OTKA 83533 (Balázs Sarkadi) - Closing scientific report (max 15 000 characters)

Investigation of human ABC multidrug transporters in normal pluripotent and cancer stem cells

The human ATP-Binding Cassette (ABC) transporter proteins play important roles in protection against xenobiotics, in drug metabolism, as well as in the regulation of numerous basic physiological functions. In addition, the multidrug (MDR) transporter ABC proteins generate wide-range drug resistance in the tumor cells. We have shown earlier that the ABCG2 multidrug transporter is present in several forms of human stem cells and is involved in the drug resistance of tumor stem cells. In this project we focused on the exploration of the function of regulation of ABC transporters expressed in normal and tumor stem cells, built an infrastructure and scientific network to study these roles and established a cell- and molecular biology capacity to support these studies at a cellular and molecular level. The project has also lead to several results applicable in stem cell technologies and yielded new assay systems for drug discovery. The main results of the project are summarized below:

In the first year of the project we have developed new model systems for studying cancer stem cells, by stably expressing the ABCG2 protein in tumor cells which are the targets of specific, new antitumor agents. We concentrated on the kinase inhibitors of the EGF receptor pathway, and expressed several variants, as well as a GFP-conjugated version of the ABCG2 protein. We found that the presence of MDR-ABC transporters significantly modifies the cellular actions of targeted anticancer agents (Brózik et al, Exp. Opin. Drug Metab. Toxic., 2011). We have also developed a new, antibody-based method for the rapid and efficient studies of the ABCG2 protein inhibitors (Telbisz et al, Eur. J. Pharmacol. Sci., 2012). We have examined the intracellular trafficking and fate of the GFP-conjugated, functionally active ABCG2 protein (Hegedüs et al, Biochem. Pharmacol., 2012) and described the role of ABCG2 in cancer stem cells in a book chapter (Arias et al, 2011).

Regarding the human normal stem cells, we have generated and characterized various model systems, including iPS cell lines. When looking at the mesenchymal stem cells, generated form human pluripotent cells, we could document their preserved immunosuppressive activity (Varga et al, BBRC, 2011). In the human pluripotent stem cells we performed the basic characterization of the calcium-dependent signaling systems (Apáti et al, Mol. Cell. Endocrin., 2012), and started to examine the role of MDR-ABC transporters in stem cells. For this purpose we have examined the expression pattern of ABCG2 both in human embryonic stem cells, their differentiated derivatives and in human pluripotent stem cells (see below).

We have published a book chapter describing the application of the transposon-based system for gene modifications in stem cells (Orban et al, 2011), a review article about the importance of gene modifications in human stem cells (Szebenyi et al, Person. Med. 2012), and

about the role of stem cell research in the development of new scientific approaches (Sarkadi and Schatten, Stem Cell Reviews, 2012).

In the second year of the project we have further investigated the role of human ABC multidrug transporters in normal and cancer stem cells and developed new stem-cell based technologies.

In the studies led by Csilla Hegedüs, Ph.D. student, we focused on the potential regulation of the ABCG2 protein in cancer stem cell models (Hegedüs et al, Biochem Pharmacol., 2012, Hegedüs et al, BBRC, 2012). We found that the expression and function of the ABCG2 multidrug transporter significantly modulates the cellular effects of targeted anticancer agents. Moreover, some of the targeted kinase inhibitors had strong inhibitory effect on MDR-ABC transporters, thus modifying the effects of other cytotoxic agents as well. In the studies led by Tamas Aranyi and Hugue deBoussac, we studied the regulation of ABCG2 expression by hormones and toxic agents in a hepatocyte model (deBoussac et al, BBRC, 2012). We found a promoter-dependent regulation of the drug transporter, significantly affecting drug metabolism.

In a systematic work, led by Agnes Telbisz, we have developed an efficient procedure to isolate and purify the human ABCG2 protein from Sf9 cell membrane preparations (Telbisz et al, Biochem J., 2013). We demonstrated the lipid and cholesterol dependence of the transport activity, and achieved a high-activity, reconstituted ABCG2 preparation first in this field.

The team lead by Tamas Hegedus developed an openly available, interactive electronic database for the mutations in the human ABC transporters (Gyimesi et al, Hum. Mut., 2012). Based on the feed-back reports, this database is now widely, internationally used for planning and controlling experimental work related to these transporters.

As a new project in this program, we have generated a new model system, the flow-cytometry based investigation of the membrane proteins the human red cell membrane. This platform was used for examining the effects of genetic variations on the expression levels of the ABCG2 transporter (Kasza et al, PLOS One, 2012). We found that the red cell membrane protein expression closely correlated with the polymorphic variants and mutations affecting ABCG2 processing.

In the human pluripotent stem cells (this work was led by Agota Apati and involved numerous Ph.D. students) we have documented a dynamic regulation of the expression and localization of the ABCG2 protein under stress conditions (Erdei et al, Eur. Biophys. J.). Both up- and down-regulation of the transporter expression in the pluripotent and differentiated human stem cells could be followed upon the applications of drugs or stress conditions.

In close connection with this work, led by Tamas Orban, Attila Sebe and Agota Apati, we have developed a new method for the generation of human induced pluripotent stem cells, based on the use of the Sleeping Beauty transposon system, developed earlier by Zsuzsanna Izsvak and

Zoltan Ivics (Grabundzija et al, Nucl. Acid Res., 2013). By using the transposon system and human pluripotent stem cells, we have also developed cell lines stably expressing an intracellular calcium indicator protein (GCaMP-2), and studied ligand induced calcium signals in the pluripotent cells as well as in the differentiated cardiomyocytes and neuronal cell types (Apati et al, Cell Signal. 2013). This new approach allows studying these cellular phenomena in cells without the need of additional loading with calcium indicator dyes.

Tamas Orban and a Ph.D. student, Anita Schamberger first reported the generation and potential role of human mirtrons (Schamberger et al, RNA Biol, 2012). These microRNAs are formed in an alternative, Drosha-independent maturation pathway, using the splicing machinery to produce pre-miRNAs from short introns during mRNA processing. A figure from the report of this new discovery was published on the cover page of the relevant RNA Biology issue.

In the third year of the project we achieved important new results in the field of stem cell related research. In the laboratory led by Ágota Apáti, Zsuzsa Erdei followed the expression of numerous ABC proteins in human stem cells and in their differentiated offspring. They have demonstrated that under stress conditions or after drug exposure the ABCG2 expression in pluripotent cells changes dynamically (Erdei et al, Eur. Biophys. J., 2013), and both up- and down-regulation of the transporter expression may occur under these conditions. They also performed detailed mRNA expression and flow cytometry studies for examining numerous ABC transporters in human stem cells and during early stem cell differentiation (Erdei et al, Cytometry, 2014). Kornelia Szebenyi, based on her previous work focused on studying the cardiac differentiation of human stem cells and the generation of early cardiac progenitors. These preparations provide an excellent model system for personalized drug screening (Szebenyi et al, book chapter, 2014). These experiments were also extended in animal models studying cardiac tissue regeneration (Paloczi et al, book chapter, 2013).

In this year we have studied in detail the molecular regulation of the ABCG2 transporter and its drug interactions. In the project led by Ágnes Telbisz, she and her colleagues examined the role of membrane lipids in isolated and reconstituted ABCG2 protein preparations (Telbisz et al, Biochem J., 2013). We have demonstrated the specific lipid and cholesterol dependence of the transport activity, and the isolated protein is now used to study the direct regulation of the transporter, as well as applied for crystallization and structural studies in a Swiss collaboration. Based on these experiments we have obtained a joint NIH (USA) grant together with a US-based drug screening laboratory.

We have also performed detailed targeted mutational studies for exploring the cholesterol and bile acid regulation of the ABCG2 transporter. Based on these experiments we proposed a molecular regulation model for the concerted action of cholesterol and bile acids on ABC transporters in the human hepatocytes (Telbisz et al, Drug Metab. Dispos., 2014).

The potential substrates and inhibitors of the ABCG2 transporter, explored in a collaboration with a French research group (Gauthier et al, Front. Pharmacol. Res, 2013, and Winter et al, J. Med. Chem, 2013) provided important new information for structural features of drug interactions. These data should provide a basis for the development of new inhibitors and modulators of this transporter.

The ABCG2 protein is medically important both in cancer drug resistance and in physiological uric acid transport. Polymorphic variants and mutations in ABCG2 are causative in the development of gout, therefore we have studied an important polymorphic variant, Q141K, of ABCG2. We have shown the processing defect of this protein and introduced "corrector" mutations which may provide important information regarding further drug screening (Saranko et al, BBRC, 2013). In collaboration with the group of Andras Varadi, we also performed detailed studies for the cellular localization and processing of the ABCC6 protein (Pomozi et al, Circulation Res, 2013).

Regarding the determination of ABC transporters in the membrane of the human red cells, we published a review describing this technology (Varady et al, Biomark. Medic., 2013) and applied for a patent protection of the technology. Moreover, this technology was extended to the ABCB6 protein (Koszarska et al, PLOS One, 2014), and is now a basis for initiating clinical diagnostic studies in metabolic diseases.

Within this project Tamas Orban and his Ph.D. students performed detailed studies for the potential role of transposons in normal and tumor stem cells (Kolacsek al, Mob. DNA, 2011; Kolacsek et al., Genomics III Book chapter, 2014). They become involved in important further studies, the generation of transgenic rat models by using the transposon technology (see below).

In the fourth year of the project we have concluded and extended some of the previously started sub-projects. We have studied the mechanisms of the tissue repair and immunosuppressive features of the mesenchymal stem cells and the role of ABC transporters in this cell type (Szepesi et al, Stem Cell Devel., 2015, Bácskai et al, Stem Cell Devel, 2015). The role of differentiated stem cells in tissue repair and drug testing has been examined both in human stem-cell-derived cardiomyocyte preparations (Szebenyi et al, Tissue Engineering, 2015), and by generating a transgenic rat model. In this latter case the totipotent stem cells of the rat were modified by transposon-based expression of a calcium indicator protein, and the resulting stable rat model was utilized to visualize calcium dynamics in the kidney both in vitro and in vivo (Szebenyi et al, JASN, 2015). In addition, human stem cell derived hepatocyte-like cells were applied for studying the expression of tight junction proteins (Erdélyi-Belle et al, POR, 2015). The transposon-based systems were further investigated regarding their excision and transgenic efficiency in various model systems (Kolacsek et al, Human Gene Ther. Methods, 2014).

Regarding the structural and functional studies of the ABCG2 protein we have performed a detailed investigation of the lipid requirements of this protein and its mutant variants (Hegedüs et al, Adv. Cancer Res., 2015), and investigated in detail the potential molecular determinants of the ABCG2-cholesterol interactions, by generating specific mutant variants of the protein (Gal et al, Biochim. Biophys. Acta, 2015). In addition, we have worked out a mathematical model for describing the transporter and drug interaction kinetics in a complex biological framework (Toth et al, PLOS One, 2015).

In general, the project was scientifically successful, altogether we have published 37 research papers in international journals, presented oral or poster presentations in more than 50 occasions, and published 5 book chapters (see list of publications supported by OTKA 83533).