Green Fluorescent Protein Monitoring of P-glycoprotein Mediated Chemoresistance and its Regulation by Glycolysis in Multicellular Tumour Spheroids

Wartenberg, M.[1], Richter, M[1], Datchev, A.[1], Günther, S.[3], Milosevic, N.*, Figulla, H.-R.[1], Aran, J. M.[2], Pétriz, J.[2], Sauer, H.[4]

[1]Department of Internal Medicine I, Cardiology Division, Friedrich Schiller University Jena, Germany
[2]Medical and Molecular Genetics Center, Institut d'Investigació Biomèdica de Bellvitge (IDIBELL), Hospital Duran i Reynals, L'Hospitalet de Llobregat, Barcelona, Spain
[3]Institute of Microbiology and Epizootics, Veterinary Faculty, Free University Berlin, Germany
[4]Department of Physiology, Justus Liebig University Giessen, Germany

ABC transporters like P-glycoprotein (P-gp/ABCB1) are membrane proteins responsible for the transport of toxic compounds out of non-malignant cells and tumour tissue. Most pharmacokinetic studies on P-gp function are currently performed in laboratory animals.

Aims: 1) to establish a green fluorescent protein (EGFP) reporter gene based in vitro model to study expression and function of P-gp in living three-dimensional tumour tissues.

2) to investigate the effect of glycolysis and the tissue redox state on P-gp expression in multicellular tumour spheroids derived from prostate adenocarcinoma cells (DU-145), glioma cells (Gli36), and the human cervix carcinoma cell line KB-3-1.

Results: During cell culture of DU-145, Gli36 and KB-3-1 tumour spheroids P-gp expression was observed as well as increased lactate and decreased pyruvate levels and expression of glycolytic enzymes. Inhibition of glycolysis for 24 h by either iodoacetate (IA) or 2-desoxy-D-glucose (2-DDG) downregulated P-gp expression which was reversed upon coincubation with the radical scavenger ebselen as shown by semi-quantitative immunohistochemisty in DU-145 and Gli36 tumour spheroids, and by EGFP fluorescence in KB-3-1 tumour spheroids. Consequently endogenous ROS generation in DU-145 tumour spheroids was increased in the presence of either IA or 2-DGG, which was abolished upon coincubation with ebselen. Exogenous addition of pyruvate significantly reduced ROS generation, increased P-gp expression as well as efflux of the P-gp substrate doxorubicin. In summary our data demonstrate that P-gp expression in tumour spheroids is closely related to the glycolytic metabolism of tumour cells and can be monitored in living multicellular tumour spheroids transfected with a EGFP-Pgp reporter gene construct.