The latest pharmacogenomics of anticancer agents

Miki Nakajima

Slide 1

I am Miki Nakajima of Kanazawa University's School of Pharmacy/School of Pharmaceutical Sciences. Today, I will be talking about the latest pharmacogenomics of anticancer agents.

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Once a drug is administered, whether it is truly efficacious is determined by how strongly its active ingredient acts on a target molecule, i.e. pharmacodynamics, and the concentration of the active ingredient that reaches the site of action, i.e. pharmacokinetics. Pharmacokinetics consists of 4 processes: absorption, distribution, metabolism, and excretion, abbreviated as ADME. Among the 4 ADME processes that determine drug disposition, drug metabolism is the most important. This fact will become apparent from the next slide.

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Shown here are the factors that determine the disposition of the top 200 pharmaceuticals in clinical use. As shown in the bar graph on the left, about 70% of pharmaceuticals disappear from the body once they are metabolized. As shown in the bar graph at the bottom, cytochrome P450 (CYP) accounts for 70% of the enzymes responsible for drug metabolism, followed by UGT, which catalyzes glucuronidation, and esterases, which catalyze hydrolysis. These enzymes play an important role in pharmaceutical disposition and efficacy.

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Even when a pharmaceutical is administered in the same dose, there are individual differences in the concentration in the blood. I'm sure you're familiar with an example that can be explained by a polymorphism found in a gene coding for a drug-metabolizing enzyme. When a genetic mutation leads to the inactivation of a metabolizing enzyme, the concentration of that enzyme in the blood increases and its effect may be sustained in heterozygotes for that variant more so than in people lacking that variant. In homozygotes for the variant, the concentration in the blood increases and adverse effects may occur. Thus, the field of pharmacogenomics seeks to make drug therapy efficacious by determining the relationship between drug efficacy and genes. This is accomplished by studying polymorphisms in genes coding for drug-metabolizing enzymes, and the field has certainly advanced. Genetic testing for drug-metabolizing enzymes allows customized medicine for people with a genetically low metabolic capacity, e.g. whether to not administer the drug or to administer it in a lower dose.

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Shown here is a dose-response curve. The horizontal axis indicates the concentration of a drug in the blood and the vertical axis indicates the intensity of action. If the concentration increases, action intensifies. There are differences in the pharmacological action, toxic effects, and lethal effects of generics, so adverse reactions can immediately occur if the concentration in the blood increases even slightly and serious toxicities that can lead to death often develop…

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The 3 dose-response curves are close together for an anticancer agent, which is a chemotherapy drug exhibiting cytotoxic action. In other words, the agent has a broad therapeutic range, so the concentration of that agent in the blood needs to be controlled. If, however, the therapeutic range is exceeded, then extremely intense toxicities will develop. A chemotherapy agent is administered in the maximum dose at which adverse reactions can occur, i.e. the maximum tolerated dose, so serious adverse reactions can occur as a result of slight variations in pharmacokinetics.

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In the field of pharmacogenomics, a well-known anticancer agent is irinotecan hydrochloride, or CPT-11. Irinotecan is a prodrug and is not efficacious itself. After intravenous administration, it is hydrolyzed by carboxylesterase in the liver, where its efficacious form, SN-38, is generated. SN-38 reaches the target site and is cytotoxic to cancer cells. However, it is toxic to normal cells as well as cancer cells, and it causes adverse reactions such as myelosuppression. UGT1A1 in the liver detoxifies SN-38. It metabolizes SN-38 into a glucuronide conjugate, detoxifying SN-38. SN-38 glucuronide is excreted into bile. It reaches the intestines and is excreted in stool. However, some SN-38 glucuronide is hydrolyzed by β glucuronidase of intestinal flora to again generate SN-38. This irritates intestinal cells and presumably induces diarrhea. In addition, enterohepatic circulation occurs whereby SN-38 is reabsorbed and again returned to the liver. Thus, UGT1A1 is crucial to the disposition of irinotecan and its active metabolite SN-38. In patients with low UGT1A1 activity, the concentration of SN-38 in the blood increases and adverse reactions can readily occur.

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Over 100 variants of UGT1A1 have been identified. The most important variants are UGT1A1*28 and UGT1A1*6. UGT1A1*28 is a variant with 5 to 7 TA repeats in the promoter region in comparison to wild-type UGT1A1. A decline in promoter activity leads to a decrease in the level of expression and enzyme activity. In UGT1A1*6, an amino acid changes as a result of an SNP in exon 1, and enzyme activity decreases as a result. UGT1A1*28 is noted at a relatively high frequency of

40% in whites, 45% in blacks, and 10% in Japanese. UGT1A1*6 is characteristic variant found in Asians, and it is noted at a frequency of 15% in Japanese.

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In people with UGT1A1*28, UGT1A1*6, or both, the AUC ratio of SN-38G to SN-38 is markedly low, i.e. their SN-38 glucuronidation capacity has been found to decrease. This means that the concentration of SN-38 in the blood increases, and the likelihood of serious neutropenia developing increases as a result. Thus, genetic testing for UGT1A1 polymorphisms is recommended. Important findings regarding UGT1A1 polymorphisms have highlighted use of pharmacogenomics in personalized medicine involving anticancer agents.

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Polymorphisms in the UGT1A1 gene affect irinotecan, but polymorphisms in metabolizing enzymes have been found to affect the efficacy and the incidence of adverse reactions to other anticancer agents as well. Indicated in red, TPMT and CYP2D6 are closely related to the genotype and phenotype, so genetic testing for them is useful. The activity of the enzymes indicated in black varies substantially depending on factors besides polymorphisms, genetic testing for metabolizing enzymes alone cannot predict the efficacy of an anticancer agent or whether adverse reactions can be avoided. Moreover, adverse reactions to irinotecan cannot be predicted solely based on UGT1A1 polymorphisms, and even some patients without a variant suffer adverse reactions.

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What factors not attributable to polymorphisms cause the activity of a drug-metabolizing enzyme to vary? This question has been studied, and concomitant medications, supplements, and dietary components that inhibit or induce metabolizing enzymes have been identified. These substances cause drug-drug interactions or drug-food interactions. As I now talk about inducing metabolizing enzymes…

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Research on a transcription factor that binds to the 5'-upstream region of a gene and that activates transcription has been actively conducted. The compounds that activate that transcription factor have been identified, revealing a mechanism causing individual differences in the level of gene expression. In addition to this mechanism of transcription regulation, post-transcriptional regulation via microRNA has recently been found to play an important role in regulation of the expression of drug-metabolizing enzymes.

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miRNA is a small single-stranded RNA with around 22 bases that does not code for a protein. miRNA binds with target mRNA, inhibiting translation and breaking down mRNA, thus negatively regulating expression. In humans, over 2,500 miRNAs have been identified thus far. Regulatory mechanisms involving miRNA are presumably involved in every biological process. Variations in miRNA expression in particular have been found to cause the development and progression of illness, and miRNAs have garnered attention as new therapeutic targets.

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Shown here are pathways of miRNA biosynthesis. First, miRNAs are transcribed from genomic DNA as primary miRNAs with several hundred to several thousand bases and then processed by proteins in the nucleus. Primary miRNAs are then cleaved into precursor miRNAs (which have around 70-100 bases) with a stem and loop configuration. Pre-miRNAs are transported to the cytoplasm via exportin 5 where they are processed and cleaved into double-stranded RNA with 22 bases. Afterwards, they are processed into single-stranded mature miRNAs.

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Thus, miRNAs control the expression of drug-metabolizing enzymes such as CYPs and transcription factors. In our Laboratory, we have continually led the way in revealing how miRNAs are involved in controlling the development and progression of cancer, how they lead to individual differences in an enzyme's ability to metabolize a drug, and how they regulate the homeostasis of substances in the body. For details, have a look at these papers.

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Expression of miRNA varies in the context of disease and it varies due to various factors. An interesting fact is that expression of miRNA also varies due to treatment with an anticancer agent. Over the past few years, studies have revealed an instance where variations in miRNA expression due to an anticancer agent facilitate pharmacological action. A leukemia treatment, imatinib is a Bcr-Able tyrosine kinase inhibitor. Expression of many miRNAs varies when leukemia cells are treated with imatinib. Expression of miR-203 in particular increases, and studies indicated that imatinib may be efficacious even though it reduces the expression of Bcr-Able tyrosine kinase.

Although not shown in the slide, studies have reported that variations in miRNA expression due to anticancer agents may be involved in the development of resistance to anticancer agents. Thus, studies have revealed an instance where miRNAs play a role in the efficacy of anticancer agents.

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Let's now return to our discussion of pharmacogenomics. SNPs are located on genes coding for microRNAs. If there is a mutation in an miRNA precursor, processing changes and the level of mature miRNA expression can change. If a mutation is present in mature miRNA or at the miRNA-binding site on mRNA, then the binding of the miRNA changes and the level of target gene expression changes. In other words, a mutation can cause some form of functional variation.

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As shown here, SNPs on genes coding for microRNAs have been found to be related to the development and progression of numerous illnesses. Although not shown here, studies have reported miRNA-SNPs that are associated with the development and progression of cancer.

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Over the past few years, studies have revealed that the efficacy of anticancer agents and the severity of adverse reactions differ in people with an SNP in a gene coding for a microRNA and in people without that SNP. Among patients with colon cancer who were treated with 5-FU and irinotecan, those with the miRNA-SNP indicated in red had a shorter time to progression.

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As shown here, patients with lung cancer who had an SNP in precursor miR-196a2 were more likely to suffer serious adverse reactions such as leukopenia, neutropenia, and a gastrointestinal disorder as a result of a platinum-based anticancer agent like cisplatin. That molecular mechanism is still unclear, but the level of expression of that miRNA changed due to the SNP and it may have caused variations in target gene expression, as shown at the bottom. Thus, the pharmacogenetics of miRNAs should lead to new biomarkers that can predict drug response and drug toxicity.

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However, the subjects of previous studies have been whites and Chinese, and no study has exhaustively analyzed miRNA-SNPs in Japanese. Thus, we identified around 2,000 miRNAs using next-generation sequencing and we exhaustively analyzed SNPs in healthy Japanese individuals and patients with cancer in an effort to identify potential biomarkers of adverse reactions and drug efficacy.

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I will now describe our results thus far. We analyzed 20 healthy individuals, 24 patients with non-small-cell lung cancer, and 13 patients with colon cancer, and we used the same sequence as the reference sequence (i.e. the wild-type) to detect mutations. The graph on the right shows the number of miR-SNPs found in each group of subjects, and the graph on the left shows the number of miRNA

variants (and not just SNPs) with deletion or insertion of a base. On average, 170 miRNA variants were found per person, and differences in that number were not noted among the groups.

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Shown here are details on genetic mutations that have been identified. Healthy individuals, patients with lung cancer, and patients with colon cancer are aligned beside each other. The homozygous wild-type is indicated in white, a heterozygous mutation is indicated in orange, and a homozygous mutation is indicated in red. We examined the miR-SNPs that differed significantly in frequency in healthy individuals and patients with cancer. The odds ratio for miR-SNPs that differed significantly in frequency in healthy individuals and patients with non-small-cell lung cancer is indicated with a green background. We compared healthy individuals and patients with colon cancer, and the odds ratio for miR-SNPs that differed significantly in frequency is indicated with a yellow background. Only 1 miR-SNP, shown in the black box, was common to both cancers. This may be a marker of susceptibility to cancer unrelated to tissue. In contrast, other SNPs that differed significantly only in the groups of patients with cancer may serve as markers of susceptibility to non-small-cell lung cancer or colon cancer.

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We then examined miRNA-SNPs that could potentially serve as biomarkers of adverse reactions to anticancer agents. The subjects of this study were all patients with colon cancer who received regorafenib. Patients were divided into those with mild or severe adverse reactions, and we looked for significant differences in the frequency of miRNA-SNPs in the two groups. The 6 SNPs shown at the top may serve as a biomarker to predict a potential adverse reaction in the form of hand and foot syndrome while the 2 SNPs shown at the bottom may serve as a biomarker to predict a potential adverse reaction in the form of hypertension. That said, the sample size was small, so increasing the number of specimens might allow more accurate identification of biomarkers.

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miRNAs are responsible for the post-transcriptional regulation of genes. Incorporating genetic analysis of miRNAs should allow further optimization of personalized medicine with more appropriate drug therapy. This can be accomplished by stratifying responders, i.e. patients who will respond appropriately without suffering adverse reactions, and non-responders, i.e. patients who will suffer intense adverse reactions, beforehand. In the future, the task is to determine the significance of mutations in individual miRNAs and to elucidate the molecular mechanisms of those mutations.

In other words, the level of miRNA expression changes due to genetic mutations. Is the key to alter the expression of a target gene or to alter the binding of an miRNA to a target gene and thus alter the

expression of that target gene? And the expression of which target genes should be altered? Finding the answers to those questions will establish the utility of miRNAs as biomarker.

With that, I will conclude my talk on the latest pharmacogenomics of anticancer agents.

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

Takeo Nakanishi

Slide 1

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

Slide 2

・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.

Slide 3

・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.

Data mining based on -omics

Hiro Takashi

Slide 1

I am Hiro Takashi of Kanazawa University, where I am responsible for data mining based on -omics.

Slide 2

Data mining based on -omics is based on the field of bioinformatics, which involves the use of computers, so I will give a basic description of bioinformatics and then provide examples of use of data mining based on -omics in medicine.

Slide 3-7

I will now describe the Human Genome Project, which heavily involved bioinformatics. The Human Genome Project was a project that started by the US Department of Energy and the National Institutes of Health (NIH) in October 1990 with a budget of $3 billion. Ultimately, 24 institutions in 6 countries participated in the project; 60% were in the US, 30% were in the UK, and 6% were in Japan (the third leading participant). Japan began participating in the project in 1991. The project yielded a rough draft of 2.86 billion base pairs, corresponding to 99% of the haploid human genome, in June 2000 and a more accurate official version in April 2003. What did the project reveal? Well, it merely revealed strings of bases. Thus, the question is how did those strings of bases lead to drug discovery and identification of biological phenomena.

Slide 8-14

As I explained a moment ago, the Human Genome Project revealed that the human genome has around 6 billion base pairs. This information is DNA. Information in DNA is conveyed by RNA. Extensive RNA information is referred to as the transcriptome. There are around 30,000 genes. Genetic information is ultimately turned into proteins that perform a function. Extensive protein information is referred to as the proteome. There are 100,000 to 200,000 proteins. Over the past few years, techniques for extensively examining genomes and identifying mRNAs and proteins have been developed and a vast amount of information has been compiled. Use of this information allows, as an example, elucidation of the causes of illness based on differences in the genomes of patients and healthy individuals. Moreover, comparing cancer tissue and normal tissue at the level of the transcriptome and proteome allows an examination of how normal tissue becomes diseased. In other words, examining various differences will allow the elucidation of the mechanisms of disease.

Slide 15-18

Such information has been obtained from humans and various other organisms. The suffix -omics is added to a term such as genome, transcriptome, or proteome to indicate the study of biology at a particular molecular level, and -omics refers to the field of study that encompasses all of those levels. A large collection of -omics data results in vast amounts of bioscience data. Humans would have difficulty visually analyzing vast amounts of bioscience data. Thus, the field of bioinformatics is crucial. Using computers to analyze vast amounts of bioscience data allows the consolidation and identification of important information from -omics. To me, gleaning findings from that mountain of data is a treasure hunt with a computer.

Slide 19-20

As I have explained thus far, one method of obtaining -omics data is known as a DNA chip or a microarray. Microarrays appeared in the late 1990s. Next-generation sequencers appeared in the 2000s and have currently garnered attention.

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How are -omics data obtained? As an example, clinical data or samples of peripheral are collected during a blood medical checkup. DNA can be obtained from white blood cells in the blood. A sample of diseased tissue can be obtained, and vast amounts of bioscience data can be obtained using a next-generation sequencer.

Slide 26-27

As I just explained, genomic information is converted into proteins via RNA, and those proteins perform a function. The transcriptome can easily and simply be examined using a DNA chip, and this approach is suitable for diagnostic markers and therapeutic targets. What can be analyzed with a DNA chip can also be analyzed with a next-generation sequencer. DNA chips are often used because of our increased familiarity with them.

Slide 28-30

I will now explain DNA chips (microarrays). A microarray is a densely packed array of several thousand to 10,000 genes on a glass or silicon chip. If we look closer, each black dot is an immobilized DNA fragment corresponding to an individual gene. Use of such a chip allows large quantities of genetic information to be processed and analyzed at one time.

Slide 31

I have thus far explained how -omics data are obtained. I will now explain how those data are used in medicine.

Slide 32-38

I will start by explaining the Millennium Genome Project, which I was involved in while at the National Cancer Center. The Millennium Genome Project assembled healthy individuals and patients and it obtained and analyzed their medical charts and blood samples. The information obtained has been analyzed bioinformatically to identify genes causing diseases and to identify predictors of drug efficacy. Susceptibility to illness and drug efficacy and safety are being determined and new drugs are being developed. This will ultimately lead to personalized medicine, i.e. treatment tailored to an individual, to thus improve patient QOL.

Slide 39-42

The Millennium Genome Project performed 2 types of genetic analysis. The first was genetic analysis of the germline, i.e. analysis of innate genes. Based on those findings, adverse reactions to anticancer agents will be predicted, therapies to prevent cancer will be identified, and drugs will be discovered. The second type of analysis was the genetic analysis of diseased tissue. The particular nature of an illness will be determined, leading to its diagnosis and treatment. These techniques are not limited to cancer and can be applied to various illnesses. I will start of by describing genetic analysis of the germline.

Slide 43

Genetic analysis of the germline involves analysis of polymorphisms.

Slide 44-46

As I just explained, genomic information is converted into proteins via RNA, and those proteins perform a function. If base sequences in the genome differ even slightly, the functions of proteins can change and the amount of proteins can change.

Slide 47-50

Polymorphisms are differences between individuals. When there are individuals with a different genotype in a group of the same species of organisms, polymorphisms refer to the different genes and DNA sequences. Typically, a gene is considered common if it occurs at a frequency higher than 1% in the population while a gene occurring at a frequency lower than 1% is called a mutation.

Blood types are an example of a well-known polymorphism. The ABO blood group system was devised by Landsteiner in 1901 and is the most widely used blood group system. The blood type phenotypes are AB, A, B, and O. Many Japanese are type A while many whites are type O.

Slide 51-54

Another example involves types of earwax. We have long been aware of the dry and wet varieties of earwax. The gene that causes those varieties have already been identified. Adenine is substituted for guanine in ABCC11 on chromosome. Many Japanese have the dry variety while many whites have the wet variety. Such ethnic differences in polymorphisms are known. Other known polymorphisms are related to eye color and nicotine dependence.

Slide 55

I will now talk about the anticancer agent irinotecan as an example of personalized medicine based on polymorphisms.

Slide 56-63

Irinotecan is administered as a prodrug. Irinotecan is metabolized in the liver by a protein such as CES2, resulting in its active form called SN38. SN38 is then inactivated by a protein known as UGT1A1, resulting in SN38G. SN38G is then excreted from the body. SN38 has antitumor action and it causes adverse reactions such as diarrhea and neutropenia, so the concentration of SN38 in the blood must be controlled. There are polymorphisms in the promoter of the UGT1A1 gene. One polymorphism has 6 TA repeats while another has 7 TA repeats. UGT1A1 activity in a person with 7 TA repeats is lower than that in a person with 6 TA repeats, and the concentration of SN38 in the blood is known to be 2 times higher even when irinotecan is administered in the same dose. In other words, whether a person has 7 TA repeats for not is determined beforehand to regulate the dose of irinotecan, thus allowing personalized medicine. The Millennium Genome Project performed an exhaustive genomic analysis and found that polymorphisms in KCNQ5 (a channel-related gene) are correlated with adverse reactions. This is one of the topics I worked on while at the National Cancer Center.

Slide 64

I have thus far talked about genetic analysis of the germline. I will now talk about genetic analysis of somatic cells.

Slide 65-69

Personalized medicine based on an analysis of gene expression involves a search for biomarkers. These substances indicate whether turning a gene on or off is associated with the degree of malignancy. As an example, CCND1 is known to be a useful biomarker for diagnosis. Expression of the CCND1 gene is analyzed in various patients. If the level of expression is low, the patient can be given a good prognosis; if the level of expression is high, the patient can be given a bad prognosis. The reality,

however, is not that simple. Expression is determined by numerous biomarkers, and new biomarkers need to be identified.

Slide 70-73

I will now talk about joint research conducted with the National Cancer Center. A DNA chip was used to analyze samples of esophageal cancer and information on transcriptome gene expression was obtained. A filtering method (S2N', using a modified signal-to-noise ratio) that I developed was then used to rank genes associated with the degree of malignancy of esophageal cancer. We identified the genes KRT7 and FOXA1 as a result. We closely examined these genes and found that FOXA1 is a transcription factor that controls KRT7 and that FOXA1 controls a gene known as LOXL2.

Slide 74-76

The figure on the left depicts the survival curve depending on whether KRT7 expression is present or absent. KRT7-negative patients have a good prognosis while KRT7-positive patients have a poor prognosis. When expression of LOXL2 is silenced, the percent of infiltrating cancer cells, i.e. the metastatic potential of cancer, is inhibited. In other words, patients with high levels of KRT7 expression can be identified by examining KRT7 expression, and personalized medicine targeting LOXL2 can be provided. I have thus used examples of research in personalized medicine to explain data mining based on -omics data.