The role of transporters in the efficacy of and adverse reactions to anticancer agents

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・In this chapter, I will be talking about the role of transporters in the efficacy of and adverse reactions to anticancer agents.

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・I would like to start by providing an overview of the effects that transporters have on pharmacokinetics.

・ Transporters consist of SLC transporters that are expressed on the cell membrane and that take up a drug and ABC transporters that conversely prevent a drug from entering cells by eliminating it.

・Previous clinical studies have actively examined ABC transporters associated with multidrug resistance to chemotherapy. In this chapter, I will briefly describe SLC transporters in relation to chemotherapy. I will then describe the relationship between ABC transporters and multidrug resistance with a focus on the breast cancer resistance protein (BCRP). I will explain its expression, function, and the effects of its polymorphisms on anticancer agents in detail.

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・When a drug is ingested, the drug is disintegrated and dissolved in the gastrointestinal tract and ultimately dissolved in water; only then is it absorbed. The portion that is not absorbed is excreted in stool.

・A drug absorbed via the gastrointestinal tract (and particularly the small intestine) travels through the portal vein to reach the liver. The portion that is not metabolized, i.e. the portion that is not subjected to the first-pass effect, enters the blood stream and is distributed in the body. Afterwards, it is eliminated from the body via the liver or the kidneys.

・Thus, the fate of a drug in the body is determined by absorption, distribution, metabolism, and excretion. These 4 processes are abbreviated ADME.

・So how are transporters involved?

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・As an example, an uptake transporter (in blue) in the SLC family and an efflux transporter (in red) in the ABC family are expressed in small intestinal epithelial cells.

・When a drug with poor membrane permeability is taken up and transported by a transporter, the absorption rate and percent absorption increase and its concentration in the blood increases.

・When, conversely, a drug is transported by an efflux transporter, its gastrointestinal absorption is

limited. Such a phenomenon is noted in drug transfer to the liver, the kidneys, and the brain. Studies have often reported that drug disposition changes substantially due to changes in the expression and function of drug transporters and due to interaction on drug transporters.

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・From the perspective of drug efficacy, the same can be said for the site of action.

・An uptake transporter facilitates drug uptake while an efflux transporter limits drug delivery, so drug efficacy and adverse reactions are known to be affected by transporters.

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・Let's look at an instance involving an SLC transporter and an anticancer agent.

・I will start by providing an overview of SLC transporters.

・SLC stands for solute carrier and it refers to a transporter that transports a solute.

・SLC transporters are currently classified into 65 subfamilies, and over 400 transporters have been identified.

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・Based on the mechanism of transport, SLC transporters are classified as those that function by facilitative diffusion and those that function by secondary active transport. The latter use the electrochemical potential created by the ion concentration gradient inside and outside of cells.

・SLC transporters regulate the concentration of various solutes that are essential to sustaining life, from inorganic ions to nutrients such as sugars and amino acids, inside and outside of cells.

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・There are clinical examples where transporters account for the intake or efficacy of a drug, but transporters are known to be involved in internal radiotherapy using radioactive iodine.

・Radioactive iodine, iodine-131, is an isotope of iodine. In radioiodine therapy, iodine-131 is drunk to treat diseases of the thyroid.

・Here, thyroid tissue remaining after thyroid cancer was completely removed has been visualized using iodine scintigraphy. As a result of taking I-131, the cancer was found to have disappeared completely after 6 months.

・So how has tissue been visualized with radioactive iodine and can it be treated?

・Studies have reported that radioactive iodine uptake involves SLC transporters.

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・Thyroid tissue synthesizes thyroid hormones in the follicular lumen, and this requires a lot of iodine.

・A gene (SLC5A5) coding for the **Na^+/I^- symporter** (NIS) is expressed in thyroid follicular cells, and it causes iodide ions from the blood to be taken up in cells. Those ions are then transported to the follicular lumen via pendrin (SLC26A4).
・NIS expression is mostly limited to thyroid tissue, so iodine in the blood is mostly brought to thyroid tissue by these 2 transporters.
・When a radioactive agent is used, cells are killed by the beta rays that it releases, so the agent should be therapeutically effective.
・Thus, internal radiotherapy using radioactive iodine is an ideal instance where tissue-specific expression of SLC transporters is used in both diagnosis and treatment.

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・Shown here are SLC transporters that are highly expressed in cancer cells and anticancer agents that serve as substrates for those transporters.
・The expression of these transporters is associated with drug intake, so these transporters should be therapeutically effective.

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・In specific terms, studies have noted a correlation between the level of expression and function of organic cation transporters, nucleoside transporters, and folate transporters in tumor tissue and the efficacy of platinum-based drugs, nucleoside analogues, and folate analogues.
・Determining the role of these transporters in a clinical setting is a topic for future research.

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・Next, I would like to turn to chemotherapy and ABC family transporters.

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・In the figure, the vertical axis indicates tumor growth and the horizontal axis indicates survival. The concept of multidrug resistance (MDR) to chemotherapy and the problem it poses is clearly evident.
・When a malignancy is attacked with chemotherapy, the tumor temporarily regresses but ultimately recurs.
・If a response to the first anticancer agent is not noted, a second round of chemotherapy is started.
・If, however, a tumor is untreated and it recurs, anticancer agents that were previously used to treat it can no longer be used.
・If tumor tissue acquires MDR, it cannot be managed by starting another round of chemotherapy.
・Thus, MDR is a major obstacle in chemotherapy.

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・Various mechanisms of MDR have been put forth.

・An ABC transporter eliminates a drug via the cell membrane. Overexpression of an ABC transporter as a cause of MDR has long been studied.

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・I will now briefly explain ABC transporters.

・An ABC transporter is expressed on the cell membrane and it has an ATP-binding site known as an ATP-binding cassette in its intracellular domain. ABC transporters function as enzymes that hydrolyze ATP.

・An ABC transporter uses energy produced by the hydrolysis of ATP. It is responsible for primary active transport, which expels xenobiotic substances and anticancer agents from cells in accordance with the concentration gradient.

・In simple terms, an ABC transporter acts as a drug pump.

・48 ABC transporters in 7 subfamilies have been identified in humans.

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・Shown here is a phylogenetic tree for the main members of the ABC transporter family.

・The transporters that are associated with MDR in cancer cells are mainly P-gp, which is coded for by the ABCB1 gene, MRP1, which is coded for by ABCC1, and BCRP, which is coded for by ABCG2. The relationship between these 3 transporters and the pharmacokinetics and efficacy of and the adverse reactions to various anticancer agents has been studied.

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・Shown here are the molecular structures of the 3 transporters.

・P-gp is a 12-transmembrane-domain protein with 2 ATP-binding sites.

・In contrast, MRP1 is a 17-transmembrane-domain protein (so it has 5 more transmembrane domains than P-gp). The main substrates of MRP1 are glutathione conjugates, glucuronide conjugates, and sulfate conjugates.

・BCRP is known as a half-size transporter. Two of its molecules form a homodimer. Like P-gp, it uses 2 ATP molecules to transport 1 substrate molecule.

・Over the past few years, BCRP has been considered to be a molecule causing MDR that is not due to P-gp. I will now explain the role of ABC transporters with a focus on BCRP.

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・BCRP is overexpressed in MCF-7/Adr Vp3000 breast cancer cells treated with adriamycin in the presence of the P-gp inhibitor verapamil, and the gene coding for BCRP has been isolated.
・If you look at the anticancer agents transported by BCRP as are listed on the left, you can see that their efficacy and structures differ.
・Therefore, if expression of BCRP is induced by a given anticancer agent, then cancer cells can acquire resistance to all of these drugs, so this is an extremely effective system.

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・So how exactly is BCRP expressed in tumor tissue?
・Overexpression of BCRP is often noted in monocytic cells from patients with myelogenous leukemia or lymphocytic leukemia.
・There are differences in the level of expression of BCRP in solid cancers, but expression of BCRP has been noted in many cancer cells, as shown here.

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・This slide shows the relationship between the expression of BCRP and the drug concentrations inside cells.
・When S1 cells are treated with mitoxantrone (a substrate of BCRP), the resulting S1-M1-80 cells overexpress BCRP.
・As you can see, red fluorescence indicating mitoxantrone is almost absent in the drug-resistant cell line.
・Epirubicin is a substrate of BCRP and an anticancer agent. Like mitoxantrone, red fluorescence indicating epirubicin is almost absent in S1-M1-80 cells.
・These findings indicate that when cancer cells overexpress BCRP, cell intake of multiple anticancer agents is limited.

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・BCRP similarly limits the intake of molecularly targeted drugs that were recently developed into cells.
・The uptake of alvocidib (a CDK inhibitor) was almost absent in African clawed frog oocytes expressing BCRP. In the presence of FTC (a selective BCRP inhibitor), the amount of uptake almost reached the level of intake in oocytes infused with water instead of BCRP cRNA.
・Intake of the IGFR1 inhibitor BMS-536924 was almost absent in cells overexpressing BCRP.

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・Thus, many other molecularly targeted drugs are transported by BCRP.

・Here is a summary of the substrates transported by BCRP and P-gp. The red circles indicate substrates that are transported and the triangles indicate drugs with inhibitory action.
・Indicated with a red circle, the anticancer agents imatinib, gefitinib, and dasatinib are widely used clinically. These agents are recognized and transported by BCRP and P-gp.
・The substrate spectrum of P-gp is similar to that of BCRP, so P-gp will presumably have a similar effect on the efficacy of anticancer agents.
・How does expression of BCRP relate to the efficacy of anticancer agents in a clinical setting?

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・In the early 2000s, numerous clinical studies reported on the level of BCRP expression in monocytic cells from patients with acute myelogenous leukemia and the efficacy of anticancer agents.
・Shown here is a typical result.
・In the 9 studies, findings regarding the expression of BCRP honestly varied. Overexpression of BCRP compared to expression in normal cells was not necessarily noted.
・The 5 studies indicated in red found no correlation whatsoever between the expression of BCRP and drug responsiveness.
・In contrast, the 3 studies indicated in blue reported that expression of BCRP was inversely correlated with drug responsiveness. In other words, drug sensitivity decreased as a result of BCRP expression.
・Thus, a definite relationship between the level of BCRP expression and anticancer agent responsiveness has yet to be established.
・The reason for this is unclear, but the 3 studies indicated in green stressed that expression of BCRP was noted in the leukemia cell fraction.
・So what exactly does this mean?

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・Previous studies have reported that expression of BCRP coincides with expression of a stem cell marker in myeloid leukemia.
・BCRP is known to be overexpressed in breast cancer cells with the ability to self-replicate.
・Therefore, I will offer a hypothesis regarding the role of BCRP in MDR.
・Cancer cells with both the ability to self-replicate and the ability to proliferate as a result of administration of an anticancer agent, i.e. stem cell-like cancer cells, overexpress BCRP. Thus, a few groups of cells will survive even if tumor tissue is attacked with an anticancer agent, and the tumor will recur.
・At this point, the cancer cells have acquired drug resistance, so they will not respond to the anticancer agent that was previously used.
・Thus, stem cell-like cancer cells will be protected from the anticancer agent as they acquire MDR

even if individual cancer cells do not overexpress BCRP. This will lead to the acquisition of MDR.

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・Thus, we have looked at how the expression of an ABC transporter, BCRP, affects the efficacy of anticancer agents.

・Expression of an ABC transporter limits drug efficacy, so inhibitors of P-gp and BCRP have actively been developed.

・Shown here is an example where 99Tc-sestamibi, which is a Pgp substrate labeled with 99Tc, proved to be efficacious as a P-gp inhibitor.

・As shown on the right, co-administration of tariquidar (a P-gp inhibitor) resulted in substrate intake in metastases in the lung.

・Several such inhibitors have been developed. Elacridar (GF120918) is well-known for its dual inhibition of P-gp and BCRP.

・Unfortunately, these inhibitors have yet to come on the market.

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・I will now be talking about the impact of ABC transporters like BCRP on the disposition of anticancer agents.

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・BCRP and P-gp are expressed in intestinal epithelial cells, so they limit the absorption of an orally administered drug.

・BCRP is expressed on the apical membrane of hepatocytes and bile duct epithelial cells and on the apical membrane of renal tubule epithelial cells. With P-gp, BCRP facilitates drug excretion.

・BCRP is also expressed in endothelial cells that form the lumen of brain capillaries, and it protects the brain by limiting delivery of a drug to the brain.

・Thus, BCRP acts to protect the body by preventing entry of a drug, i.e. a xenobiotic substance, into the body and by promoting its excretion.

・Therefore, decreased expression of BCRP and functional defects may intensify systemic exposure to an anticancer agent.

・I will now provide an example of that.

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・Topotecan is a substrate for P-gp and BCRP. Shown here are changes in the concentration of topotecan in plasma over time when it was administered to mice and humans.

・On the left you can see the efficacy of the P-gp and BCRP inhibitor GF120918 when topotecan was

orally administered to mdr1a/1b, i.e. P-gp gene knockout, mice. Cmax markedly increased in the group administered GF120918 compared to Cmax in the control group. This means that absorption of topotecan was limited by BCRP expressed in the gastrointestinal tract.

・On the left are results of a clinical study in humans. As expected, Cmax and the AUC increased in the group administered GF120918, so absorption of topotecan was found to be limited by BCRP and P-gp, much as it was in mice.

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・Shown here are the plasma kinetics of a molecularly targeted drug and its delivery to the brain.

・TKO stands for triple knockout, and mdr1a/1b and BCRP have been knocked out in these mice.

・The concentration of sorafenib and ceritinib in plasma was found to increase in the wild-type (WT) compared to that in the TKO.

・Moreover, delivery to the brain markedly increased in the TKO compared to that in the WT, so animal experiments verified that these ABC transporters limited drug delivery to the brain.

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・Thus, the expression of and functional variations in ABC transporters cause pharmacokinetics to vary.

・Hazardous drugs and anticancer agents have a broader therapeutic range than generics, so a correct understanding of the determinants of drug disposition is crucial to proper drug use.

・Over the past few years, clinical studies have reported the effects of P-gp and BCRP polymorphisms on drug disposition.

・Therefore, polymorphisms in transporters and their interaction with anticancer agents transported by ABC transporters need to be understood, as is the case with drug-metabolizing enzymes.

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・BCRP polymorphisms are summarized here.

・BCRP is known to have numerous polymorphisms. The mutations V12M and Q141K severely suppress the expression of BCRP.

・These SNP alleles occur at a higher frequency in Asians than in Europeans, so they should be taken into account in order to properly use drugs.

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・Expression of ABC transporters limits drug absorption by the small intestine and delivery to the brain, it promotes drug excretion via the liver and kidneys, and it inhibits drug intake in tumor tissue, so it affects drug disposition.

・Therefore, an understanding of the expression and function of these ABC transporters will help to use drugs properly and overcome MDR.

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・SLC transporters overexpressed in cancer cells increase the cell intake of substrates (anticancer agents) and diagnostic reagents. Therefore, cancer cell-specific transporters may serve as target molecules for anticancer agents.

・ABC transporters expressed in cancer cells function as a factor for MDR to chemotherapy. The heterogeneity of cancer cells hampers the avoidance of MDR, but effective inhibitors of these transporters should be developed in the future.

・With that, I will conclude this chapter.