Toward Therapies Tailored to Patient Characteristics

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Although considerable progress has been made in the diagnosis and treatment of non-small cell lung cancer (NSCLC) in recent years, this disease is still associated with a dismal prognosis. Some improvements have been reported recently after the implementation of third-generation drugs into the clinic. Realistically, we have probably reached a plateau in terms of clinical outcomes with the ways in which we are currently administering cytotoxic chemotherapy. Further steps could be the implementation of molecularly targeted therapies and pharmacogenomics into the therapeutic armamentarium. Pharmacogenomics is the branch of pharmaceutics that deals with the influence of genetic variation on drug response in patients by correlating gene expression or single-nucleotide polymorphisms with a drug's efficacy or toxicity. In this way, pharmacogenomics aims to develop rational means of optimizing drug therapy with respect to the patient's genotype, to ensure maximal efficacy with minimal adverse effects. Such approaches suggest the advent of "personalized medicine," in which drugs and drug combinations are optimized for each individual's unique genetic makeup.

Pharmacogenomics, the whole genome application of pharmacogenetics that examines the single gene interactions with drugs, holds the promise that drugs might one day be tailored to the individual and adapted to each person's genetic makeup. Environment, diet, age, lifestyle, and state of health can all influence a person's response to medicines, but understanding an individual's genetic makeup is thought to be the key to creating personalized drugs with greater efficacy and safety.

What Are the Anticipated Benefits of Pharmacogenomics?

Pharmaceutical companies will be able to create drugs based on the proteins, enzymes, and RNA molecules associated with genes and diseases. This will facilitate drug discovery and allow drug makers to produce a therapy more targeted to specific diseases. This accuracy will not only maximize therapeutic effects, but also decrease damage to nearby healthy cells. Instead of the standard trial-and-error method of matching patients with the right drugs, physicians will be able to analyze a patient's genetic profile and prescribe the best available drug therapy from the very beginning. This will not only take the guesswork out of finding the right drug, but also speed up recovery time and increase safety as the likelihood of adverse reactions is eliminated.

Pharmacogenomics has the potential to dramatically reduce the estimated 100,000 deaths and 2 million hospitalizations that occur each year in the United States as the result of adverse drug response. Current methods of dosing based on weight and age will be replaced with dosages based on a person's genetics, which will maximize the value of the therapy and decrease the chance of overdose.

The decrease in the number of adverse drug reactions, the number of failed drug trials, the time it takes to get a drug approved, the length of time patients receive medication, the number of medications patients must take to find an effective therapy, the effects of a disease on the body (through early detection), and an increase in the range of possible drug targets will promote a net decrease in the cost of health care.

GENETIC POLYMORPHISMS AND LUNG CANCER

Although the genome of individuals is 99.9% identical, that small 0.1% difference predicts as many as 3 million polymorphisms, which have almost no effects. However, many of them are able to influence protein expression and function, resulting in phenotypes that affect disease or drug response. The genomic approach may lead us to a new world of gene-based medicine, although it is unlikely to materialize for at least another decade. Considering the genetic variation, we can theoretically predict how a patient will respond to a single drug, but the expectations are more relevant, and they include the optimization of drug dosing, reduction of adverse events, improvement of clinical outcomes, and reduction of costs.

Polymorphisms are particularly attractive because they are easy to investigate using any source of DNA, including peripheral blood. For this reason, it is not surprising that the birth of pharmacogenomic research was linked to the identification of specific gene polymorphisms involved in altered drug metabolism or transport; this information could be used to identify groups of patients with a high risk of developing severe drug toxicity and/or poor anticancer response with specific cytotoxic drugs, leading to the principle of individualized chemotherapy.

The concept of specific genetic markers that can predict response to each of the main cytotoxic drugs is closely linked to DNA damage and repair. Radiation therapy and alkylating

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agents, including cisplatin, show the same indiscriminate mechanism of cell damage. Each gray of ionizing radiation causes thousands of single-strand DNA breaks in each cell, cross-links in DNA and with DNA-associated proteins, and 30 to 40 double-strand DNA breaks. Most of this damage is repaired. One of the earliest events after DNA strand breakage by radiation is the poly(ADP-ribosylation) of surrounding proteins by poly(ADP-ribose) polymerase (PARP). PARP binds to single-strand and double-strand breaks to catalyze the transfer of successive units of ADP-ribose from NAD+[1] covalently with itself and with other nuclear acceptor proteins, including p53. PARP-deficient cells are more radiosensitive and exhibit genomic instability, and PARP cycling may be important in apoptosis, DNA replication, and DNA repair.[2]

Several studies have documented that a polymorphism in the DNA base excision repair gene XRCC1 (Arg399Gln) is associated with reduced DNA repair capacity as assessed by the persistence of DNA adducts. This polymorphism is associated with the incidence of several common cancers: head and neck, breast, lung, bladder, stomach, and colorectal. XRCC1 directly participates in both base excision and single-strand break repair. The arg399gln polymorphism occurs at a conserved residue in the PARP-binding domain of XRCC1 and may alter the efficiency of repair processes.[3] Hence, the XRCC1 arg399gln polymorphism could be a good sensor of response to radiation therapy and cisplatin, although few studies have examined the real value of this marker in the clinical setting.

We have recently genotyped 203 patients with non-small cell lung cancer (NSCLC) and 45 patients with small cell lung cancer (SCLC) for the XPD Lys751Gln and XRCC1 Arg399Gln single nucleotide polymorphisms and correlated the results with clinical outcome. Most of the patients (81%) received platinum-based chemotherapy. The patients' genotype frequencies did not significantly differ from controls, and both groups were in Hardy-Weinberg equilibrium for the two polymorphisms. The XRCC1399 variant Gln/Gln genotype was associated with higher median survival time (80 vs 54.6 weeks for the Arg/Gln heterozygous and 55.6 weeks for the wild-type Arg/Arg genotype; $p = 0.09$). At multivariable analysis adjusted for histology, stage of disease, performance status, age, and gender, the Gln/Gln genotype was associated with a better survival of borderline significance in the subgroup of patients treated with cisplatin (HR = 0.55, 95%CI = 0.30–1.00); this association became significant for those with grade III or IV clinical toxicity (HR = 0.46, 95%CI = 0.22–0.98). No association between XPD Lys751Gln genotype and clinical outcome was found.

This prospective investigation provides suggestive evidence of a favorable effect of the XRCC1399 Gln/Gln genotype on survival for platinum-treated NSCLC and, for the first time, for SCLC.[4]

GENETIC PROFILING OF LUNG CANCER

Unfortunately, in NSCLC, as in most malignancies, there are polygenic variations: the influence of environment, lifestyle (e.g., smoking habits), and health status play a relevant role in the pathogenesis, prognosis, and response to treatment. These are good reasons to believe that the evaluation of a single gene will not be sufficient, but it is a good starting point.

Cisplatin remains a milestone in the treatment of NSCLC, and its activity is mainly the result of the formation of DNA adducts, the removal of which is performed by the nucleotide excision repair pathway (NER). Cisplatin resistance is also related to an increased removal of these adducts; therefore, NER plays a key role in this event.

The NER pathway consists of several steps: damage recognition, dual incision/excision, repair synthesis, and ligation. Approximately 30 proteins participate in this repair process; in particular, excision repair cross-complementing 1 (ERCC1) has a crucial role in the incision process, which is the rate-limiting step of the pathway. ERCC1 is a 15-kb repair gene located on human chromosome 19. ERCC1 forms a heterodimer with XPF, and the ERCC1/XPF complex is responsible for the incision cleaving the damaged strand at the phosphodiester bonds between 22 and 24 nucleotides 5′ to the lesion.

ERCC1 defective cells have the most severe DNA repair-deficient phenotype. High ERCC1 mRNA levels are a good prognostic factor after surgery for early-stage NSCLC and predict poor outcome from platinum-based therapies in different cancers.[5]

In a pivotal study, the role of ERCC1 expression in patients with NSCLC treated with gemcitabine/cisplatin was investigated in tumor samples by using quantitative polymerase chain reaction. The median ERCC1 expression in the 56 patients analyzed was 6.7 relative to the expression of the control β-actin. Patients with ERCC1 expression greater than 6.7 had a median survival of 5 months, in contrast with those with lower levels, for whom the median survival was 15 months. This difference was statistically significant, and, more importantly, ERCC1 levels were as an independent predictive variable in a Cox multivariable analysis.

The same type of phenomenon was observed in our laboratory experience, in which we found an inverse relationship between ERCC1 expression level in the tumor and median survival in patients with advanced NSCLC; the predictive information provided by ERCC1 was also maintained at multivariate analysis ($p < 0.0026$).

Recently, the results of a prospective clinical study in which patients with advanced NSCLC were randomized to receive standard chemotherapy (cisplatin/docetaxel) or customized treatment according to ERCC1 intratumoral levels (low ERCC1 level: cisplatin/docetaxel, high ERCC1 levels: gemcitabine/ docetaxel) have been reported. More than 400 patients were included, and the main objective of the study (response rate) was reached: the response rate for patients with low ERCC1 expression (56.6%) was shown to be significantly higher than that for the patients in the control arm (40.4%; $p = 0.02$).[6]

In the adjuvant setting, a retrospective study performed by the IALT investigators assessed the role of ERCC1 expression as evaluated by immunohistochemical analysis. They found that the benefit of adjuvant chemotherapy was

confined to those tumors that were ERCC1 negative, and a paradoxical effect was observed in ERCC1-positive tumors.[7]

BCRA1 is a part of the DNA repair pathway and is considered a molecular marker of response to a range of DNA-damaging and antimicrotubule agents. In some types of cancers, including NSCLC, the BRCA1 function is abrogated, which increases sensitivity to cisplatin and resistance to antimicrotubule drugs.[8] Rosell et al. reported a correlation between BCRA1 and ERCC1 mRNA expression and a role of BCRA1 mRNA level in predicting outcomes for patients with locally advanced NSCLC treated with neoadjuvant gemcitabine plus cisplatin followed by surgery.[9]

The chromosomal aberrations in cancer could consist of deletion, translocation, and insertion. The technique most frequently used to assess these aberrations is loss-of-heterozygosity (LOH) analysis. Many chromosomal regions with frequent LOH were detected in lung cancer, leading to the identification of tumor-suppressor genes with potential involvement in the development and progression of this disease. For instance, a part of chromosome 11 was mapped and sequenced, and the corresponding tumor suppressor gene named ribonucleotide reductase subunit M1 (RRM1) was identified within this area. Overexpression of RRM1 results in a more benign phenotype: for patients with resectable NSCLC, survival was better in cases of high RRM1 expression.[10]

Ribonucleotide reductase has a key role in DNA synthesis and repair, and RRM1 is the most likely intracellular target for gemcitabine. Davidson et al. identified increased expression of RRM1 as the major determinant of gemcitabine resistance.[11]

Two retrospective studies identified RRM1 as a predictive marker of survival for patients with advanced NSCLC treated with cisplatin and gemcitabine. In our experience, when patients with advanced NSCLC treated with cisplatin/gemcitabine were analyzed for RRM1 expression, we found an inverse correlation with survival at univariate analysis ($p < 0.00390$) and a close correlation between ERCC1 and RRM1 levels.[12]

As previously observed for ERCC1, there is an apparently contradictory role of these markers in early versus advanced disease. High levels of ERCC1 and RRM1 predict better survival in early disease but a worse one in advanced NSCLC treated with cisplatin-based chemotherapy. Actually, this is simply the expression of a different prognostic and predictive role of these two genetic markers in NSCLC.

An ongoing study performed at the Moffitt Cancer Center in Tampa, Florida, is selecting chemotherapy treatment on the basis of a differential expression of RRM1 and ERCC1 in fresh tumor specimens.

Thymidylate synthase (TS) is an enzyme with an important role in DNA biosynthesis that is the target enzyme for many antimetabolite agents, including pemetrexed. In many studies, an adverse effect of higher TS levels on prognosis has been documented in different types of human cancers. For patients with colorectal cancer, it was reported that low microsatellite instability, the genotype 2R/2R of the 5′-untranslated region-enhancer region of the TS gene, and lower intratumoral expression levels were associated with a good response to chemotherapy.[13] TS protein expression correlates significantly with higher proliferative activity of NSCLC cells and with poor prognosis. We designed a study to investigate the detection of TS gene activity and protein expression in patients with chemotherapy-naive NSCLC. We included 56 patients from stage I to IIIA, most with adenocarcinoma histology. The quantification of mRNA from formalin-fixed, paraffin-embedded specimens was performed in real-time polymerase chain reaction, and the median expression level for adenocarcinoma was 1.16, whereas the median expression level was 2.17 for squamous cell carcinoma ($p < 0.0001$) (Figure 1). Comparable results were documented in the IHC analysis. This could explain the unique data regarding UFT activity in the adjuvant setting, as previously reported by Japanese investigators: 84% of patients included in the UFT Japanese meta-analysis had a diagnosis of adenocarcinoma, and no benefit was reported for patients with squamous carcinoma. We also found a strong correlation between TS mRNA and protein levels. The analysis of TS transcript regulation in snap-frozen specimens by polymerase chain reaction showed that TS expression levels in tumors were considerably higher than levels in normal tissues.[14]

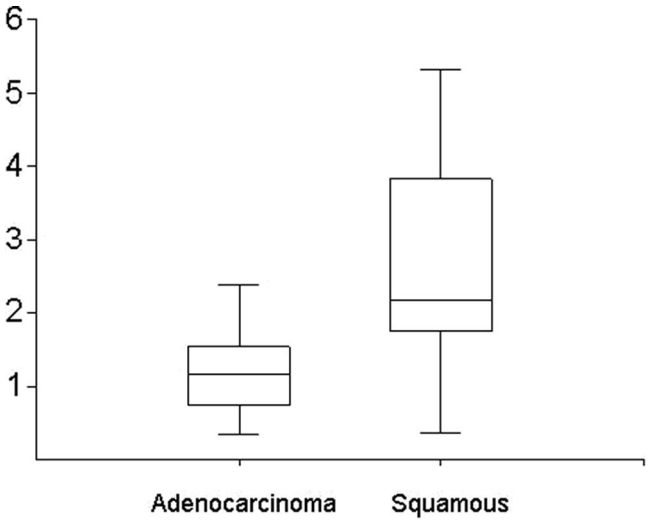

FIGURE 1. Thymidylate synthase mRNA levels in adenocarcinoma compared with squamous cell carcinoma. The *horizontal lines* in the *boxes* represent median values; upper and lower bars represent the distance of the 10th and 90th percentile from the median. $p < 0.0001$.

CONCLUSION

Pharmacogenomics may represent a major step forward in improving the outcomes of cytotoxic chemotherapy in many solid tumors, but there is urgent need for the prospective validation of the experimental hypothesis. In addition to the benefits, a number of potential barriers to the implementation should be recognized. First, the correlation is not always 100% because of polygenic and environmental influences. Second, the correlation requires biological plausibility, which may be possible with a haplotype but not with a specific mutation. Finally, the concept requires clinical vali-

dation through specifically designed clinical studies, and the development of a specific test may not be a sufficient economic incentive. In fact, patents can be appealing from a financial point of view but at the same time be a cause of stagnation.

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