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The Role of Transporters in Future Chemotherapy

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Abstract: The expression of membrane transporter is often altered in cancer cells compared to their corresponding healthy cells. Since these proteins, classified into solute carriers (SLCs) and ATP-binding cassette (ABC), can carry not only endogenous compounds, nutrients, and metabolites, but also drugs across the cell membranes, they have a crucial role in drug exposure and clinical outcomes of chemotherapeutics. Curiously, up-regulation of SLCs can be exploited to deliver chemotherapeutics, their prodrugs, and diagnostic radiotracers to gain cancer cell-selective targeting, as exemplified with L-type amino acid transporter 1 (LAT1). SLCs can also be inhibited to limit the nutrient uptake of cancer cells and thus, cell growth and proliferation. Furthermore, LAT1 can be utilized to deliver ABC-inhibitors selectively into the cancer cells to block the efflux of other chemotherapeutics suffering from acquired or intrinsic efflux transport-related multidrug resistance (MDR). Taking into account the current literature, compounds that can affect up- or down-regulation of transporters in a cancer cell-selective manner could be a valuable tool and promising chemotherapy form in the future.

Keywords: ATP-binding cassette (ABC) · L-type amino acid transporter 1 (LAT1) · multidrug resistance (MDR) · prodrug · solute carrier (SLC)

Introduction

Cancer is one of the leading causes of death globally and it affects millions of people every year worldwide.[1] Although cancer therapies have greatly developed in the last few decades, some unsolved issues have remained, such as non-specific targeting causing side effects in the healthy cells, and the ability of cancer cells to develop multi-drug resistance (MDR) against chemotherapeutics.[2] Endogenous membrane transporters, solute carriers (SLCs) and ATP-binding cassette (ABC), have a major role in both above-mentioned cases.[3,4] SLC can carry essential substances and nutrients not only into the cancer cells but also into corresponding healthy cells. As they can also carry drugs, selective delivery and targeting of chemotherapeutics into the cancer cells is a great challenge.[4] Contrarily, ABC transporters are responsible for pumping toxins, including chemotherapeutics and other drugs, out of the cells.[3] Importantly, they are considered one of the main MDR mechanisms in cancer chemotherapy.[2] Therefore, both SLCs and ABC transporters, and their functional modulation are promising targets in future drug research and development.

MDR can be classified as either intrinsic or acquired chemoresistance.[1,5] In intrinsic resistance, the mechanisms diminishing the effects of the anti-cancer agents already exist in the cancer cells, while in acquired resistance, the cancer cells develop different mechanisms to decrease the effects of chemotherapeutics during the treatment. For example, cancer cells can avoid apoptosis by mutating the apoptotic factors, or by increasing the repair of damaged DNA.[6] Since many traditional anti-cancer agents aim to damage DNA that can lead to apoptosis, these mutated mechanisms can effectively inhibit their action. Cancer cells may also develop mechanisms to inactivate anti-cancer agents (e.g., increased enzymatic metabolism). However, with a prodrug approach, in which the inactive prodrugs are needed to be bioconverted to their active species, increased enzymes activity may be exploited in cancer-targeting. Lastly, cancer cells can also alter complex processes that are related to the proteins and their signaling pathways (inactivation), which the anti-cancer agents are supposed to attack. These changes in the target pathways can, in turn, decrease the chemotherapeutic efficacy. Nevertheless, MDR caused by increased expression of ABC proteins is one of the most common mechanisms and it has a significant role in the clinical outcome of chemotherapy.[1, 2] Thus, in this mini-review roles of SLCs and ABC transporters in future drug development of chemotherapies are discussed.

Possibilities and Challenges of Solute Carriers (SLCs) in Chemotherapy

SLC transporter superfamily includes more than 400 members that are classified to date into 65 subfamilies.[7] Although the responsibility of SLCs is to transport various essential substances, such as sugars, amino acids, neurotransmitters, and vitamins across the plasma membranes, they also have a crucial role in the absorption and distribution of different drugs.[8] SLCs share several common structural and functional features, despite their diversity; *e.g.*, most of them have 7-14 flexible transmembrane domains (TMDs).[9] Majority of SLCs also mediate passive facilitative or secondary active transport, which is energy-independent. Some SLCs have quite strict substrate specificities, while others can accept a wide variety of different compounds. Therefore, the substrate specificities can overlap among different SLC members.[8,9]

SLCs have a significant role also in the delivery of anti-cancer drugs across the plasma membranes, and therefore, they are major determinants of the pharmacological response to these compounds.[4] Numerous SLCs are expressed relatively ubiquitously throughout the body, and therefore targeting via specific transporters may be a great challenge. However, in many different types of cancer cells, specific SLCs can be highly overexpressed, creating a possibility to utilize these transporters for cancer cell-targeted drug delivery.[4] Although the targeting efficacy in the cancer cells *versus* healthy

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cells will not be 100%, the proportions of anti-cancer agents in the cancer cells can be greatly enhanced, while the amounts of them in the healthy cells can be simultaneously decreased. For example, the expression of organic anion transporting polypeptide 1A2 (OATP1A2, *SLCO1A2*, which is a transporter that can carry a wide range of anti-cancer agents among other drugs, including atrasentan, chlorambucil taurocholate, docetaxel, imatinib, and methotrexate, is upregulated in several cancers, (breast, pancreas, bone, and some lung cancer cell lines).[10] More importantly, OATP1A2 expression has been detected on the plasma membrane of breast carcinoma cells but not in the adjacent non-cancerous cells.[11] Thus, these kinds of differential expression profiles could be exploited in novel chemotherapeutic approaches to selectively destroy the cancer cells, while minimizing the damage to healthy cells. However, the expression profiles may vary among patients and thus, successful personalized therapy requires a detailed understanding of pharamcoproteomics, *i.e*, how the transporter expression differences affect drug pharmacokinetics and selective distribution.

Curiously, transporters can also act as tumor suppressors or promoters in cancer cells.[12] Those transporters that carry essential nutrients, such as SLCs, are often up-regulated and therefore, they can also serve as tumor promoters to fulfill the increased needs for nutrients. These examples include a well-known glucose transporter 1 (GLUT1, *SLC2A1*) and L-type amino acid transporter 1 (LAT1, *SLC7A5*) to name a few (Table 1). On the other hand, those transporters that carry the produced metabolites and thus, can remove the excess of cancer cells' waste products, are often tumor suppressors. So far, at least three SLC proteins have been identified as tumor suppressors (Table 1) [13-15]. Therefore, methods and compounds that could downregulate the activity of tumor-promoting transporters and on the other hand, upregulating tumor growth suppressing transporters may serve as novel chemotherapy in the future. Moreover, multiple signaling mechanisms have been identified that could be exploited for transporter expression regulation, including oncogenic protein c-MYC, hypoxia-inducible factor 1α, mammalian target for rapamycin (mTOR), and histone deacetylases, depending on the transporter and its localization.[12]

Role of L-Type Amino Acid Transporter 1 (LAT1) in Anti-Cancer Therapy

L-Type Amino Acid Transporter 1 (LAT1, SLC7A5) that forms a heterodimeric complex with type II membrane glycoprotein 4F2hc (SLC3A2) via a disulfide bond is a pH- and sodium-independent SLC transporter.[16,17] LAT1 carries essential L-type large and neutral amino acids, such as L-leucine, L-tyrosine, and L-phenylalanine, but it also transports thyroid hormones (T3 and T4) and some amino acid-mimicking drugs, like the antiparkinsonian L-dopa and anticonvulsant gabapentin.[18] LAT1 functions as an antiporter as it exchanges the extracellular substrate for an intracellular amino acid, such as L-glutamine that is in turn transported into the cells via some other transporter, often via sodium-dependent neutral amino acid transporter 1 or 2 (ASCT1, SLC1A4 or ASCT2, SLC1A5). Therefore, LAT1 is a secondary active transporter. It is mainly found in the brain, testis, placenta, and bone marrow and its expression profile correlates with the high demand for amino acids.[16,19] Noteworthy, it is expressed at the apical (luminal) as well as basolateral (abluminal) sides of endothelial cells of the blood-brain barrier (BBB).[17,20]. Moreover, LAT1 expression has also been detected in brain parenchymal cells, including neurons, astrocytes, and microglia, and therefore, it is a suitable carrier for brain drug delivery of compounds.[21] Curiously, LAT1 is also overexpressed in various cancer cell types including breast, lung, and prostate cancers to support the high need for building blocks for protein synthesis that guarantees continuous growth and proliferation.[19,22] Therefore, LAT1 has been widely studied as a promising target to be inhibited in cancer starvation therapy as well as in targeted and increased delivery of chemotherapeutics and diagnostic markers.

LAT1-inhibitors, such as BCH (2-aminobicyclo[2.2.1] heptane-2-carboxylic acid) and JPH203 have been studied as anti-cancer agents that could reduce cell growth and proliferation (Figure 1).[23] Unfortunately, neither BCH nor JPH203 are selective LAT1 inhibitors over other transporters and therefore, relatively high amounts of BCH are needed to achieve antiproliferative effects, and interactions of JPH203 with OATs and OATPs have raised questions about its off-target effects.[24] However, JPH203 has already proceeded to clinical trials in Japan,[25] although a structural optimization of JPH203 could yield a more selective LAT1-inhibitor with greater chances to be commercialized as a novel anti-cancer agent. In addition to these inhibitors, irreversible LAT1-inhibitors 1,2,3-dithiazoles have been reported (Figure 1).[26]

We have also serendipitously created a LAT1-inhibitor that structurally resembles JPH203. This inhibitor (KMH-233) is a LAT1-selective, slowly reversible inhibitor (Figure 1) and it has potentiated antiproliferative efficacy in breast cancer cells (MCF-7) together with DNA-damaging agent, cisplatin, and protease inhibitor, bestatin, via reduction of mTOR and NF-κB signaling pathways.[27] However, we have concluded that LAT1 inhibition as a single medication is not likely to be sufficient as chemotherapy, since the deprived cancer cells can develop other compensating routes for amino acid supply. For example, via autophagy and ubiquitin–proteasome pathway, peptides are degraded into amino acids by intracellular aminopeptidases. Therefore, LAT1-inhibitor KMH-233 together with protease inhibitor bestatin will most likely result in greater clinical outcomes than sole LAT1 inhibition, as demonstrated by our pre-clinical data.

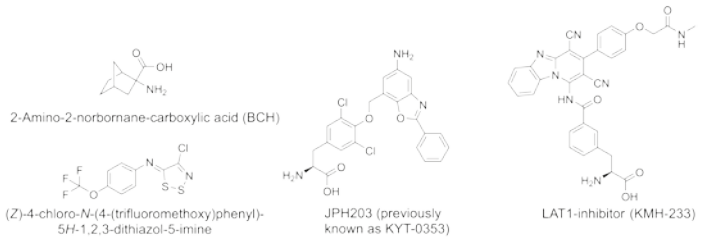

Figure 1. Chemical structures of reported LAT1-inhibitors.

Creating an amino acid-mimetic drug without losing the potency of the drug for its final target can be very challenging. Therefore, the prodrug approach can serve as a potential fine-tuning method to improve the delivery and targeting properties of compounds. However, a successful LAT1-substrate requires the presence of amino as well as carboxylic acid groups, in addition to a large neutral side group, such as an aromatic ring.[18,28,29] Therefore, promoieties that are favorable to be attached to the active parent drug include *e.g.*, L-Phe and L-Trp amino acid derivates, although other attempts have also been proposed, however without proper evaluation of LAT1-mediated transport. Curiously, it has been long thought that LAT1 is mainly stereoselective, preferring L-amino acids, but recently it has been shown that it can also transport D-enantiomers.[28,30]

Interestingly, several brain-targeted LAT1-utilizing prodrugs of neuroprotective agents have been reported to date, but not so many cancer-targeted LAT1-utilizing prodrugs of chemotherapeutics. Anti-cancer and alkylating agent, melphalan, is a known LAT1-substrate, however, it is not a prodrug. One

Table 1. Transporter proteins that can function as tumor promotors or suppressors with their literature references.

SLC short name / Gene name	Protein full name	Function in tumors
ASCT2, SLC1A5	Sodium-dependent neutral amino acid transporter 2 (for alanine, serine, and cysteine,	Promoter [12]
GLUT1, SLC2A1	Glucose transporter 1	Promoter [12]
SGLT1, SLC5A1	Sodium-coupled concentrative glucose transporters 1	Promoter [12]
SGLT2, SLC5A2	Sodium-coupled concentrative glucose transporters 2	Promoter [12]
$ATB^{0,+}$, SLC6A14	Sodium- and chloride-dependent neutral and basic amino acid transporter B(0+)	Promoter [12]
LAT1, SLC7A5	L-Type amino acid transporter 1	Promoter [12]
NHE1, SLC9A1	Sodium/hydrogen exchangers 1	Promoter [12]
NHE3, SLC9A3	Sodium/hydrogen exchangers 3	Promoter [12]
xCT, SLC7A11	Sodium-independent cystine/glutamate antiporter	Promoter [12]
MCT1, SLC16A1	Monocarboxylate transporter 1	Promoter [12]
MCT4, SLC16A3	Monocarboxylate transporter 4	Promoter [12]
SNAT2, SLC38A2	Sodium-coupled neutral amino acid transporters 2	Promoter [12]
SNAT5, SLC38A5	Sodium-coupled neutral amino acid transporters 5	Promoter [12]
SMCT1, SLC5A8	Sodium-coupled monocarboxylate transporter 1	Suppressor [13]
CLD, protein DRA, SLC26A3	chloride anion exchanger	Suppressor [15]
ZIP1, SLC39A1	zinc transporter	Suppressor [14]

of the few LAT1-utilizing prodrug examples is the L-aspartate derivative of doxorubicin (Figure 2).[31] Due to the aromatic nature of doxorubicin itself, L-aspartic acid is sufficient to fulfill structural requirements for LAT1. Moreover, LAT1 is known to deliver L-borono-phenylalanine (L-BPA) (Figure 2) into brain tumors in the boron neutron capture therapy (BNCT). In BNCT, L-BPA (^{10}B) containing cancer cells are irritated with low energy thermal neutron beam, which produces a fission reaction of ^{10}B (L-BPA) that yields high-energy α-particles (^{4}He) and ^{7}Li.[32] This selectively destroys cancer cells without affecting non-boron-containing healthy cells. In addition to these, second generation's positron emission tomography (PET) -tracers, including, *O*-(2-[^{18}F]-fluoroethyl)-L-tyrosine ([^{18}F]-FET) and 3-[^{18}F]-fluoro-α-methyl-L-tyrosine ([^{18}F]-FMT) (Figure 2), have reported to utilize LAT1 for their cancer cell accumulation.[33] Before these LAT1-utilizing tracers, 2-deoxy-2-[^{18}F]-fluoro-D-glucose ([^{18}F]-FDG) was more commonly used as a tool in the diagnosis of cancers. [^{18}F]-FDG instead utilizes GLUT1 for its cellular accumulation that is also upregulated in several different cancer cell types, as mentioned earlier. However, utilizing GLUT1 for PET suffers also from complete cancer cell targeting efficacy over healthy cells. As LAT1 has been seen as a more selective carrier, several other LAT1-utilizing radiotracers for PET diagnostics of cancers have been reported after [^{18}F]-FET and [^{18}F]-FMT development.

Since LAT1 has an important role in amino acid homeostasis, its utilization for targeted/improved drug delivery must not disturb these vital functions. We have previously shown that inhibiting LAT1 at the BBB by the slowly reversible inhibitor (KMH-233, Figure 1) does not affect the brain amino acid homeostasis or modulate the function of LAT1 on the cell surface.[34] However, the situation can be *vice versa*; food that contains high amounts of amino acids that are transported via LAT1 and therefore high plasma concentrations (millimolar concentrations) of these amino acids can also compete for the interaction with LAT1-utilizing compounds or inhibitors. Moreover, there are also overlapping substrate specificities among LAT1 and other amino acid transporters and it is well known that there are species differences in LAT1 expression, *e.g.*, between humans and rodents. Therefore,

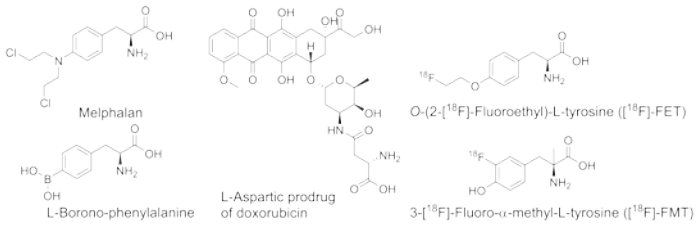

Figure 2. Chemical structures of anti-cancer agents melphalan, L-borono-phenylalanine, and L-aspartate prodrug of doxorubicin, as well as radio-tracers O-(2-[18F]-fluoroethyl)-L-tyrosine ([18F]-FET) and 3-[18F]-fluoro-α-methyl-L-tyrosine ([18F]-FMT).

the design and development of LAT1-utilizing prodrugs must be carried out with caution. Interestingly, since efficient and selective ABC-inhibitors are in great demand in cancer chemotherapy, LAT1 has the potential to be utilized in targeted drug delivery of ABC-inhibitors that are discussed in the next chapter.

Possibilities and Challenges of ABC Transporters in Chemotherapy

ABC proteins, contrary to SLCs, comprise a well-known superfamily with 48 transporter members classified into seven subfamilies.[2,35,36] All ABC transporters have two cytoplasmic nucleotide-binding domains (NBDs), whose function and structures share similarities across all subfamilies. In addition, ABC proteins have two TMDs, which in turn are more heterogenous and therefore distinct transporters have different substrate specificities. ATP is required for the function of ABC transporters and therefore they are considered to be energy-dependent proteins. The binding sites of ATP are in the NBDs, whereas the substrates bind to the TMD. The substrate is released from the transporter protein after hydrolysis of ATP.

The most studied ABC transporters in cancer cells are P-glycoprotein (P-gp, *ABCB1*), breast cancer-resistant protein (BCRP, *ABCG2*), and multidrug-resistant protein 1 (MRP1, *ABCC1*). Numerous anti-cancer agents have been identified as substrates of at least one of these efflux transporters.[2,35] P-gp, MRP1, and BCRP share substrate specificities, particularly with neutral and positively charged hydrophobic compounds.[36] In addition, these transporters are upregulated in numerous cancer types and their increased expression correlates with poor prognosis. Moreover, their increased expression has been demonstrated to be followed by chemotherapy.[37]

Thus, compounds that could either inactivate or block ABC transporters have been developed for a few decades now in attempts to increase the amounts of other conventional chemotherapeutics in the cancer cells. Examples of the "first-generation" P-gp modulators were verapamil and quinine (later also found to inhibit other ABC transporters) (Figure 3).[37] Unfortunately, these compounds showed efficacy in pre-clinical trials but were not effective enough or showed off-target toxicity in clinical trials.[2] The further developed "second-generation" structural analogs, *e.g.*, for verapamil proved to be more potent and less toxic in combinations with chemotherapeutics.[37] Nevertheless, these compounds had issues with inhibition of hepatic and intestinal enzymes that resulted in systemic toxicity. Finally, the "third-generation" inhibitors, including elacridar and tariquidar that were designed to inhibit BCRP and MRP1 in addition to P-gp, were significantly more effective and safer than their ancestors.[2, 37] Despite the success gained so far in pre-clinical trials, these inhibitors have not yet been able to reduce multidrug resistance in clinical chemotherapy. Therefore, one potential solution to revert MDR could be the development of inhibitors of ABC transporters and their prodrugs that would be selectively delivered into the cancer cells.

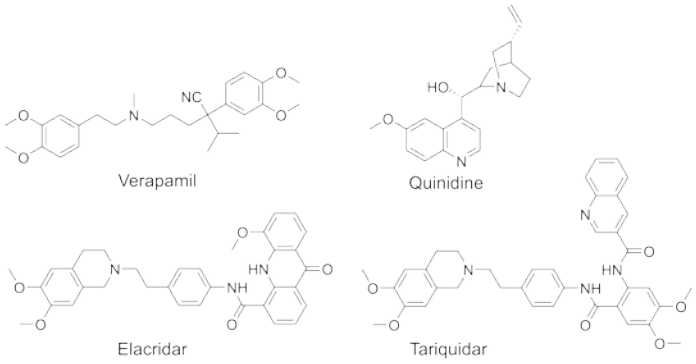

Figure 3. Chemical structures of "first-generation" P-gp modulators, verapamil and quinidine, and "third-generation" multi-targeting efflux inhibitors elacridar and tariquidar.

We have recently developed LAT1-utilizing prodrugs of probenecid that itself is an inhibitor of several efflux transporters, including MRP1-5, P-gp, and BCRP (Figure 4) [38,39] When given the targeted efflux inhibitor together with a cytotoxic anti-cancer agent and *vinca* alkaloid, vinblastine that in turn suffers from several efflux transporters-mediated MDR,[40] increased vinblastine accumulation was achieved with human breast cancer cells (MCF-7). This subsequently resulted in increased apoptotic and antiproliferative effects of vinblastine.[38] Moreover, it was also demonstrated that by utilizing LAT1, probenecid can be targeted not only into cancer cells but also into the brain, to improve the brain uptake of vinblastine.[39] Hence, this kind of combination therapy could be particularly effective in the treatment of brain tumors.

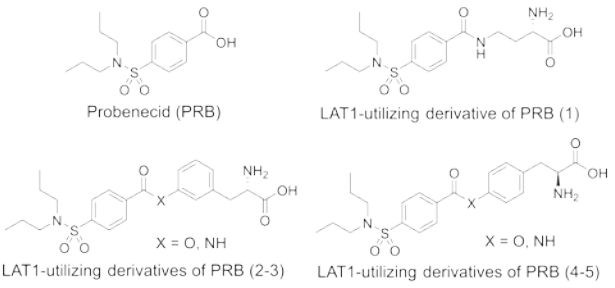

Figure 4. Chemical structures of LAT1-utilizing probenecid (PRB) prodrugs as multi-targeted efflux inhibitors.

Conclusions and Future Prospects

In conclusion, both SLCs and ABC transporters present interesting and promising targets in future cancer chemotherapy. Since many SLCs serve as either tumor promotors or suppressor, their down- or up-regulation, respectively, could have anti-proliferative effects. In turn, ABC (so-called efflux pumps) can be inhibited or down-regulated to combat efflux transporter-mediated MDR. In addition, SLC overexpression in several different cancer cell types can be exploited to improve the delivery of chemotherapeutics or their prodrugs, and thus, improve the treatment outcomes. Furthermore, SLCs can be utilized to improve the selective delivery of radiotracers in the PET-diagnostics, as

exemplified with LAT1-utilizing applications. However, there are still unsolved issues, particularly with targeting efficacy between cancer and healthy cells, since SLCs and ABC transporters are expressed also in the healthy tissues. Moreover, SLCs have overlapping substrate specificities across the subfamilies, which may impair cancer-cell targeting.

Recently, structural biology has advanced greatly and cryo-electron microscopy (cryo-EM) technique has increased our understanding of protein structures of both SLCs *(e.g., LAT1)*[41,42] and ABC transporters.[43] However, the function of transporters is a very dynamic process, and therefore these static models can describe only partly the interactions of these so-called "moving barriers". Therefore, it is very important to understand those interactions that are crucial for the outcome of the compounds. For example, transporter inhibitors are needed to be bound tightly to the protein and not translocated to the other side of the plasma membrane via the transporter cavity, while the transporter substrate needs to induce conformational changes in the transporter cavity that results in translocation. For the latter one, computational techniques, such as molecular dynamics simulations, are needed to understand the kinetic process of substrates.[44] For the former, it is very important to utilize the right conformations in the computational inhibitor design, *i.e.*, the outward-open state and not the inward-open state, unless it is confirmed that these inhibitors are transported into the cell via other mechanisms and they bind and inhibit to the target transporter intracellularly. Moreover, with correct computational methods, issues of overlapping substrates specificities could be fine-tuned while designing novel transporter-utilizing compounds, if several proteins are studied simultaneously.

Lastly, it has been already reported that P-gp is affected by circadian rhythms at the BBB, having lower expression during the nighttime and higher expression during the daytime. [45] Therefore, some drugs have demonstrated to gain higher brain exposure during the sleeping period compared to the waking period.[46] If this same phenomenon occurs also in the cancer cells, it could be taken into account when administering the chemotherapeutics to achieve the highest possible outcomes. Therefore, the importance of circadian biology that is affected by genetic and environmental factors, should be considered very carefully in pre-clinical studies.

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[1] S. Hussain, A. Singh, S. U. Nazir, S. Tulsyan, A. Khan, R. Kumar, N. Bashir, P. Tanwar, R. Mehrotra, *J. Cell. Biochem.* **2019**, *120*, 14213, https://doi.org/10.1002/jcb.28782.

[2] W. Li, H. Zhang, Y. G. Assaraf, K. Zhao, X. Xu, J. Xie, D. H. Yang, Z. S. Chen, *Drug Resistance Update* **2016**, *27*, 14, https://doi.org/10.1016/j.drup.2016.05.001.

[3] J. I. Fletcher, R. T. Williams, M. J. Henderson, M. D. Norris, M. Haber, *Drug Resistance Updates* **2016**, *26*, 1, https://doi.org/10.1016/j.drup.2016.03.001.

[4] M. D. Nyquist, B. Prasad, E. A. Mostaghel, *Molecules* **2017**, *22*, https://doi.org/10.3390/molecules22040539.

[5] C. Holohan, S. Van Schaeybroeck, D. B. Longley, P. G. Johnston, *Nat. Rev. Cancer* **2013**, *13*, 714, https://doi.org/10.1038/nrc3599.

[6] a) M. M. Gottesman, *Annu. Rev. Med.* **2002**, *53*, 615, https://doi.org/10.1146/annurev.med.53.082901.103929; b) G. Housman, S. Byler, S. Heerboth, K. Lapinska, M. Longacre, N. Snyder, S. Sarkar, *Cancers (Basel)* **2014**, *6*, 1769, https://doi.org/10.3390/cancers6031769.

[7] A. Cesar-Razquin, B. Snijder, T. Frappier-Brinton, R. Isserlin, G. Gyimesi, X. Bai, R. A. Reithmeier, D. Hepworth, M. A. Hediger, A. M. Edwards, G. Superti-Furga, *Cell (Cambridge, MA, U. S.)* **2015**, *162*, 478, https://doi.org/10.1016/j.cell.2015.07.022.

[8] W. W. Wang, L. Gallo, A. Jadhav, R. Hawkins, C. G. Parker, *J. Med. Chem.* **2020**, *63*, 3834, https://doi.org/10.1021/acs.jmedchem.9b01237.

[9] M. D. Pizzagalli, A. Bensimon, G. Superti-Furga, *Febs. J.* **2021**, *288*, 2784, https://doi.org/10.1111/febs.15531.

[10] R. R. Schulte, R. H. Ho, *Mol. Pharmacol.* **2019**, *95*, 490, https://doi.org/10.1124/mol.118.114314.

[11] R. Sutherland, A. Meeson, S. Lowes, *Cancer Metastasis Rev.* **2020**, *39*, 919, https://doi.org/10.1007/s10555-020-09879-6.

[12] Y. D. Bhutia, E. Babu, S. Ramachandran, S. Yang, M. Thangaraju, V. Ganapathy, *Biochem. J.* **2016**, *473*, 1113, https://doi.org/10.1042/BJ20150751.

[13] H. Li, L. Myeroff, D. Smiraglia, M. F. Romero, T. P. Pretlow, L. Kasturi, J. Lutterbaugh, R. M. Rerko, G. Casey, J. P. Issa, J. Willis, J. K. Willson, C. Plass, S. D. Markowitz, *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 8412, https://doi.org/10.1073/pnas.1430846100.

[14] R. B. Franklin, P. Feng, B. Milon, M. M. Desouki, K. K. Singh, A. Kajdacsy-Balla, O. Bagasra, L. C. Costello, *Mol. Cancer* **2005**, *4*, 32, https://doi.org/10.1186/1476-4598-4-32.

[15] C. W. Schweinfest, K. W. Henderson, S. Suster, N. Kondoh, T. S. Papas, *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90*, 4166, https://doi.org/10.1073/pnas.90.9.4166.

[16] a) P. D. Prasad, H. Wang, W. Huang, R. Kekuda, D. P. Rajan, F. H. Leibach, V. Ganapathy, *Biochem. Biophys. Res. Commun.* **1999**, *255*, 283, https://doi.org/10.1006/bbrc.1999.0206; b) Y. Kanai, H. Segawa, K. Miyamoto, H. Uchino, E. Takeda, H. Endou, *J. Biol. Chem.* **1998**, *273*, 23629, https://doi.org/10.1074/jbc.273.37.23629.

[17] R. J. Boado, J. Y. Li, M. Nagaya, C. Zhang, W. M. Pardridge, *Proc. Natl. Acad. Sci. U. S. A.* **1999**, *96*, 12079, https://doi.org/10.1073/pnas.96.21.12079.

[18] H. Uchino, Y. Kanai, D. K. Kim, M. F. Wempe, A. Chairoungdua, E. Morimoto, M. W. Anders, H. Endou, *Mol. Pharmacol.* **2002**, *61*, 729, https://doi.org/10.1124/mol.61.4.729.

[19] O. Yanagida, Y. Kanai, A. Chairoungdua, D. K. Kim, H. Segawa, T. Nii, S. H. Cha, H. Matsuo, J. Fukushima, Y. Fukasawa, Y. Tani, Y. Taketani, H. Uchino, J. Y. Kim, J. Inatomi, I. Okayasu, K. Miyamoto, E. Takeda, T. Goya, H. Endou, *Biochim. Biophys. Acta.* **2001**, *1514*, 291, https://doi.org/10.1016/S0005-2736(01)00384-4.

[20] a) R. Duelli, B. E. Enerson, D. Z. Gerhart, L. R. Drewes, *J. Cereb. Blood Flow Metab.* **2000**, *20*, 1557, https://doi.org/10.1097/00004647-200011000-00005; b) T. Kageyama, M. Nakamura, A. Matsuo, Y. Yamasaki, Y. Takakura, M. Hashida, Y. Kanai, M. Naito, T. Tsuruo, N. Minato, S. Shimohama, *Brain Res.* **2000**, *879*, 115, https://doi.org/10.1016/S0006-8993(00)02758-X.

[21] a)J. Huttunen, S. Peltokangas, M. Gynther, T. Natunen, M. Hiltunen, S. Auriola, M. Ruponen, K. S. Vellonen, K. M. Huttunen, *Sci. Rep.* **2019**, *9*, 12860, https://doi.org/10.1038/s41598-019-49009-z; b) E. Puris, M. Gynther, S. Auriola, K. M. Huttunen, *Pharm. Res.* **2020**, *37*, 88, https://doi.org/10.1007/s11095-020-02826-8.

[22] Q. Wang, J. Holst, *Am. J. Cancer Res.* **2015**, *5*, 1281.

[23] a) K. Oda, N. Hosoda, H. Endo, K. Saito, K. Tsujihara, M. Yamamura, T. Sakata, N. Anzai, M. F. Wempe, Y. Kanai, H. Endou, *Cancer Sci.* **2010**, *101*, 173; b) D. B. Shennan, J. Thomson, *Oncol. Rep.* **2008**, *20*, 885, https://doi.org/10.1111/j.1349-7006.2009.01386.x.

[24] J. Toyoshima, H. Kusuhara, M. F. Wempe, H. Endou, Y. Sugiyama, *J. Pharm. Sci.* **2013**, *102*, 3228, https://doi.org/10.1002/jps.23601.

[25] N. Okano, D. Naruge, K. Kawai, T. Kobayashi, F. Nagashima, H. Endou, J. Furuse, *Invest. New Drugs* **2020**, *38*, 1495, https://doi.org/10.1007/s10637-020-00924-3.

[26] L. Napolitano, M. Scalise, M. Koyioni, P. Koutentis, M. Catto, I. Eberini, C. Parravicini, L. Palazzolo, L. Pisani, M. Galluccio, L. Console, A. Carotti, C. Indiveri, *Biochem. Pharmacol.* **2017**, *143*, 39, https://doi.org/10.1016/j.bcp.2017.07.006.

[27] a) K. M. Huttunen, M. Gynther, J. Huttunen, E. Puris, J. A. Spicer, W. A. Denny, *J. Med. Chem.* **2016**, *59*, 5740, https://doi.org/10.1021/acs.jmedchem.6b00190; b) M. Markowicz-Piasecka, J. Huttunen, A. Montaser, K. M. Huttunen, *Apoptosis* **2020**, *25*, 426, https://doi.org/10.1007/s10495-020-01603-7.

[28] H. C. Chien, C. Colas, K. Finke, S. Springer, L. Stoner, A. A. Zur, B. Venteicher, J. Campbell, C. Hall, A. Flint, E. Augustyn, C. Hernandez, N. Heeren, L. Hansen, A. Anthony, J. Bauer, D. Fotiadis, A. Schlessinger, K. M. Giacomini, A. A. Thomas, *J. Med. Chem.* **2018**, *61*, 7358, https://doi.org/10.1021/acs.jmedchem.8b01007.

[29] J. Kärkkäinen, T. Laitinen, M. Markowicz-Piasecka, A. Montaser, M. Lehtonen, J. Rautio, M. Gynther, A. Poso, K. M. Huttunen, *Bioorg. Chem.* **2021**, *112*, 104921, https://doi.org/10.1016/j.bioorg.2021.104921.

[30] J. Tampio, S. Löffler, M. Guillon, A. Hugele, J. Huttunen, K. M. Huttunen, *Int. J. Pharm.* **2021**, *601*, 120565, https://doi.org/10.1016/j.ijpharm.2021.120565.

[31] W. Wu, Y. Dong, J. Gao, M. Gong, X. Zhang, W. Kong, Y. Li, Y. Zeng, D. Si, Z. Wei, X. Ci, L. Jiang, W. Li, Q. Li, X. Yi, C. Liu, *Cancer Sci.* **2015**, *106*, 747, https://doi.org/10.1111/cas.12672

[32] a) A. Detta, G. S. Cruickshank, *Cancer Res.* **2009**, *69*, 2126, https://doi.org/10.1158/0008-5472.CAN-08-2345; b) T. Fujimoto, M. Suzuki, T. Sudo, I. Fujita, T. Sakuma, Y. Sakurai, T. Hirose, M. Morishita, T. Takata, Y. Tamari, H. Tanaka, T. Andoh, T. Kawamoto, H. Hara, N. Fukase, Y. Kawakami, R. Shigemoto, T. Matsumoto, H. Ichikawa,

K. Ono, R. Kuroda, T. Akisue, *Appl. Radiat. Isot.* **2020**, *166*, 109324, https://doi.org/10.1016/j.apradiso.2020.109324.
[33] a) K. Kaira, N. Oriuchi, Y. Otani, K. Shimizu, S. Tanaka, H. Imai, N. Yanagitani, N. Sunaga, T. Hisada, T. Ishizuka, K. Dobashi, Y. Kanai, H. Endou, T. Nakajima, K. Endo, M. Mori, *Clin. Cancer Res.* **2007**, *13*, 6369, https://doi.org/10.1158/1078-0432.CCR-07-1294; b) V. Makrides, R. Bauer, W. Weber, H. J. Wester, S. Fischer, R. Hinz, K. Huggel, T. Opfermann, M. Herzau, V. Ganapathy, F. Verrey, P. Brust, *Brain Res.* **2007**, *1147*, 25, https://doi.org/10.1016/j.brainres.2007.02.008.
[34] M. Markowicz-Piasecka, J. Sikora, L. Mateusiak, E. Mikiciuk-Olasik, K. M. Huttunen, *Eur. J. Pharmacol.* **2017**, *811*, 208, https://doi.org/10.1016/j.ejphar.2017.06.011.
[35] R. W. Robey, K. M. Pluchino, M. D. Hall, A. T. Fojo, S. E. Bates, M. M. Gottesman, *Nat. Rev.. Cancer* **2018**, *18*, 452, https://doi.org/10.1038/s41568-018-0005-8.
[36] J. I. Fletcher, M. Haber, M. J. Henderson, M. D. Norris, *Na. Rev. Cancer* **2010**, *10*, 147, https://doi.org/10.1038/nrc2789.
[37] R. J. Kathawala, P. Gupta, C. R. Ashby, Jr., Z. S. Chen, *Drug Resist. Updat.* **2015**, *18*, 1, https://doi.org/10.1016/j.drup.2014.11.002.
[38] J. Huttunen, M. Gynther, K. M. Huttunen, *Int. J. Pharm.* **2018**, *550*, 278, https://doi.org/10.1016/j.ijpharm.2018.08.047.
[39] A. Montaser, M. Markowicz-Piasecka, J. Sikora, A. Jalkanen, K. M. Huttunen, *Int. J. Pharm.* **2020**, *586*, 119585, https://doi.org/10.1016/j.ijpharm.2020.119585.
[40] a) K. M. Pawłowski, J. Mucha, K. Majchrzak, T. Motyl, M. Król, *BMC Vet Res* **2013**, *9*, 119, https://doi.org/10.1186/1746-6148-9-138; b) Y. Honda, F. Ushigome, N. Koyabu, S. Morimoto, Y. Shoyama, T. Uchiumi, M. Kuwano, H. Ohtani, Y. Sawada, *Br. J. Pharmacol.* **2004**, *143*, 856, https://doi.org/10.1038/sj.bjp.0706008.
[41] R. Yan, X. Zhao, J. Lei, Q. Zhou, *Nature* **2019**, *568*, 127, https://doi.org/10.1038/s41586-019-1011-z.
[42] Y. Lee, P. Wiriyasermkul, C. Jin, L. Quan, R. Ohgaki, S. Okuda, T. Kusakizako, T. Nishizawa, K. Oda, R. Ishitani, T. Yokoyama, T. Nakane, M. Shirouzu, H. Endou, S. Nagamori, Y. Kanai, O. Nureki, *Nat. Struct. Mol. Biol.* **2019**, *26*, 510, https://doi.org/10.1038/s41594-019-0237-7.
[43] D. Januliene, A. Moeller, *FEBS Lett.* **2020**, *594*, 3776, https://doi.org/10.1002/1873-3468.13989.
[44] A. Schlessinger, M. A. Welch, H. van Vlijmen, K. Korzekwa, P. W. Swaan, P. Matsson, *Clin. Pharmacol. Ther.* **2018**, *104*, 818, https://doi.org/10.1002/cpt.1174.
[45] a) R. W. Logan, C. A. McClung, *Nat. Rev. Neurosci.* **2019**, *20*, 49, https://doi.org/10.1038/s41583-018-0088-y; b) E. Esposito, W. Li, T. M. E, J. H. Park, I. Sencan, S. Guo, J. Shi, J. Lan, J. Lee, K. Hayakawa, S. Sakadzic, X. Ji, E. H. Lo, *Nature* **2020**, *582*, 395, https://doi.org/10.1038/s41586-020-2348-z.
[46] S. L. Zhang, N. F. Lahens, Z. Yue, D. M. Arnold, P. P. Pakstis, J. E. Schwarz, A. Sehgal, *Nat. Commun.* **2021**, *12*, 617, https://doi.org/10.1038/s41467-020-20795-9.