NQO1 ENZYMATIC ACTION ALLEVIATES ADRIAMYCIN-INDUCED CARDIAC DYSFUNCTION IN MICE

D Khadka*, GS Oh, HJ Kim, A Shen, SHYang, HS So.

1Wonkwang University, Center for Metabolic Function Regulation and Microbiology, Iksan, South Korea;
2Wonkwang University, Internal Medicine, Iksan, South Korea

Introduction Adriamycin (ADR) is a potent anticancer drug widely used to treat a variety of human neoplasms. Nevertheless, its clinical application is hampered because of severe side effects such as cardiotoxicity and heart failure. ADR-induced cardiomyopathy (AIC) has been reported to be caused by myocardial damage and dysfunction through oxidative stress, DNA damage, and inflammatory responses. But, the remedy for ADR cardiomyopathy is still not developed. We describe the effect of NAD+/NADH modulation by NAD(P)H quinone oxidoreductase 1 (NQO1) enzymatic action on AIC. 

Material and methods AIC was established by three times intraperitoneal injections of ADR (cumulative dose of 12 mg/kg i.p.; 4 mg/kg every day for 3 consecutive days) in C57BL/6 wild-type (WT) and NQO1 knockout (NQO1−/−) mice. Before and after exposure to ADR, the mice were orally administered dunnione (20 mg/kg), a substrate of NQO1. Cardiac biomarker levels in the plasma, cardiac dysfunction, oxidative biomarkers, and mRNA and protein levels of pro-inflammatory mediators were determined to compare the cardiac toxicity of each experimental group.

Results and discussions All biomarkers of Cardiac damage and oxidative stress, and mRNA levels of pro-inflammatory cytokines, including cardiac dysfunction were significantly increased in ADR-treated both WT and NQO1−/− mice. However, this increase was significantly reduced by dunnione in WT, but not in NQO1−/− mice. In addition, a decrease in SIRT1 activity due to a decrease in the NAD+/NADH ratio by PARP-1 hyperactivation was associated with ADR-induced cardiotoxicity through increased nuclear factor (NF)-κB p65 and p53 acetylation in both WT and NQO1−/− mice, whereas an increase in NAD+/NADH ratio by NQO1 enzymatic action using dunnione as a substrate recovered SIRT1 activity and
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

1SG Pelaz*, 2M Gómez de Cedrón, 3M Taberner, 4A Ramirez de Molina, 5JM Medina, 1A Taberner. 1Instituto de Neurociencias de Castilla y León, Neurobiochemistry Lab. 15, Salamanca, Spain; 2IMDEA Food Institute- CEB UAM + CSIC, Precision Nutrition and Cancer Program- Molecular Oncology and Nutritional Genomics of Cancer Group, Madrid, Spain

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 that mimics the effect of Cx43 on GSC phenotype. GSCs reprogram their metabolism to compete for glucose resources through HIF-1-alpha, 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 spectrofluorimetry and normalised to mg of protein.

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-a), 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.