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Research paper

A novel gene delivery system for stable transfection of thiopurine-*S*-methyltransferase gene in versatile cell types

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Abstract

A novel gene delivery system termed artificial viral particles (AVPs) containing a plasmid coding for a recombinant fusion protein of enhanced green fluorescent protein (EGFP) with thiopurine-*S*-methyltransferase (TPMT) was designed for transfection of selected cell lines to establish stable clones which express recombinant EGFP–TPMT protein for further in vitro investigation of toxic effect of thiopurine drugs. Various AVPs based on a complex of the cationic polymer polyethylenimine (PEI) and anionic liposomes were formulated and transfection conditions were adapted in order to transfect the human Jurkat, HepG2 and HEK 293 cell lines. An adequate transfection rate was achieved with AVP containing branched low molecular weight PEI at a PEI:DNA charge ratio of 4.5:1 and liposomes composed of DOPS, DLPE, cholesterol and an activated *N*-glutaryl-DOPE membrane anchor. Stably transfected clones were successfully established and expression of recombinant EGFP–TPMT in homogeneous cell populations was demonstrated by flow cytometry, fluorescence microscopy and immunoblotting. The level of the expressed protein in stable clones was highest in HEK 293, followed by HepG2 and Jurkat. The enzymatic activity of the TPMT moiety was demonstrated by decreased sensitivity to 6-thioguanine and increased sensitivity to 6-mercaptopurine in HEK 293 cells expressing EGFP–TPMT. Formulation of AVP as transfection vector succeeded in establishing human cell lines stably expressing EGFP–TPMT, thereby proving a successful delivery system and providing an initial step to enable investigation of the role of the clinically important drug metabolizing enzyme TPMT.

Keywords: Artificial viral particle; TPMT; Non-viral vector; Transfection; Gene delivery; Plasmid DNA; Nanoparticles; Thiopurine-*S*-methyltransferase

1. Introduction

The introduction of foreign genetic material into target cells is a critical step for gene therapy as well as for basic research. While application of viral vectors leads to high levels of genetically modified cells, their origin predisposes individuals to immune reactions and raises other safety issues [1]. Thus the development of non-viral vectors composed of cationic polymers [2,3] and lipids [4] is despite their inferior effectiveness of great importance. A new generation of non-viral vectors have been designed in an attempt to reproduce the success of natural viruses [5–7].

Artificial viral particles (AVPs) mimic principally the design of some natural viruses, with a condensed core and a lipid envelope [8,9]. They consist of a cationic polymer (polyethylenimine; PEI) that packages the genetic material, in combination with anionic liposomes to imitate the viral envelope. They have already been used successfully to transfect cells with plasmid DNA and siRNA [8,10].

Thiopurine-*S*-methyltransferase (TPMT) is a drug-metabolizing enzyme widely expressed in mammalian and non-mammalian cells [11]. It catalyzes the inactivation of thiopurine drugs such as 6-mercaptopurine (6-MP) and 6-thioguanine (6-TG), which are used in the treatment of various disorders, most notably acute lymphoblastic

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leukaemia [12]. The TPMT gene contains genetic polymorphisms in its coding region [13,14], some of them responsible for non-synonymous amino acid substitutions that lead to faster degradation and consequently lower activity of TPMT [15,16]. Patients with decreased TPMT activity treated with standard doses of thiopurine drugs experience increased formation of active 6-thioguanine nucleotides responsible for cytotoxic effects, which results in severe therapy-related toxicity [17,18]. For patients with clinically relevant mutant TPMT genotypes, doses have to be reduced to 50–100% (for heterozygous) and 6–14% (for homozygous) of the standard dose regimens to minimize unwanted side effects [19–21].

Although the pharmacogenetic and clinical aspects of TPMT have been studied in depth, its endogenous role and endogenous substrate are unknown. TPMT cDNA has been introduced into mammalian cells in attempts to clarify the role of TPMT in thiopurine drug metabolism. Specifically, plasmids coding for TPMT have been stably transfected into CCRF-CEM leukemic cell line by retroviral transduction [22], and into the human embryonic kidney cell line HEK 293 [23].

We have aimed at clarifying the endogenous role of TPMT by transfecting cell lines using AVP containing a plasmid coding for the fusion protein between the C-terminal domain of green fluorescent protein (EGFP) and the enzyme TPMT. Stable cell lines expressing EGFP–TPMT protein have been established in order to explore the role of TPMT in the cell and to monitor and localize the protein (Fig. 1). The present investigation was aimed at establishing a basis for further biochemical research on TPMT and dedicated to experimental testing of a new generation of gene delivery systems not only for transient but also for stable transfection of hard-to-transfect cells.

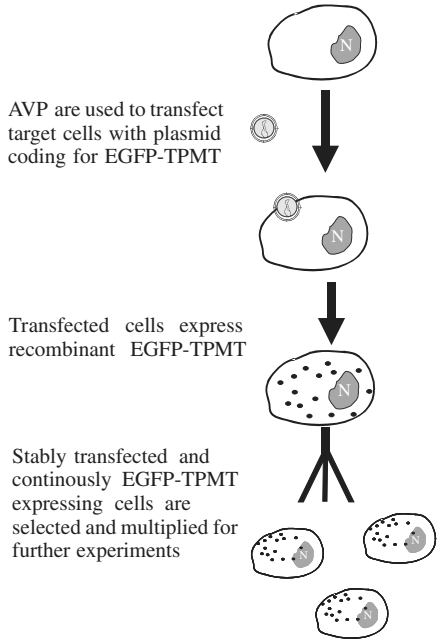

Fig. 1. Schematic presentation of pEGFP–TPMT delivery by AVP to produce stable transfected cells expressing recombinant EGFP–TPMT protein.

2. Materials and methods

2.1. Preparation of artificial viral particles AVP

Liposomes were composed of DOPS (1,2-dioleoyl-*sn*-glycero-3-[phospho-L-serine], *N*-glutaryl-DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*(glutaryl) as (sodium salt)) (both Avanti Polar Lipids Inc. Alabaster, AL, USA), cholesterol (Genzyme, Cambridge, UK) and DLPE (1,2 dilauroyl-*sn*-glycero-3-phosphoethanolamine (research material Sygena, Liestal, Switzerland). Liposomes with the composition DOPS:DLPE:Chol in equal molar ratio were prepared by a thin film method, hydrated by 1 ml sterile Tris buffer (10 mM pH 7. 4), resulting in a total lipid concentration of 10 μmol/ml. The multilamellar liposome suspension was ultrasonicated, extruded through polycarbonate membrane filters with a pore size of 50 nm in a Liposofast mini extruder (Avestin, Ottawa, Canada) and liposome size and size distribution characterized by photon correlation spectroscopy (Zetasizer 3000, Malvern Instruments, UK).

Additionally, liposomes with an anchor of *N*-glutaryl-DOPE, composed of DOPS:DLPE:Chol:*N*-glutaryl-DOPE in molar ratio 3:3:3:1, were prepared as described above. The *N*-glutaryl anchor was in this case activated by incubating with EDC (*N*(-3-dimethyl-aminopropyl-)-*N*′-ethyl-carbodiimide hydrochloride, Merck, Darmstadt, Germany) for 8 h at 37 °C. EDC was removed by column gel chromatography with Sepharose G 25 (Pharmacia). All liposomes were stored at 4 °C after filtration through 0.22 μm filters under aseptic conditions.

The final AVP vectors were prepared freshly prior to each transfection following the previously described procedure [8]. Plasmids coding for EGFP or recombinant EGFP–TPMT (described below) and polyethyleneimine (PEI) – either Lupasol® G100 (BASF, Ludwigshafen, Germany) or jetPEI® (Polyplus San Marco, CA, USA) – were mixed in Tris buffer 10 mM pH 7.4 resulting in a nitrogen:phosphate ratio of 20.7 and a positive:negative charge ratio of ~4.5:1 for standard AVP. After 15 min ripening time, liposomes were added, mixed by vigorous pipetting and, after incubation for 20 min, the resulting particles were used directly for transfection.

2.2. Plasmids and cloning

Human TPMT cDNA from T84 colon carcinoma cells, provided by Dr. Weinshilboum [24], was subcloned into the mammalian expression vector pEGFP-C1 (Clontech Mountain Valley, CA, USA). BglII and PstI restriction sites were created on the 5′- and 3′-ends of the cDNA, respectively, by amplifying TPMT cDNA by polymerase chain reaction, using 5′-ACCGAGATCTGAAACTATG

GATGG-3′ (forward) and 5′-CCTAGCTGCAGCATTTA CTTTTCTGTA-3′ (reverse) primers. The amplified PCR product and plasmid were digested and ligated to obtain the plasmid coding for TPMT protein fused with the C-terminal of EGFP. After *Escherichia coli* transformation, TPMT-positive colonies were selected on the basis of restriction fragment length polymorphism analysis and direct sequencing of the plasmid DNA (data not shown). For the extraction of large quantities of the wild-type pEGFP and pEGFP–TPMT constructs, PureYield plasmid MidiPrep System (Promega) was used according to the manufacturer's instructions. The isolated plasmids were stored at −20 °C.

2.3. Cell culture

HEK 293 and HepG2 cells (American type culture collection ATCC, Manassas, VA, USA) were grown to 60 to 90% confluency in DMEM (Sigma Chemical Co., St. Louis, MO) supplemented with 10% fetal calf serum (Gibco, Grand Island, NY), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin. Jurkat cells (ATCC) were grown as described [25] in RPMI 1640 (Sigma) supplemented with 10% fetal calf serum (Gibco, Grand Island, NY), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 50 μM 2-mercaptoethanol (Sigma). All cells were grown in a humidified atmosphere at 37 °C and 5% CO_2.

2.4. Transient and stable transfection

HEK 293 or HepG2 cells ($5*10^4$ cells/well) were seeded in 24-well plates the day before transfection. Jurkat cells ($5*10^5$ cells/well) were centrifuged and re-seeded in fresh media directly before transfection. Transfection was carried out using an AVP sample containing 1 or 2 μg DNA per well in normal cell growth media, including 10% fetal calf serum unless stated otherwise. Six hours after transfection, the medium of the attached HEK 293 or HepG2 cells was changed. RPMI 1640 complete growth media were added to Jurkat cells, with or without phorbol 12-myristate 13-acetate (PMA, Sigma), resulting in a final concentration of 25 ng PMA/ml. Twenty-four hours after transfection an additional 500 μl RPMI 1640 was added to the Jurkat cells.

For stable transfection, cells (0.2 to $1*10^6$) were transfected as described above. Twenty-four hours after transfection, the standard growth medium was changed to medium containing 1.0 mg/ml of the selection agent G418 (Geneticin®, Invitrogen, Carlsbad, CA, US) and incubated for ~1 week. Surviving cells were pelleted and re-seeded into 96-well plates in medium containing 500 μg G418/ml. Discrete colonies were picked after 1–2 months, regrown, and tested for expression of green fluorescent protein by flow cytometry. A maintenance concentration of 500 μg G418/ml was used for the growth of stably transfected clones.

2.5. Flow cytometry

HepG2 cells were detached by trypsinization (Trypsin 0.05%, EDTA 0.02%, ~3 min), HEK 293 by severe pipetting and Jurkat cells harvested directly from suspension. Cells were pelleted, washed, resuspended in phosphate-buffered saline (pH 7.4) and stored on ice until measurement on an Epics® Altra (Beckman Coulter, Fullerton, California). Forward and sideward light scattering of $2*10^4$ events were plotted. Green fluorescence (525 nm FITC detector) of cells was measured and displayed in a histogram. A threshold for transfected green fluorescent cells was set in the histogram at less than 1%, negative control cells being false positive.

2.6. Microscopy

Cells were grown on coverslips and fixed with 4% formaldehyde solution. Nuclei were stained with Hoechst 33342 (Sigma). Pictures were taken in z-stacks using the 60-fold objective on an Olympus IX 81 fluorescence microscope (Olympus, Tokyo, Japan), using its Disc Scanning Unit (DSU) to avoid out of focus stray light, and processed using CeîlR Software (Olympus).

2.7. Immunoblotting

Cells were lysed, sonicated in ice-cold TNE lysis buffer (1% Triton X-100, 50 mM Tris–HCl, pH 8, 115 mM NaCl, 2 mM EDTA) and centrifuged (12,000*g*, 4 °C). The supernatant was stored at −80 °C until further use. Total protein content was determined using the Bio-Rad DC Protein Assay (Biorad, Hercules, CA, US) according to the manufacturer's instructions. Equal amounts of protein were loaded on 10% polyacrylamide gels and separated by gel electrophoresis (SDS–PAGE). Proteins were transferred to a nitrocellulose membrane using electro-transfer. After incubation in blocking solution (5% non-fat dry milk, 0,2% Tween 20, 25 mM Tris, 137 mM NaCl, 3 mM KCl, pH 7.4) for 1 h at room temperature, the membrane was incubated at 4 °C overnight with an anti-TPMT monoclonal antibody (Abnova, Taipei, Taiwan) diluted to 2 μg/ml, washed 3 times in TTBS (0,2% Tween 20, 25 mM Tris, 137 mM NaCl, 3 mM KCl, pH 7.4) and incubated for 1 h at room temperature with HRP-conjugated goat anti-mouse IgG antibody (0.7 μg/ml) (Upstate, Lake Placid, NY, US). After washing, chemiluminescence was activated using Lumiglo® (Upstate, Lake Placid, NY, US) and the bands were visualised on an X-ray film (Sigma). To detect β-actin, the membranes were stripped and re-blotted with an anti-β-actin monoclonal antibody (Sigma) following the procedure described above.

2.8. Metabolic activity

Following treatment with 6-MP or 6-TG (all from Sigma) the metabolic activity of transfected and non-trans-

fected cells was tested using the Cell titer 96® Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI, US) according to the manufacturer's instruction. Cells were seeded in 96-well plates, treated with the chemicals of interest or pure media as control. After 48 h, the supplied tetrazolium compound was added to the media (1:10), incubated for 2 h and absorbance of the formazan product was measured at 490 nm on a plate reader (Tecan, Mannedorf/Zürich, Switzerland).

3. Results and discussion

3.1. Optimization of artificial viral particles and transfection conditions for target cells

Success of gene delivery depends on the compatibility of vector and cell. The composition of AVP was optimized for transfection of HEK 293 kidney cells, HepG2 liver carcinoma cells and the T-lymphoma cell line Jurkat, all derived from organs expressing TPMT [11,26–28]. Effects of changes in AVP composition and transfection conditions were assayed by transfection of cells with MOCK EGFP plasmid coding for wild-type EGFP.

While gene delivery to embryonic kidney cells (HEK 293) is relatively easily achieved, lymphocytes are particularly difficult to transfect [29]. Nevertheless, lymphoblasts represent an important target since they not only express endogenous TPMT [30] but are the site of action of thiopurine drugs [31]. Different AVP formulations were thus tested on Jurkat cells, while HEK 293 cells served as a control.

First, cell-specific optimization of AVP composition employed the charge ratio of the positively charged polyethylenimine (PEI) reagent used to condense the DNA to that of the DNA. In previous studies AVPs, with a PEI/DNA charge ratio of 4.5:1, have been successfully applied on endothelial and primary neuron cells [8–10]. This charge ratio led repeatedly to successful transfection on Jurkat and HEK 293 cells. In preliminary experiments either decrease or increase of the charge ratio (2.25:1 or 6.75:1), respectively, led to lower transfection efficiency in Jurkat cells, but only slightly affected it in HEK 293. Second, we have evaluated the use of jetPEI®, a PEI based transfection reagent consisting of a linear polyethylenimine derivative, instead of the low molecular weight branched PEI (Lupasol® G100). DNA condensation by jetPEI®, followed by standard processing with liposomes to produce AVP, resulted repeatedly in greater transfection of HEK 293 cells than using low molecular weight branched PEI. In Jurkat cells the same exchange of PEI led to a decrease of transfection rate from $50.59 \pm 18.73\%$ to $6.10 \pm 0.43\%$ transfected cells.

In a further step the anionic liposome composition has been varied. AVPs derived from anionic liposomes (DOPS, DLPE and Chol) were compared to AVPs derived from liposomes containing, in addition, *N*-glut-DOPE which functions as a membrane anchor and has been used to couple proteins such as antibodies to liposomes after activation with a carbodiimide [32]. The latter liposomes largely increased the transfection rate in all cell lines, most notably in Jurkat cells (Fig. 2).

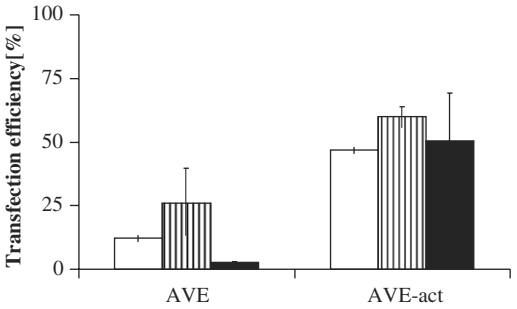

Fig. 2. Transfection efficiency of AVP based on normal AVE liposomes and AVE-act on HepG2 (white bars), HEK 293 (striped bars) and Jurkat (filled bars) cells. AVPs based on AVE-act were superior on all three cell lines. Transfection efficiency is expressed as percentage of EGFP positive cells as determined by flow cytometry (mean ± SD, $n = 3$).

A satisfactory transfection rate of Jurkat cells was only achieved with AVP containing branched low molecular weight PEI at a PEI/DNA charge ratio of 4.5:1 and liposomes including an activated *N*-glutaryl-DOPE membrane anchor. This composition was therefore used for transfection in all further experiments. The resulting mean hydrodynamic diameter of AVP was in the range of 100 to 200 nm. The role of the activated anchor in anionic liposomes is not clear, but could be attributed to higher binding of the liposomes to cell surfaces [33]. Such non-specific interaction may increase the internalization of AVP into cells, and present an advantage for *in vitro* experiments.

Further optimization involved transfection conditions, including cell density and AVP concentration. No differences in transfection rates were observed when suspended Jurkat cells were seeded at densities of 0.25 to $1*10^6$ cells. While on HEK 293 cells transfection rate was repeatedly highest at an AVP dose of 2 μg DNA/well, transfection rate of Jurkat cells reached already the maximum at 1 μg DNA/well ($50.59 \pm 18.73\%$) and did not profit from an increase to 2 μg ($53.32 \pm 1.87\%$).

3.2. Expression of recombinant fusion EGFP–TPMT protein and establishment of stable expressing cell lines

Cells were transfected by AVP carrying a plasmid construct coding for the fusion protein EGFP–TPMT. Successful transfection and expression of EGFP–TPMT in initial experiments was monitored as fluorescence intensity of the EGFP and by specific anti-TPMT antibodies.

To establish stable cell lines expressing the recombinant protein constitutively, Jurkat, HEK 293 and HepG2 cells were transfected with plasmids coding for the recombinant EGFP–TPMT fusion protein and for EGFP as control (MOCK). Stable clones were selected utilizing the G418-resistance determinant (Neo^R) encoded by the plasmids. The degree of expression of the recombinant protein in

selected clones was determined by measuring intensity of EGFP emitted fluorescence using flow cytometry. Cell populations showing monoclonal characteristics and high fluorescence intensity were selected (Fig. 3). HEK 293 clones displayed the highest fluorescence levels, followed by HepG2 and Jurkat clones. Fluorescence intensity of transfected HEK 293 clones exceeded the average detection range. Therefore, intensity profiles of HEK 293 are depicted on a fractured log scale (Fig. 3, left panel).

Selected clones were also tested for expression of the recombinant fusion EGFP–TPMT protein by immunoblotting. Cell proteins were separated by SDS–PAGE, transferred to membranes and immunoblotted with anti-TPMT antibodies, with β-actin as loading control (Fig. 4). In clones stably transfected with p-EGFP–TPMT, the presence of a ~56 kDa expressed recombinant protein, corresponding to the fusion of ~27 kDa EGFP and ~29 kDa TPMT, was detected. The expression was higher in HEK 293 and HepG2 than in Jurkat. The weak band of 58 kDa in the control and MOCK samples, which was also detected in anti-TPMT immunoblots, could represent a dimer of endogenous TPMT, recently observed in similar experimental conditions by Wu et al. [14], or else it could be the consequence of non-specific TPMT-antibody binding. Endogenous TPMT at ~29 kDa was also detected and appeared to be at the highest level in HepG2 cells.

Stably transfected and monoclonal cell populations derived from all three cell lines transfected with AVP were characterized by flow cytometry and immunoblotting. Detected expression levels of recombinant protein correspond to fluorescence intensity of different clones. In all three cell lines, the fluorescence intensity of MOCK cells expressing EGFP was higher than for cells expressing recombinant EGFP–TPMT. This could be explained perhaps by altered conformation of EGFP in the recombinant fusion EGFP–TPMT protein.

3.3. Intracellular localisation of recombinant EGFP–TPMT

To detect the possible effect of expression of TPMT on cell morphology, stably transfected cells, expressing the recombinant protein, were examined by fluorescence microscopy. In parallel, MOCK control cells expressing EGFP were monitored to differentiate between TPMT-specific effects and those due to the expression of EGFP or to the transfection and selection conditions.

Stably expressing clones of HEK 293 and HepG2 showed high fluorescence intensity (Fig. 5), while the fluorescence of Jurkat clones was relatively weak. Furthermore, the fluorescence signal was distributed evenly across all cellular compartments, including the Hoechst-stained nucleus, indicating that the expression of the recombinant protein is not restricted to a single compartment or organelle, but was detected both in cytoplasm and nucleus. Changes in growth rate or structural integrity of HEK 293 and HepG2 cells expressing recombinant EGFP–TPMT were not observed. Jurkat cells with stable EGFP–TPMT expression showed a lower tendency to grow in clusters and were bigger in size than non-transfected cells. However, a similar effect was observed in Jurkat

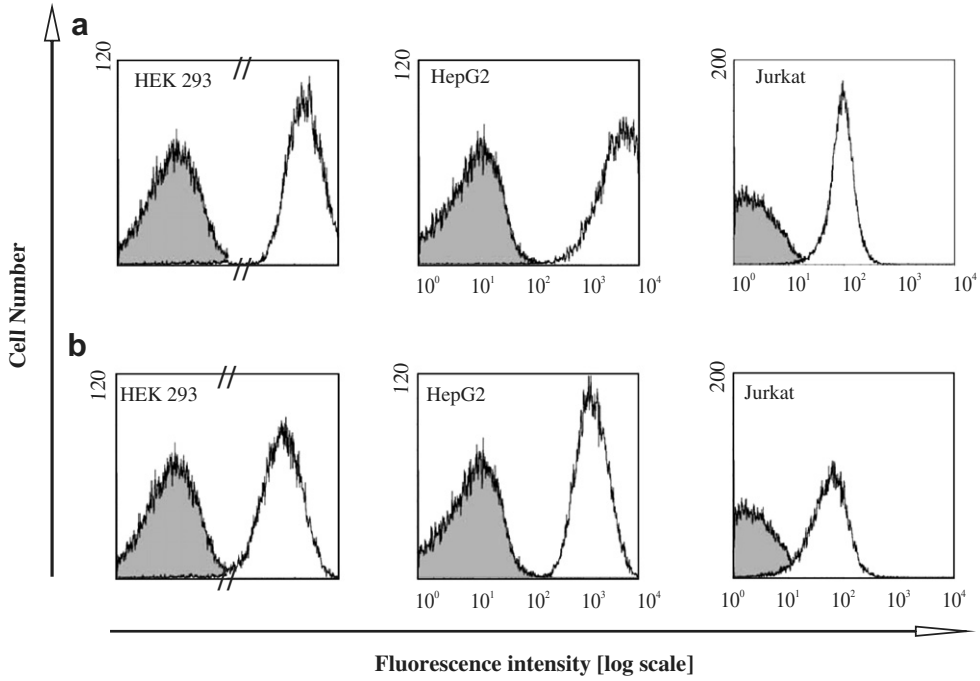

Fig. 3. Selection of stably expressing clones. Flow cytometry histograms of green fluorescence of selected stable clones expressing either green fluorescent protein EGFP (a), or recombinant EGFP–TPMT protein (b). The selected stable clones show homogeneous populations of cells with intensive fluorescence compared to the weak autofluorescence of untransfected cells (grey). Intensity of stable transfected HEK 293 cells exceeded average detection range and is therefore depicted on a fractured log scale.

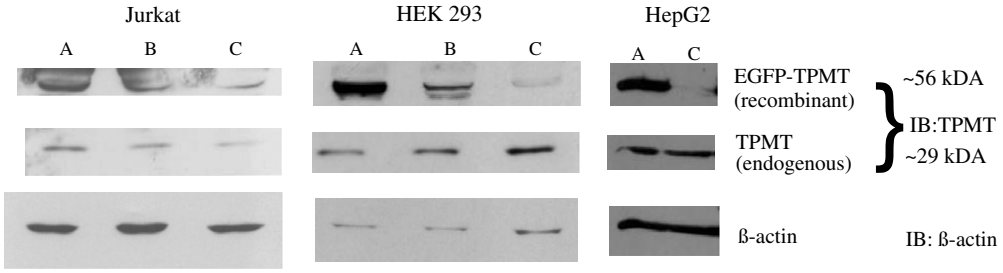

Fig. 4. Immunodetection of recombinant EGFP–TPMT. Immunoblots of selected clones detecting recombinant EGFP–TPMT and endogenous TPMT expression levels in (A) stable EGFP–TPMT transfected clones, (B) stable EGFP transfected clones (MOCK) and (C) non-transfected cells. Secondly expression of the endogenous housekeeping protein β-actin is compared on the same membranes.

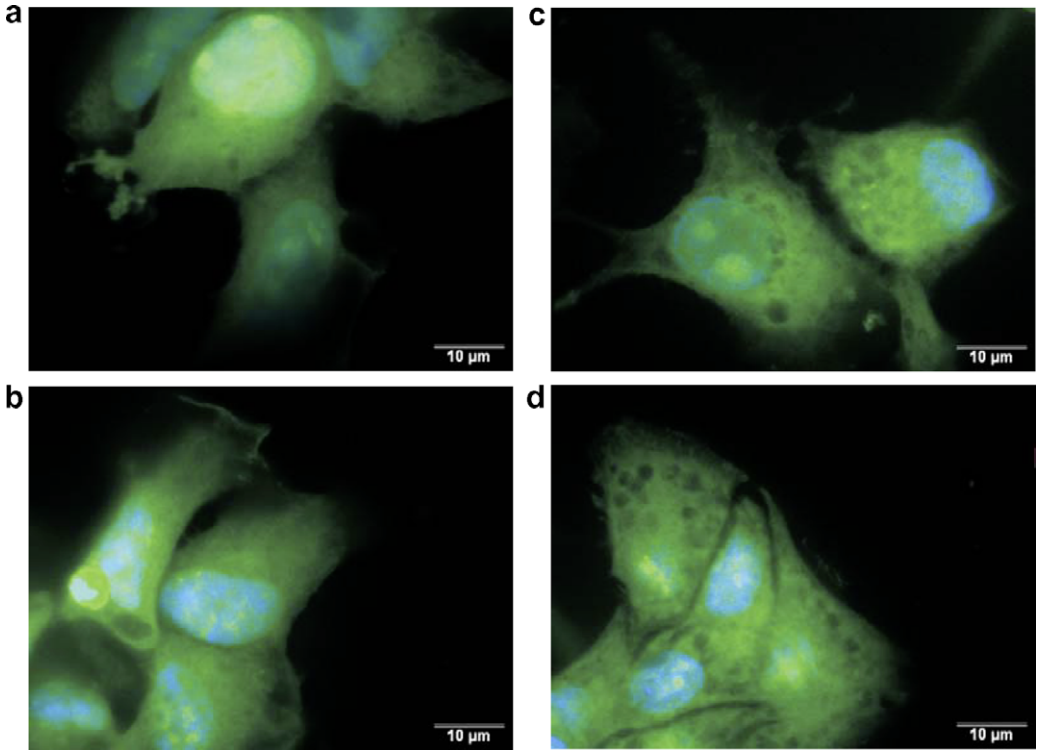

Fig. 5. Indetermined expression of EGFP–TPMT protein. Green fluorescence of HEK 293 clones stably expressing EGFP (a) or the recombinant EGFP–TPMT (b) and HepG2 clones also stable expressing EGFP (c) or EGFP–TPMT (d) observed with 60-fold objective in fluorescence microscopy. Nuclei were additionally stained blue.

MOCK control cells and can therefore be attributed to transfection and/or selection conditions. The observed lack of a TPMT-specific influence on the morphological appearance of stable transfected cells is in accordance with earlier findings by Dervieux et al. who also did not observe changes of phenotype [22].

3.4. Influence of TPMT expression on sensitivity to thiopurine drugs

To investigate whether the TPMT moiety of the expressed EGFP–TPMT is enzymatically active we assayed the sensitivity of cells to thiopurine drugs. Stable cell lines and non-transfected cells were incubated with 6-mercaptopurine (6-MP) or 6-thioguanine (6-TG) for 48 h. While incubation of both non-transfected and stable MOCK transfected HEK 293 cells with 6-TG (5 μM) resulted in reduction of cell proliferation to 30–40% of the control value, EGFP–TPMT HEK 293 clones were resistant to the thioguanine assault (Fig. 6a). In contrast incubation of HEK 293 cells expressing EGFP–TPMT with 6-MP (10 μM) led to a decrease in metabolic activity of approximately 50%, while similar treated MOCK and non-transfected HEK 293 cells showed only a slight decrease in proliferation (Fig. 6b).

The differing responses of EGFP–TPMT expressing HEK 293 cells to 6-MP and 6-TG are in accordance with observations of Coulthard et al. and Dervieux et al. [22,23]. They reported that the decreased cell sensitivity to 6-TG and increased sensitivity to 6-MP of both HEK

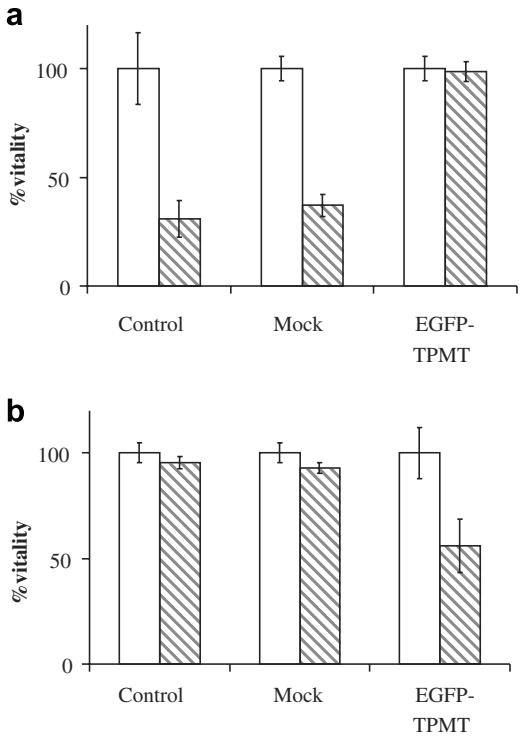

Fig. 6. Influence of expression of EGFP–TPMT in HEK 293 cells on the sensitivity to thioguanine drugs. Metabolic activity of untransfected (Control), stable GFP transfected (Mock) and stable EGFP–TPMT transfected (EGFP–TPMT) HEK 293 cells was measured after incubation with 5 μM 6-thioguanin (a) or 10 μM 6-mercaptopurin (b) for 48 h by MTS assay. Bars represent the percentage of vitality of treated cells (stripes) compared to untreated control cells (empty) and show the average of 3 wells of an representative experiment. Error bars depict coefficient of variation in %.

293 and CCRF-CEM cells with stable TPMT expression are the consequence of the different metabolic pathways of the two drugs. In the case of 6-TG, the decreased cell sensitivity is a result of more extensive detoxification of the drug by TPMT-mediated *S*-methylation. On the other hand, the metabolic pathway of 6-MP involves additional conversion catalyzed by TPMT that is responsible for increased cell sensitivity. The expressed enzyme namely causes the production of higher amounts of *S*-methyl-thioinosine 5′-monophosphate, a metabolite exerting its antiproliferative effects by inhibition of *de novo* purine synthesis, the main mechanism of action of 6-MP in stable TPMT overexpressing cells.

The observed changes in sensitivity of HEK 293 cells in our experiments are thus consistent with previous observations and indicate that the TPMT moiety of the expressed EGFP–TPMT protein can actively catalyze thiopurine *S*-methylation reactions.

4. Conclusion

Artificial viral particles were successfully modified and used to express recombinant EGFP–TPMT protein in Jurkat, HepG2 and HEK 293 cells. Selection of stably transfected clones resulted in homogeneous populations of cells expressing recombinant protein detected by anti-TPMT immunoblotting and by emitted fluorescence. HEK 293 clones expressing recombinant EGFP–TPMT displayed modified sensitivity to thioguanine drugs compared to non-transfected cells, indicating that the TPMT moiety is enzymically active. The potential of using AVP in molecular and cell biology studies was demonstrated, providing a valuable beginning of detailed research on the biochemistry and function of the TPMT enzyme involved in thiopurine drug metabolism.

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