TOWARDS MOLECULAR MEDICINE: CANCER PHARMACOGENOMICS, PERSONALIZED MEDICINE, AND OUR RECENT APPROACHES

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Abstract

Recent advances in genomics and molecular technologies have given a potential to revolutionize cancer therapy. Most of the existing chemotherapeutic treatments are palliative in the advanced solid tumors, and further responses to the therapies significantly vary among the patients. Genomic data and genome-wide analysis of gene expression derived from high throughput DNA sequencing and DNA chip have shown great promise for hunting novel drug targets in the broadest range and selecting optimal cancer therapy for individual patients through better diagnosis of an “at risk” subgroup. However, these challenges have not been always successful. To determine the critical genes from the large number of candidates, a tremendously large amount of data is statistically required. The methodology or the experimental design to exploit the full power of a global perspective is still controversial. Recent challenges for drug discovery and development of personalized medicine, including ours, are briefly reviewed.

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Introduction

Severe systemic toxicity and unpredictable efficacy are still hallmarks of existing cancer chemotherapies (Watters and McLeod 2003). At present chemotherapeutic treatments are palliative in most of the advanced solid tumors, particularly in the case of the common epithelial tumors. Although these cancers have a few chemotherapeutic regimens yielding prolonged survival, it is clear that new therapeutic options and selection of a better regimen are necessary. Recent progress in genome science and biomedical technology presents the opportunities for novel drug discovery and creating a novel therapeutic strategy, personalized medicine, which would allow selection of optimal regimens to each individual based on the gene expression profile or the genomic make-up (Evans and Relling 1999). High-throughput DNA and mRNA analyses enable us to hunt novel drug targets in the broadest range and understand a basis underlying variable drug response in individual patients, and such analytical approaches and technologies have been actively introduced into cancer research.

Molecular Targeting Drug Development and Identification of Novel Drug Target

Upon the discovery some 20 years ago of the first oncogene defects in cancer, it was envisioned that the genetic information could be translated into therapeutics that could selectively ablate tumors without the systemic side effects often associated with cancer drugs (Gibbs, 2000). Much effort has been directed towards identifying cancer specific targets, and provided a variety of potent candidates for drug development: the mutated genes such as ras, raf, p53, bcr-abl, myc and p16, and the overexpressed gene products such as HER-2, epidermal growth factors (EGF), cyclines, and vascular endothelial growth factors (VEGF) (Sherr, 1996, Shapiro and Harper, 1999, Hahn, Stewart, et al., 1999, Neufeld, Cohen, et al., 1999). The target discovery was translated into new drug and several drugs such as a monoclonal antibody of HER-2 (trastuzumab, Herceptine®), an EGFR-tyrosine kinase inhibitor (gefitinib, Iressa®), and an inhibitor of protein tyrosine kinase family comprising Abl (imanitib mesylate, Glivec®) have been widely used as novel active agents.

These successful results in the drug development clearly show that the understanding of the fundamental mechanisms underlying cancer can lead to drug discovery, and newly developed molecular tools are indispensable for such drug target
identification and its validation. Among a variety of these tools, gene chip technology is considered to be one of the most powerful ways to elucidate critical pathways that might serve to identify targets for drug development (Debouck and Goodfellow, 1999). There are multiple biological pathways and a variety of genes are involved in each of the pathways, but gene chip enables us to simultaneously analyze expression of tens of thousands of genes, generating clues to gene function that can help to identify appropriate targets for therapeutic intervention. A variety of studies are now going on in various laboratories including ours. The recent methods for target validation run the gamut from massively parallel differential expression (and proteomics) screens using microarray technology, which yield relatively limited validation information on many targets in an efficient manner (U’Prichard, 2000).

The goal of such studies is to discover a cancer specific target, and immortalization and uncontrollable growth activity yielding metastasis and invasion are apparently distinguished characters of cancer cells from normal tissue cells. Our recent attention therefore has focused on these mechanisms and their critical regulators. Among them, the regulators responsible for immortalization of cancer cells are our intense study. The key enzyme in cell immortalization, telomerase, is present in nearly all immortal cells, germ-line cells, stem cells, and ~90% of human tumors but seldom in normal somatic cells, and well known to be an attractive therapeutic target (Mo, Gan, et al., 2003). Though, recent studies have suggested that telomerase activation alone may be insufficient to induce transformation, and further additional changes to telomerase activation may be equally important to achieve cellular immortality (Lindvall, Hou, et al., 2003). The factors involved in the additional mechanisms to telomerase activation could be critical and specific drug targets in cancer treatment. The identification might help to understand the cancer metastasis mechanisms. The comparison of gene expression profiles using gene chip in various combination settings of 4 different types of cells—mortal normal, immortal normal, mortal malignant, and immortal malignant cells—enable us to identify genes specifically responsible for immortalization and uncontrollable cell growth of cancer cells (Figure 1). We have already established such analysis system through introduction of human telomerase reverse transcriptase (hTERT) into normal
human cells, and found several possible candidates for novel drug targets, cancer immortalization factors.

**Personalized medicine**

The ultimate goal of drug therapy is to give the right drug at the right dose to the right patient at the right time, but there are significant variations in individual response to anti-cancer drugs in terms of both toxicity and efficacy. Pharmacogenomics is the study of the link between an individual’s genetic make-up and their response to drug therapy, and the progress should enlighten our use of many medications toward the goal. Though, very few critical prediction markers of drug response have been validated to date despite extensive efforts (Mancinelli, Cronin et al., 2000, Diasio and Johnson, 2000, Scherf, Ross, et al., 2000).

1) **Prediction of drug toxicity**

Most anti-cancer drugs are biotransformed or detoxified by polymorphic enzyme systems for each drug, and the clearance of cytotoxic drugs is believed to vary by ten-fold or more among individual patients (Iyer and Ratain, 1998). Since pharmacokinetics mainly depends on the activity of rate-limiting enzymes in liver, genetic polymorphisms of these drug-metabolizing enzymes are thought to be useful in the prediction of drug toxicity. In fact, the prediction of toxicity based on genetic polymorphisms is becoming possible for many classes of chemotherapeutic agents. The most clinically significant examples of such polymorphism-toxicity relationships are: dihydropyrimidine dehydrogenase gene (DPYD) for 5-fluorouracil (5-FU); uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) for irinotecan (CPT-11); and thiopurine S-methyltransferase (TPMT) for thiopurines (Watters and McLeod, 2003). DPD is the initial, rate-limiting catabolic enzyme of 5-FU, and 80-90% of an administered dose of 5-FU is rapidly converted into biologically inactive metabolites through this catabolic pathway. Deficiency in DPD activity is well known as a significant cause of the life-threatening toxicity of 5-FU, and the exon 14-skipping mutation (DPYD*2) appears the most prominent genetic change related to severe DPD deficiency (Diasio and Johnson, 2000). UGT1A1 enzyme would be responsible for the glucuronidation of SN-38, and interindividual difference in the pharmacokinetics of SN-38 and SN-38 glucuronide was strongly suggested to be dependent on the UGT1A1 genotype (Iyer, King, et al., 1998).
Figure 1. Immortalization, transformation, and cancer: in vitro experimental system for drug discovery composed of 4 different types of cells. (Annals of Cancer Research & Therapy, 16-331-Vol. 11-Tannimoto et al.-© 2003 by PJD Publications Ltd.)
Figure 4. Drug sensitivity genes selected by cDNA microarray analysis. Expression analysis of 20,784 clones and cytotoxic assay for 8 anticancer drugs were performed, respectively, by cDNA microarray and MTT assay in 19 human cancer cell lines, and rank correlation analysis between the expression levels and IC50 values were performed to select genes which closely correlated with sensitivity to the drugs. The genes correlated with cellular sensitivity to each drug were colored according to the correlation significance (red, $P<0.01$; green, $0.01\leq P<0.05$; yellow, $0.05\leq P<0.1$; and blue, $0.1\leq P$), and plotted on the spotted place of the target cDNA on the array as a visualized image using computer. All expression analysis using cDNA microarray was performed in duplicate, and three individual experiments were done in the cytotoxicity assay. (Annals of Cancer Research & Therapy, 16-331-Vol. 11-Tanimoto et al.-© 2003 by PJD Publications Ltd.)
Figure 2. Pathway of CPT-11 disposition and related genes.
Figure 3. Cloned 5' region (-2918→+83 bp) of *DPYD*. In the upper scheme, transcription start site was indicated as +1, and the translation initiation site for the DPD protein located at +99. The 5' region (-2918→+83 bp) of *DPYD* was subcloned into a pGL3 basic plasmid vector (Middle), and contained a total of 57 CpG sites indicated as a bar (Top).
More than 30 genetic variations of UGT1A1 have been reported to date, and the variant genotype in the promoter region, UGT1A1*28, is known to yield significant reduction of the gene expression and the enzymatic activity, and thus relates to severe toxicity induced by irinotecan. UGT1A1*28 is a 2-bp insertion (TA) in the TATA box in the promoter [normal (TA)$_6$TAA], resulting in the sequence (TA)$_7$TAA, which have been reported to reduce UGT1A1 activity through decreased expression. Thiopurines are commonly used in the maintenance therapy of acute lymphoblastic leukemia (ALL) in children, and TPMT catalyses the thiopurines to the inactive metabolites. Genetic polymorphism of this enzyme appears to cause a deficiency of TPMT activity, and TPMT deficient patients may require up to 15-fold reduction in thiopurine doses to prevent hematotoxicity (Krynetski and Evans, 1998).

These variant alleles, however, have been increasingly recognized not to always explain the majority of cases in cancer patients with drug toxicity (Collie-Duguid, Etienne, et al., 2000). For 5-FU toxicity prediction, recent attention has been focused on the regulatory mechanisms of DYPD expression, since various reports clearly demonstrated that DPD activity correlates more closely to the mRNA levels (Shestopal, Johnson, et al., 2000, Collie-Duguid, Etienne, et al., 2000). Pharmacokinetics and metabolism of CPT-11 are extremely complex and have been the subjects of intensive investigation in recent years (Mathijssen, van Alphen et al., 2001, Mathijssen, Marsh et al., 2003) (Figure 2). Both CPT-11 and SN-38 are known in an active lactone form and an inactive carboxylate form, between which an equilibrium exists that depends on the pH and the presence of binding proteins, and CPT-11 is subjected to extensive metabolic conversion by various enzyme systems, including esterases to form SN-38, UGT1A1 mediating glucuronidation of SN-38, as well as CYP3A4, which forms several pharmacologically inactive oxidation products. Adverse drug reactions to thiopurines occur in 20-30% of inflammatory bowel diseases, but polymorphisms in TPMT have been shown to predict intolerance in a minority of these patients (Marinaki, 2003).

For most of chemotherapeutic agents, it is likely that drug response is most often a complex trait, with multiple polymorphic genes and environmental factors contributing with various strengths to overall treatment outcome. We are now investigating the molecular mechanisms controlling activity of the key metabolizing enzymes, such as
DPYD, and interaction of multiple polymorphic genes responsible for drug toxicity, such as CYP3A4, CES1, 2, and UGT1A1 for CPT-11 toxicity (submitted) (Figure 3).

2) Prediction of drug efficacy

Differing from the toxicity, drug efficacy is mainly determined by the characters of target cancer cells, which are composed of multiple disorders of genes that occurred in the carcinogenesis steps. The existence of a large number of mutated genes indicates that genetic alterations alone are insufficient to explain all of the biological behavior of cancer cells including their sensitivity to drugs. Furthermore, drug sensitivity mechanisms are multifactorial, and significantly vary among tumors (Tsuruo, 2003, Maliepaard, van Gastelen, et al., 1999, Cresteil, Monsarrat, et al., 2002, Diasio and Johnson, 1999, Zhang, Mack, et al., 1998, Okamoto, Takano, et al., 2002, Nishiyama, Suzuki, et al., 1997, Belcourt, Hodnick, et al., 1996). It is clear that understanding of the interaction of multiple genes is indispensable in the prediction of drug efficacy, and comprehensive gene expression analysis using DNA chip is considered to be one of the possible approaches (Clarke, te Poele, et al., 2001). Though, an approach to predict individual drug response by a certain expression pattern, “a snapshot profile”, provided by cDNA microarray analysis has been increasingly recognized to be limited (Staunton, Slonim, et al., 2001, McLeod and Evans, 2001). DNA chip technology presents the opportunity to overview a huge number of gene expressions simultaneously, but gene expression profiles responsible for drug sensitivity considerably vary even in the same drug efficacy.

However, a variety of genes have been shown to closely correlate with cellular sensitivity to anticancer drugs with clear evidences (Adlard, Richman, et al., 2002, Watters and McLeod, 2003). These findings led us to hypothesize that expression analysis of a set of the key genes could allow us to predict the therapeutic responses of drugs, when we could select truly significant genes in the sensitivities of the drugs and understand their interplay in the expression. We have attempted to select the potent prediction marker genes from various candidates which were previously proved to be a drug sensitivity determinant, and construct an incipient model for simultaneous response prediction of 8 anticancer agents using expression data of a set of the genes. In these studies, we used cDNA microarray as the screening tool of a better prediction marker
Among the candidate genes, and quantified expression levels of the selected genes by real-time RT-PCR to perform multivariate analysis, which can embrace the variable expression levels of the genes and arranged in order to predict those drug efficacy. As the first step, we have developed such a prediction model in in vitro experimental system (submitted). Then, using the same set of genes, we have constructed a prediction system of therapeutic response to 5-fluorouracil (5-FU) as a clinical application model through the investigation of clinical samples and their response data.

Nevertheless, it is obvious that more critical marker genes exist and the identification contributes to the accurate prediction of drug efficacy. To identify them, DNA chip technology is now widely used including our laboratory, but there are no definitive ways to identify such marker genes at present (Kihara, Tsunoda, et al., 2001, McLeod and Evans, 2001). In order to determine the critical marker genes by expression-sensitivity correlation analysis, tremendously large number of data is statistically required: at least, several hundreds or thousands of sample data sets are necessary for the study. A huge number of function-unknown genes are also an obstacle to determine the critical markers from the large number of candidates. cDNA microarray can provide a huge number of the candidates, but most of them are the function- or the relationship-unknown genes, or expression sequence tags (ESTs) (Figure 4). However, global view of gene expression provided by cDNA microarray is a most powerful way for the maker discovery. The methodology or the experimental design to exploit the full power of a global perspective is also our intensive study (Staunton, Slonim, et al., 2001, Quackenbush, 2002).

Conclusions and perspective

Recent advances in molecular technologies have given a potential to revolutionize cancer therapy. Genomics, particularly high-throughput sequencing and characterization of expressed human genes, and pharmacogenomics have created new opportunities for drug discovery and personalization of drug therapy (Emilien, Ponchon, et al., 2000). Among a variety of newly developed technologies, microarray analysis has shown great promise for identifying novel drug targets and individualizing cancer therapy either through better diagnosis of an “at risk” subgroup or via direct markers of chemosensitivity. However, improvements in technology and informatics are necessary
to increase the potential application of this method to the clinic. To challenge these issues, we are establishing a unique in vitro experimental system to determine cancer specific drug targets and developing an incipient model to predict efficacy of anti-cancer drugs. This strategy would increase therapeutic options and improve clinical chemotherapy with the predicted dosage of effective drug.

References


