P-glycoprotein conformational changes detected by antibody competition

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INTRODUCTION

One of the unresolved problems of cancer chemotherapy is the resistance of cancer cells to a broad range of cytotoxic drugs, referred to as multidrug resistance (MDR). This phenomenon is frequently associated with the expression of P-glycoprotein (Pgp/ABCB1). The P-glycoprotein-mediated multidrug resistance was the first discovered and probably the most widely observed mechanism in clinical multidrug resistance. After the cloning and characterization of Pgp, it became evident that other efflux-pumps may also play a significant role in transport-associated drug resistance. There are two other transporters, which have been definitively demonstrated to participate in the multidrug resistance of tumors: the multidrug resistance protein 1 (MRP1/ABCC1), and the mitoxantrone resistance protein (MXR/BCRP/ABCG2). Pgp and these other two MDR related proteins belong to the ABC (ATP binding cassette) transporter superfamily of transport ATPases. The ABC transporters are characterized by the presence of evolutionary conserved ATP binding motifs.

Pgp is expressed in more than 50 % of diagnosed human cancer cases, intrinsically, or after chemotherapy. Pgp is able to recognize structurally diverse, hydrophobic/amphiphylic substrates (among them cytotoxic drugs) in the size range of 500-1000 Da and to pump them out of the cells at the expense of ATP hydrolysis. Important questions about the mechanism of action of P-glycoprotein: How does P-glycoprotein recognize so many different substrates? How is substrate binding and/or ATP hydrolysis linked to conformational changes of Pgp?

The Pgp molecule is comprised of two homologous halves connected by a linker peptide of approx. 75 amino acids. Each half spans the plasma membrane six times forming transmembrane (TM) helices as well as extra- and intracellular loops, according to hydropathy plot predictions.

Several monoclonal antibodies (mAb) were developed against external and internal epitopes of Pgp and applied for its detection in clinical samples (e.g. UIC2, MRK16, MM12.10, MC57). It is unresolved based on the membrane topology of Pgp why some of these mAbs (MC57, MM12.10) binding to intact cells also recognize peptide motifs found in the putative intracellular loops of Pgp, rather than exclusively

outside. Alternative conformers of Pgp, perhaps associated with the different stages of the transport cycle, have been suggested to account for these findings.

The substrates appear to reach the pump primarily from within the membrane lipid bilayer. The nucleotide binding domain (NBD) comprising the ABC composite motif is situated on the intracellular side of the membrane. The two NBDs bind and hydrolyze ATP in an alternate manner. The conformational change elicited by ATP hydrolysis is thought to propagate, via the "signature" element of the ABC, to the 5^{th}, 6^{th}, 11^{th} and 12^{th} TM helices probably involved in substrate recognition and translocation. Changes in the avidity or number of binding sites of certain mAbs (UIC2) accompany the conformational changes that occur upon drug binding/transport. Labeling with UIC2 e.g., is increased by ~2-5-fold in the presence of substrates/modulators. This enhanced binding of UIC2 is applied for detection of functional, i.e. modulatable, Pgps (referred to as the "UIC2-shift" assay). The absence or dissociation from the NBDs, of ATP/ADP seems to be the critical event eliciting the conformational change responsible for enhanced UIC2 binding. UIC2 is also unique among the mAbs directed against Pgp because it blocks drug pumping rather efficiently, apparently by trapping Pgps in a certain conformational state of its enzymatic cycle. It is generally assumed that substrate transport must involve transitions between substrate-binding and substrate-releasing conformational states and must be intimately coupled to ATP hydrolysis induced by substrate binding. The suggested transitions in enzyme conformation during the consecutive steps of the catalytic cycle appear to be reflected by the changing reactivity of Pgp with a conformation sensitive mAb, UIC2.

The plasma membrane lipid composition is also related to MDR and affect Pgp function and expression in the membrane. This idea is further supported by the fact that drug-sensitive cell lines have altered levels of neutral lipids, phospholipids, cholesterol and fatty acids as compared to normal cell lines. In addition, MDR cell membranes appear to be more fragile, most likely due to the increase in complex phospholipids and higher rate of membrane turnover.

The reliable detection and quantitative determination of the clinically relevant levels of the MDR proteins would be extremely important in the clinical treatment of various cancerous diseases. Combination chemotherapy treatment protocols could be adjusted and drug-resistance reversing agents could be applied accordingly.

A large number of laboratory methods have been devised to estimate the expression and/or surface appearance of P-glycoprotein by molecular biological or immunodetection methods. Still, intra and interlaboratory differences are disturbingly large in such estimations. Currently, the most commonly used fluorescence assay method to discriminate between drug-resistant and -sensitive cells is based on the efflux of rhodamine 123 or Calcein AM. The pump selectivity of these assays can be achieved by using the fluorescent substrate compounds in combination with selective inhibitors.

Prevention of clinical MDR should significantly improve therapeutic response in many tumor patients. A better understanding of the drug transport and reversion mechanism is expected to help in working out more successful protocols for the treatment of drug resistant tumors.

OBJECTIVE

Based on a novel phenomenon (modulator-dependent antibody-competition), we hoped to develop an assay for the immunological detection of functional Pgp molecules and also applicable in screening of potential modulators with a sensitivity largely exceeding that of the UIC2-shift assay. Analysis of the phenomenon by various fluorescence-based approaches allowed us to get further insight into the conformational changes accompanying drug transport.

Specific goals:

1. Development of a simple and sensitive assay for detection of conformational changes upon Pgp modulation utilizing competition of mAbs

2. Correlation studies between conformational changes and catalytic cycle of Pgp

3. Topological characterization of Pgp in the presence and absence of modulators

4. Utilization of the antibody-competition phenomenon

    4.1. as an assay of functional and quantitative measurement of Pgp molecules

    4.2. as a method to screen drugs for more effective modulator characteristics.

MATERIALS AND METHODS

Cell lines

The drug sensitive human epidermoid carcinoma cell line KB-3-1 and its multidrug resistant relative KB-V1 obtained by vinblastine selection, and mdr1 transfected NIH 3T3 (NIH 3T3 MDR1) cells were used.

Flow cytometric assays

Labeling the cells with monoclonal antibodies

Antibodies against Pgp were the following: UIC2, 4E3, MRK16, MM12.10, MM6.15 and MM4.17. The 6-(fluorescein-5-carboxamido)hexanoic acid succinimidyl ester (sFx), fluorescein-5-isothiocyanate (FITC) and sulfo-indocianid succinimidyl ester conjugates (Cy3 and Cy5) of the above mentioned mAbs and the UIC2 Fab fragments were prepared.

Cells were trypsinized and washed twice with PBS before antibody labeling. The antibody competition test was performed as follows. Cells were pre-incubated in the absence or presence of different drugs/modulators at 37 ^{o}C for 10 min., then the first ("A") mAb was added, without washing the cells. After further 20 min. incubation at 37^{o}C, the FITC or 5-sFx conjugated second mAb ("B") was added (again without washing the cells) and incubation followed at 37 ^{o}C for another 20 min. The extent of competition between mAbs "A" and "B" were expressed as $R_{competition}$, the difference of mean fluorescence intensities of cell-bound mAb "B", in the absence and in the presence of mAb "A", divided by the fluorescence intensity obtained in the absence of mAb "A". In the "UIC2 shift assay", the cells were pre-treated with drugs, labeled first with UIC2 then with the secondary antibody on ice for 45 min. The UIC2-shift was calculated as the ratio of indirect immunofluorescence, R_{shift}, measured in the presence and absence of modulators.

Detergent solubilization assay

To examine the association of the Ppg to the cytoskeleton and rafts, we have conducted time dependent monitoring of detergent resistance of the cell surface Pgps labeled with fluorescent dye-conjugated UIC2 in a flow-cytometer. Cells were then occasionally (for cross-linking) fixed with 2 % formaldehyde or were cross-linked with a secondary antibody, isotype specific anti-Ig for 30 min., on ice. After labeling,

we have treated the cells with 0.5 % Nonidet P-40 (NP-40) or 0.1 % Triton X-100 (TX-100) on ice and measured their mean fluorescence at 0, 5, 15 and 30 minutes.

Daunorubicin and calcein accumulation assays

Calcein and daunorubicin (DNR) accumulation was measured by preincubation of cells with Pgp modulators for 10 min and then with calcein-AM for further 15 min, at 0.5 μM final concentration or with daunorubicin for further 45 min, at 2 μM final concentration. All the incubations were carried out at 37 ºC. The cells were washed with ice-cold PBS and propidium-iodide was added to the samples prior to measurement. The extent of reversal elicited by the different modulators was characterized quantitatively by an $R_{activity}$ value, calculated as the ratio of mean calcein or daunorubicin fluorescence intensities measured in the presence and absence of modulators, respectively. In combined UIC2-shift and dye accumulation assays, UIC2 labeling in the absence and presence of modulators was followed by fluorescent dye accumulation, after washes in PBS containing 1 % BSA.

The mean cellular fluorescence in each sample was determined using a Becton Dickinson FACStar Plus instrument. Dead cells, stained with propidium iodide, were excluded from the analysis. Fluorescence signals were collected in logarithmic mode and the two-color cytofluorimetric data were analyzed by the BDIS CellQuest or FloWin softwares.

Confocal microsopy

After labeling with mAbs, the cells were fixed with cold 2% formaldehyde, mounted on coverslips and imaged by a Zeiss 420 and Bio-Rad MRC 1024ES laser scanning fluorescence confocal microscope.

Fluorescence Resonance Energy Transfer measurements

In Fluorescence Resonance Energy Transfer (FRET), excitation energy is transferred in a radiation-less process from an excited donor molecule to an acceptor molecule under favorable conditions. The fluorescence intensities were measured for single and double-labeled cells using Cy3- as donor and Cy5-conjugates as acceptor, and these values were used to determine the FRET efficiency on a cell-by-cell basis, applying procedures established in our institute.

RESULTS

1. Development of a sensitive assay for detection of conformational changes upon Pgp modulation: antibody competition test (ACT)

MAb competition studies on NIH 3T3 MDR1 cells

The effect of pre-labeling of NIH 3T3 MDR1 cells with UIC2 on the subsequent binding of the (fluorescent dye-conjugated) MM12.10 mAb was studied first. Pre-labeling of cells with UIC2 only mildly (up to 40 %) decreased the subsequent binding of MM12.10. When cells were pre-labeled with UIC2 in the presence of cyclosporin A (CSA) MM12.10 reactivity was dramatically suppressed, while there was no similar effect of verapamil. Pre-treatment with drugs or modulators by itself did not significantly affect the binding of MM12.10. A number of other compounds behave like verapamil, and some like CSA (see 4.2., below).

The finding that pre-incubation with UIC2 suppressed subsequent binding of MM12.10 in the case of cells pre-treated with certain modulators, is indicative of conformational changes elicited by interaction of Pgp with these modulators.

This methodology can be used as an assay of functional Pgp molecules. We named our **t**est, since it is based on **a**ntibody **c**ompetition: ACT.

MAb competition studies using different mAb combinations

Further mAb combinations were also investigated: MM12.10, MRK16, MM6.15, MM4.17 or 4E3 were all completely inhibited by pre-incubation with UIC2 in CSA pre-treated samples. At the same time, MM12.10 pre-incubation completely prevents subsequent UIC2 binding.

Correlation between simultaneously analyzed UIC2/MM12.10 mAb competition ($R_{competition}$), UIC2-shift (R_{shift}), and Pgp function ($R_{activity}$, based on DNR accumulation measurements) in NIH 3T3 MDR1 cells.

The possible correlation between the extent of UIC2/MM12.10 competition, Pgp function and changes in UIC2-shift were also investigated. The effects of reversing agent pre-treatment on $R_{competition}$, $R_{activity}$ and R_{shift} were simultaneously studied and the results plotted against one-another. $R_{competition}$ was increased to values

tightly packed at ≤ 1 in the presence of CSA (or vinblastine), as compared with the widely scattered R_{shift} values (between 1.25-1.75), often not well resolved from the control.

The mAb competition phenomenon appears to be closely related to UIC2-shift. $R_{competition} \cong 1$ values are not seen without an accompanying UIC2-shift. On the other hand, UIC2-shift can occur at its usual magnitude without fully suppressing MM12.10 binding. Pre-treatment of the cells with verapamil elicits UIC2-shift but doesn't manifest in high (≅1) values of $R_{competition}$. Thus, the conformational changes accompanying UIC2-shift and those leading to strong UIC2/MM12.10 competition appear to be closely related, but not completely identical.

The conformational change responsible for the above competition phenomenon probably involves an element additional to what is involved in the UIC2 shift, bringing about the concomitant suppression of MM12.10 binding. UIC2 binding, rather than MM12.10 affinity, is altered upon CSA treatment, since the binding of the latter mAb is not (or very mildly) affected by the presence of modulators.

MAb competition with UIC2-Fab fragments

The conformational changes of Ppg reflected by the suppression of MM12.10 binding are well reproducible by the Fab fragments of UIC2. This suggests that either a single variable region engages with the whole complex epitope, or that the variable regions can belong to distinct antibody molecules.

Epitope segments involved in the conformational changes of Pgp

MAb MM12.10 is known to recognize a complex epitope involving segments of the 4^{th}-6^{th} extracellular loops. Recently, the UIC2 epitope was also mapped and it includes amino acid sequences localized in the 4^{th} and 6^{th} extracellular loops, in addition to a diffuse region in the 1^{st} loop. The fact that pre-incubation with MM12.10 as well as with MRK16 and MM6.15, completely prevented UIC2 binding, is in agreement with the involvement of the 4^{th} and/or 6^{th} loops in UIC2 binding (in addition to the previously recognized indispensable role of the 1^{st} extracellular loop). Suppression of the binding of the 4^{th} loop-specific MM4.17 by UIC2 pre-incubation

suggests that the 4th extracellular loop is essentially involved in the conformational change related to ACT binding.

The effects of CSA and verapamil on UIC2 mediated inhibition of drug-pumping

It was previously shown that UIC2 binding also decreases the active efflux of drugs, significantly increasing their cytotoxicity. To further investigate the functional corollaries of the ACT phenomenon, we have examined the effects of CSA and verapamil on the inhibition of drug pumping by UIC2 binding. The simultaneous incubation of cells with UIC2 mAb and CSA strongly increased calcein accumulation. When CSA was removed by washes 1 % BSA, the inhibition of calcein pumping was decreased only mildly. At the same time, the inhibitory effect of CSA was completely removed by these washes. In contrast, the pump activity of the verapamil treated cells was totally restored after washing. Similar results were obtained in daunorubicin accumulation experiments.

Thus, CSA may act in collaboration with UIC2 in freezing Pgp in a particular conformation of the catalytic cycle. UIC2 probably binds to Pgp differently in the presence of verapamil.

The simple procedure above involving the combined application of modulators and UIC2 mAb – followed by removal of the modulator – can be applied to achieve near 100 % inhibition of pump activity by the antibody.

Fluorescence resonance energy transfer measurements in ACT

The high FRET efficiency values obtained for pairs of anti-Pgp mAbs (UIC2 vs. MRK16, etc.) are in aggrement with the notion that two anti-Pgp antibody may be accomodated by a single Pgp, or Pgp molecules may exist as dimers or oligomers in the cell membrane or that.

2. Relationships between conformational changes and catalytic cycle

Effect of ATP depletion on UIC2-MM12.10 mAb competition in NIH 3T3 MDR1 cells

Since ATP depletion is known to increase UIC2 reactivity (UIC2-shift), we investigated if treatment of cells with oligomycin, or sodium azide together with 2-deoxy-D-glucose, elicit a similar effect on UIC2/MM12.10 competition. In ATP-

depleted cells, UIC2 completely abolished MM12.10 labeling. Thus, ATP depletion affects UIC2 binding and UIC2/MM12.10 competition in a parallel manner.

The effect of CSA on Pgp conformation was shown to be indistinguishable from that of ATP depletion, suggesting that CSA (and also vinblastine, etc. see below) block the pump in the same, or a very similar conformational state that is part of the catalytic cycle.

Time dependence of UIC2-MM12.10 mAb competition

We studied the possible dependence of the extent of antibody competition on the length of incubation with drugs (CSA, verapamil) and UIC2 mAb.

The binding of UIC2 to the drug-untreated and verapamil-treated samples (containing 8 mM glucose) was low – compared to the CSA-treated sample – and did not change significantly in time.

The lack of further increment in labeling during prolonged incubations with UIC2 is in variance with the possibility that the different conformations of Pgp reflect different phases of the catalytic cycle and shows that the conformational states are distinct and once established, are not readily convertible.

3. Characterization of the membrane topology of Pgp

Cell surface localization of Pgp by confocal laser scanning microscopy

We have performed co-localization experiments by confocal microscopy. Cells were labeled with fluorescent dye-conjugated UIC2/MM12.10 and UIC2/MRK16 antibody pairs, with UIC2 applied before CSA treatment, and the other mAb used to label Pgp molecules not recognizable by UIC2 in the absence of modulators. Similar experiments were performed at 4 ^{o}C, or with Fab fragments of UIC2 and with cells HCHO-fixed prior to labeling, to prevent antibody-induced patching.

Heterogeneous distribution of cell surface Pgp molecules was observed. The extent of separation is demonstrated in the correlation histogram. In control experiments, the complete colocalization was shown when different fluorescent dye-conjugated MM12.10 mAbs were mixed and used for labeling, or MM12.10 labeled cells were stained with FITC-RamIgG.

Intramembrane and cytoskeletal association

To examine the association of Ppg with the cytoskeleton, we have conducted time dependent monitoring of detergent resistance of the proteins labeled with fluorescent dye-conjugated UIC2 in a flow-cytometer. After labeling, we have treated KB-V1 cells with Nonidet P-40 and measured their mean fluorescence at different time intervals.

In the absence of CSA, the majority of UIC2 labeled Pgp molecules are NP-40-soluble and no significant difference was observed when the cells were labeled in the presence of CSA. Cross-linking the antibody with RamIgG or formaldehyde, most of the Pgp molecules were immobilized in the cell membrane. No cytoskeletal associations of MHC I molecules were apparent from these experiments, while the transferring receptor appears to exhibit a significant extent of cytoskeletal anchorage, although below that of their raft-association. Upon cross-linking, immobilization of all the above receptors were conspicuous.

Raft association of Pgp expressed on the surface of KB-V1 cells was significant, and the ratio of Triton X-100 insoluble molecules was different for Pgps labeled with UIC2 in the absence of CSA, and for the rest of cell surface Pgp molecules. These data are suggesting the heterogeneity of Pgps with respect to lipid microdomain (raft) association. Cross-linking with RamIgG or formaldehyde immobilized most cell surface Pgp molecules.

The enhanced detergent resistance upon cross-linking with antibody may be interpreted in terms of cross-linking and immobilization of Pgp molecules to those that are already lipid raft associated.

4. Application of the antibody-competition phenomenon

4.1. Utilization of the mAb competition phenomenon on an assay of functional and quantitative measurement of Pgp molecules

The UIC2-shift test was introduced into clinical studies as an assay of functional Pgp molecules. The reproducibility of the present assay exceeds that of the UIC2-shift assay when CSA is used as modulator, at least certain cell lines.

The ACT method was well reproducible on vinblastine selected KB-V1, expressing Pgp at comparable level to NIH 3T3 MDR1 and on low Pgp expressing, colchicine selected KB-8-5 cells.

It is expected that similarly well reproducible results will be obtained for clinical samples.

4.2. Utilization of the ACT phenomenon as a method to screen drugs for more effective modulator characteristics

The mAb competition phenomenon described above sharply distinguishes between two classes of modulators: verapamil belongs to drogs having no (or small) influence on mAb competition, and CSA, vinblastine belong to drogs interacting with Pgp in such a manner that the antibody added first will completely suppress the subsequent binding of the other.

We extended these observations to a panel of Pgp substrates, modulators and found that out of 30 agents examined, 7 fall into the group inducing a conformational state characterized by enhanced UIC2/MM6.15 competition.

The working concentrations of the tested Pgp substrates and modulators were determined by measuring the changes in calcein accumulation, as well as cytotoxicity by propidium iodide, in response to a wide concentration range of the agents. The concentrations of the agents with the most effective Pgp reversing effect, but <10% increase in propidium iodide-positivity, were selected and compared in the ACT assay.

This panel of Pgp substrates and modulators could also be divided into two sharply distinguishable groups, depending on the $R_{competition}$ values measured (assuming a value of 0 when UIC2 pre-treatment does not affect the binding of the second mAb (e.g. FITC-MM12.10) at all, and 1 at absolute UIC2 dominance). The $R_{competition}$ values were tightly packed at ~1 in the case of CSA, ivermectin, o-amsacrine, m-amsacrine, PSC-833, vinblastine and valinomycin. These agents (termed ACT-positive) are also strong Pgp modulators in the sense that they completely block Pgp function at a relatively low concentration. In contrast, treatment with verapamil, quinine, quinidine, tetracaine, emetine etc. affected (usually increased) mAb competition only mildly, as compared with the drug untreated samples. $R_{competition}$ values of these ACT-negative compounds

scatter in the range of 0.2-0.8. Most of these latter agents raised calcein accumulation significantly, although to a lesser extent than the CSA-like compounds. Within the group of ACT-negative agents, there were compounds that affected neither calcein accumulation nor mAb competition significantly (e.g. lidocaine, taxol, gramicidin D).

The above findings demonstrate the utility of a conformation-sensitive method in testing and screening of MDR modulators. The fact that the ACT-positive agents are also strong MDR modulators in the calcein assay, raises the possibility that agents with $R_{competition}$ values close to 1, are generally strong Pgp inhibitors.

SUMMARY AND APPLICATIONS

1. A sensitive and reproducible assay was developed based on a combined use of mAbs for the immunological detection of functional Pgp molecules. The conformational changes of Pgp that occur in the presence of certain substrates/modulators or ATP depletion, are accompanied by the up-shift of UIC2 monoclonal antibody binding, concomitant with the near-complete suppression of labeling with several other anti-Pgp mAbs specific for epitopes overlapping those of UIC2.

2. The fact that pre-incubation with MM4.17 (in addition to MRK16, etc.) completely prevented UIC2 binding, is in agreement with the essential involvement of the 4^{th} loops in UIC2 binding. The effect of CSA on Pgp conformation was shown to be indistinguishable from that of ATP depletion, suggesting that these treatments block the pump in the same, or a very similar conformational state that may be part of the catalytic cycle.

3. We have developed a simple procedure involving the combined application of modulators and UIC2, followed by removal of the modulator, achieving 100 % inhibition of pump activity by the antibody.

4. Cell surface heterogeneity of Pgp molecules appear to be present on NIH 3T3 MDR1 cells based on confocal microscopy studies. Likewise, the ratio of detergent resistant Pgps in the presence and absence of CSA appears to be

different, which suggests that these are two populations of Pgps, differentiated by CSA, which inhabit different lipid microdomains. Upon cross-linking, immobilization of Pgp to those already raft associated occurs.

5. The reproducibility of the ACT assay exceeds that of the UIC2-shift assay when CSA is used as a modulator, on cells with different levels of Pgp expression. Therefore, the test may be suitable for clinical studies as an assay of functional Pgp molecules.

6. Having extended our observations to a panel of Pgp substrates and modulators in the ACT assay and found that they may be classified into distinct modalities based on the type of the conformational change they elicit.

7. The antibody competition assay has proved to be a useful tool in dissecting the steps of the catalytic cycle. The data presented here demonstrate its utility as a novel screening method, sensitive uniquely for a subgroup of mdr modulators that happen to be the most effective ones. Using mAb competition to monitor subtle conformational changes of enzymes or receptors may provide a useful approach that could be generally applied in studying receptor-ligand interactions.

PUBLICATION LIST

Publications used in the Thesis

1. **Nagy, H.,** Goda, K., Arceci, R., Cianfriglia, M., Mechetner, E. and Szabó, G.
   P-Glycoprotein conformational changes detected by antibody competition
   Eur. J. Biochem. 268:2416-2420. 2001. **IF: 2.849**

2. **Nagy, H.,** Goda, K., Fenyvesi, F., Bacsó, Zs., Szilasi, M., Kappelmayer, J., Lustyik, Gy., Cianfriglia, M., Szabó, G.
   Distinct groups of multidrug resistance modulating agents are sharply distinguished by competition of P-glycoprotein specific antibodies
   (*under publisher review*)

3. Goda, K., **Nagy, H.**, Mechetner, E., Cianfriglia, M., Szabó, G.
   Effects of ATP depletion and phosphate analogues on P-glycoprotein conformation in live cells
   Eur J Biochem. 269(11):2672-7. 2002. **IF: 2.849**

4. Szabó, G., **Nagy, H.,** Goda, K. Quantitative detection of the conformation changes of P-glycoprotein
(*Patent No.:* 02707028.3-2404-PCT/HU0200025)

Other publications

1. Goda, K., **Nagy, H.**, Bene, L., Balázs, M., Arceci, R., Mechetner, E., Szabó, G. Conformational heterogeneity of P-glycoprotein
Cancer Detect Prev. 24(5):415-21. 2000. **IF: 1.324**

2. Gombos, I., Bacsó, Zs., Detre, C., **Nagy, H.**, Goda, K., Andrásfalvy, M., Szabó, G., Matkó, J.
A Rapid Flow Cytometric Detergent-Resistance Assay (FCDRA) Detecting Raft-Association of Membrane Proteins in Cells (*in process*)

3. Márián, T., Szabó, G., Goda, K., **Nagy, H.**, Szincsák, N., Juhász, I., Galuska, L., Balkay, L., Mikecz, P., Trón, L., Krasznai, Z.
In vivo and in vitro multitracer analyses of P-glycoprotein expression-related multidrug resistance
Eur J Nucl Med Mol Imaging 30(8):1147-54. 2003. **IF: 3.464**

4. Goda, K., Szakács, G., **Nagy, H.,** Szabó, G. and Sarkadi, B.
Flow cytometric functional assaysfor the detection of P-glycoprotein and multidrug resistance-associated protein
In: Practical guide to physical analysis of cell surface receptors. (ed. Krasznai Z. and Mátyus L.)
ISBN: 963 9070 31 9, pp. 110-125, 1998.

5. Goda, K., Szakács, G., **Nagy, H.,** Szabó, G. and Sarkadi, B.
Flow cytometric functional assays for the detection of P-glycoprotein and multidrug resistance-associated protein
In: EMBO conference handbook, "Practical guide to physical analysis of cell surface receptors"
ISBN: 963 9070 31 9, pp.64-92, 1998.