Moving Towards Personalized Medicine

Naravat Poungvarin, M.D., Ph.D.

Department of Clinical Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Siriraj Med J 2010;62:182-184

E-journal: http://www.sirirajmedj.com

Personalized medicine is a relatively new concept in medical practice. Traditionally, physicians perform medical and family history taking and physical examination, followed by laboratory and imaging investigation, to diagnose and treat patients. In many circumstances, it is difficult to predict responses to treatment. Some patients may not be getting the full benefit of the same treatment. Patients vary in the degree to which the side effect of drugs occurs. Serious adverse effects resulting from medication may occur in some patients. These variability factors pose challenges to physicians, especially those who are new to a practice, in tailoring individual care. Personal observation is generally used to guide what might be suitable for each patient.

Laboratory Medicine in the Post-genome Era

The human genome contains genes, regulatory sequences which control gene expression and vast regions of DNA that serve no obvious biological function. The Human Genome Project was initiated in the late 1980s to determine the entire sequence of the human genome, including both the genes and the non-coding sequences of the DNA. A draft of the human genome was completed in early 2001.[1] The study of the global properties of the genome is usually referred to as "Genomics". It is hoped that detailed knowledge of the human genome will provide biomedical benefits.

Recent developments in a number of molecular assays allow medical practitioners to provide individualized care for their patients. These molecular diagnostic tests detect genomic variations, mutations and single nucleotide polymorphisms (SNPs), which are associated with diseases or drug metabolisms. Test results will be used to provide a specific therapy for an individual patient by determining disease status, predicting disease risk and response to medications, and suggesting appropriate drug dosages.

Molecular Tests for Personalized Therapy

***KRAS* mutation.** The epidermal growth factor receptor (EGFR) is a relevant target for anticancer therapy because it regulates important tumorigenic processes, including cell proliferation, protection from apoptosis, enhanced survival, angiogenesis and invasion. The mutation of the *KRAS* gene (v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog) is a well-established predictive biomarker for response to EGFR-inhibiting drugs, such as panitumumab and cetuximab.[2] The *KRAS* gene encodes K-Ras protein which transmits growth signals from EGFR. Somatic mutations of the *KRAS* gene are common in pancreatic, pulmonary non-small cell, and colorectal carcinomas. In colorectal cancers, a single nucleotide substitution in exon 1 (codons 12 and 13) or exon 2 (codon 61) of the *KRAS* gene is responsible for an activating mutation. The mutated *KRAS* gene is transcribed and translated into continuously active K-Ras protein, which constitutively activates downstream signaling and impedes treatment response to EGFR inhibitors. Currently, *KRAS* gene testing is required for all metastatic colorectal cancer patients by the National Comprehensive Cancer Network (NCCN) guideline and American Society of Clinical Oncology (ASCO) Provisional Clinical Opinion.[3] An EGFR inhibitor is not recommended if the *KRAS* gene mutation is detected in a tumor.

The *KRAS* mutation test may be performed on fresh, frozen, or formalin-fixed paraffin-embedded (FFPE) tumor tissue. Several molecular techniques are currently in use for the detection of the *KRAS* gene mutations. The followings are a few examples of available diagnostic methods for *KRAS* testing in Thailand.

1. Direct sequencing after PCR (polymerase chain reaction) amplification

- Principle: Patient sequence data are aligned for comparison with corresponding wild-type (normal) sequence.
- Advantages: Most widely accessible method; covers all possible mutations in the target region; high specificity.
- Limitations: Low sensitivity (presence of 10-20 percent mutant sequences is the limit of sensitivity by direct sequencing), and it requires a large amount of mutated DNA present in the sample in addition to which it is time-consuming and cumbersome.

2. Allele-specific real-time PCR (DxS Ltd)

- Principle: Mutation specific PCR detection using Amplification Refractory Mutation System (ARMS) and Scorpion real-time PCR technology.
- Advantages: High sensitivity (can detect 1 percent of mutant in a background of wild type genomic DNA); kit based; fast method.

● Limitations: Expensive; detects 7 common mutations in codons 12 and 13 (Gly12Asp, Gly12Ala, Gly12 Val, Gly12Ser, Gly12Arg, Gly12Cys, and Gly13Asp).

3. Strip Assay (ViennaLab Diagnostics)

● Principle: Reverse-hybridization of biotinylated PCR products to a parallel array of allele-specific oligonucleotides immobilized on membrane test strips.

● Advantages: Highly sensitive (can detect 1 percent of mutant in a background of wild type genomic DNA), kit based; visible enzymatic color reaction.

● Limitations: Limited to 10 common mutations codons 12 and 13 (Gly12Ala, Gly12Arg, Gly12Asp, Gly 12Cys, Gly12Ile, Gly12Leu, Gly12Ser, Gly12Val, Gly 13Asp, and Gly13Cys).

***BRAF* mutation.** Even when patients with the mutated *KRAS* gene are excluded, only 40 percent of patients with colorectal cancers respond to anti-EGFR therapy. A Val600Glu (V600E) mutation in the *BRAF* (V-raf murine sarcoma viral oncogene homolog B1) gene which codes for B-raf, a protein downstream of K-Ras, could explain another 10 percent of nonresponse.[4] However, the routine use of *BRAF* testing for patients with metastatic colorectal disease is not recommended. The updated NCCN guidelines suggest that testing for the *BRAF* mutation is considered when the *KRAS* gene is not mutated.

Mutations in the *BRAF* oncogene are also found in other human cancers, such as melanomas, lung cancer and thyroid cancer. Specimen material for the *BRAF* mutation test is genomic DNA from tumor samples extracted from fresh, frozen or FFPE tissue. Over 90 percent of mutations are the Val600Glu mutation.

***EGFR* mutation.** Detection of *EGFR* mutations has become an essential molecular tool when deciding on the proper treatment for non-small cell lung cancer (NSCLC) patients. The American Society of Clinical Oncology has recently updated clinical practice guidelines for the treatment of patients with stage IV NSCLC to include *EGFR* mutation testing in laboratory workup.[5] Somatic mutations found in the first four exons (18-21) of the tyrosine kinase domain cause constitutive activation of the tyrosine kinase property of the EGFR. The EGFR tyrosine kinase inhibitors bind to the tyrosine kinase domain of the mutated EGFR with high specificity and affinity resulting in potent inhibition of the signalling pathways. Therefore *EGFR* mutations may predict sensitivity to the EGFR tyrosine kinase inhibitors such as gefitinib and erlotinib. *EGFR* mutations are more common in never-smokers, patients with adenocarcinoma histology and Asian patients.

The *EGFR* mutation test may be performed on fresh, frozen, or FFPE tumor tissue by PCR amplification of exon 18-21 followed by direct sequencing of the PCR products. Real-time PCR assay based on Scorpion technology (DxS Ltd) enables highly sensitive detection of 29 *EGFR* mutations (T790M, L858R, L861Q, S768I, G719S, G719A, G719C, 3 insertions in Exon 20, and 19 deletions in Exon 19).

***CYP2C9* and *VKORC1* polymorphisms.** Warfarin is the most commonly prescribed anticoagulant drug in the world. Warfarin is a mixture of two active enantiomers, S-warfarin and R-warfarin. S-warfarin is approximately 5 times more potent than R-warfarin. S-warfarin is metabolized by cytochrome P450 2C9 enzyme (CYP2C9) in the liver. Two SNPs in the *CYP2C9* gene, *CYP2C9*2* (Arg144Cys) or *CYP2C9*3* (Ile359Leu), result in reduced enzymatic activity. Patients with a *CYP2C9* variant allele have an increased risk of major bleeding complications when standard warfarin dosing algorithms are used. The prevalence of each variant varies by race. Both variants are rare in Asian.[6]

Warfarin exerts its anticoagulant effect by inhibiting the protein vitamin K epoxide reductase complex, subunit 1 (VKORC1). The variation at the *VKORC1* promoter (-1639), where the common G allele is replaced by the A allele, has a significant impact on expression of the gene. Patients with the *VKORC1* A allele exhibit increased warfarin sensitivity and generally require a lower dose of warfarin than average. The sensitive variants of *VKORC1* are common (89 percent) in the Asian population.[7,8]

The SNP testing for warfarin sensitivity can be used prior to warfarin initiation to reduce bleeding episodes and enable patients to reach the target international normalized ratio (INR) sooner. A variety of methods can be used to detect *CYP2C9* and *VKORC1* polymorphisms, including sequencing and real-time PCR techniques. The source of DNA for warfarin genotyping may be peripheral blood or buccal swab.

Although molecular diagnostics offers the potential to improve the quality of patient care, the results of these tests must be interpreted carefully. Physicians cannot rely solely on the genetic information obtained from test results. Molecular testing results may be inaccurate due to limitations of the method used in each laboratory. Therefore molecular diagnostic tests should be used as tools to guide treatment decisions in parallel to clinical evaluation.

Future perspective

The biological function cannot be inferred from the genome sequence information alone. Most genes are transcribed and their transcripts will eventually be translated into the proteins. Cell functions are controlled by and through these proteins. Various -omics have been developed, such as "Transcriptomics", "Proteomics" and "Metabolomics", to further explore complex networks of molecular interactions. Recent advances in the -omics have enabled a more detailed understanding of human genetics, gene-environment relationships, and the molecular basis of human diseases. New insights discovered by these latest technologies are being translated to clinical practice.

REFERENCES

1. Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, et al. The sequence of the human genome. Science. 2001 Feb 16;291(5507): 1304-51.
2. Meriggi F, Di Biasi B, Abeni C, Zaniboni A. Anti-EGFR therapy in colorectal cancer: how to choose the right patient. Curr Drug Targets. 2009 Oct;10(10):1033-40.
3. Allegra CJ, Jessup JM, Somerfield MR, Hamilton SR, Hammond EH, Hayes DF, et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol. 2009 Apr 20;27(12):2091-6.

4. Di Nicolantonio F, Martini M, Molinari F, Sartore-Bianchi A, Arena S, Saletti P, et al. Wild-type BRAF is required for response to panitumumab or cetuximab in metastatic colorectal cancer. J Clin Oncol. 2008 Dec 10;26(35):5705-12.
5. Azzoli CG, Baker S Jr., Temin S, Pao W, Aliff T, Brahmer J, et al. American Society of Clinical Oncology Clinical Practice Guideline update on chemotherapy for stage IV non-small-cell lung cancer. J Clin Oncol. 2009 Dec 20;27(36):6251-66.
6. Yoon YR, Shon JH, Kim MK, Lim YC, Lee HR, Park JY, et al. Frequency of cytochrome P450 2C9 mutant alleles in a Korean population. Br J Clin Pharmacol. 2001 Mar;51(3):277-80.
7. Moyer TP, O'Kane DJ, Baudhuin LM, Wiley CL, Fortini A, Fisher PK, et al. Warfarin sensitivity genotyping: a review of the literature and summary of patient experience. Mayo Clin Proc. 2009 Dec;84(12):1079-94.
8. Gage BF, Lesko LJ, Pharmacogenetics of warfarin: regulatory, scientific, and clinical issues. J Thromb Thrombolysis. 2008 Feb;25(1):45-51.