as a result of regulating tumor cell differentiation. It is possible that regulation of calcium cellular influx may modulate mitogenic signal transduction pathways in SCLC tumors, potentially inhibiting tumor cell proliferation.[5]

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Differences of Gene Expression in Non-small Cell Lung Cancer

Are Histology, Tumor Site, and Methodology Relevant?

To the Editor:

A number of studies have been carried out to identify predictive biomarkers of chemotherapy activity in non-small cell lung cancer (NSCLC). Unfortunately, the results obtained are contradictory, and tailored treatment of patients on the basis of gene expression profiles is still far from being standardized. A possible explanation could reside in differences of gene or protein expression depending on tumor histology or site of biopsy. Because only few studies have tried to address this critical point, we appreciated the article by Kang et al.[1] By means of immunohistochemistry (IHC), they observed that the expression of ERCC1 and XRCC1 was similar in adenocarcinoma and squamous cell carcinoma.

Accordingly, in our study,[2] looking at the expression level of genes involved in gemcitabine and cisplatin activity, with quantitative real time (RT)-polymerase chain reaction (PCR) analysis, we found that *ERCC1* expression is not influenced by NSCLC histology, suggesting that adenocarcinoma and squamous cell carcinoma have a similar genetic susceptibility to platinum compounds. Indeed, a recent clinical study[3] showed that the outcome after cisplatin-gemcitabine treatment is similar in patients affected by adenocarcinoma or squamous cell histology tumors. Kang et al. also reported that ERCC1 and XRCC1 were overexpressed in neoplastic lymph nodes compared with primary tumors. On the contrary, we did not observe any significant difference in expression with respect to the origin of the specimens. The possible reasons for data discrepancy could be due to not only the different ethnicity of the patients (Asian versus Caucasian) but also the different methods and targets.

Although the study included three different proteins investigated in a relatively small sample size, the authors did not perform correction for multiple variables in their statistical method. Another important point is the different technique; in the work by Kang et al., protein expression was evaluated by IHC in paraffin-embedded specimens, whereas we measured mRNA expression by quantitative RT-PCR in laser-microdissected frozen tumors. IHC is a sensitive and versatile method, but it is largely empirical; the outcome depends on the antibody used and on the expertise of the pathologists. In the specific case, there is not yet a consensus about the antibody 8F1, which was used by Kang et al.[1] to detect the ERCC1 protein.[4]

Quantitative RT-PCR method is a sensitive and specific technique, with appropriate controls that can be used for intralaboratory or interlaboratory validation. We are aware that mRNA can differ from protein expression, and we believe that optimization of both these methodologies and standardization of technical procedures are necessary before larger retrospective and prospective studies can address the same pharmacogenetic question. Other critical points of the study regard the heterogeneity of treatments and the different stage and lymph node infiltration. All these factors could be confounding elements in the analysis of correlation with outcome.

In conclusion, we agree with Kang et al.[1] that retrospective observational studies on histology and tumor site can provide strong rationale for future trials, but we believe that optimization or standardization of technical procedures and the use of larger and uniformly treated populations are crucial before prospective studies can identify the best markers for tailored treatment of NSCLC patients.

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In Response:

We thank Dr. Toffalorio and coworkers for their comments and interest in our article. All their questions were valuable, and they reminded us of the potential problems of our study. As Dr. Toffalorio commented, standardization and optimization are big issues in immunohistochemistry (IHC). This is one of the major obstacles preventing generalization of IHC data. To maintain the quality of tests and standardization, we performed several pilot tests with different antibody concentrations in normal lung tissue and other cancer tissues. After this, we chose the optimal concentration level, and this was used in this study. To minimize the technical error, we made a tissue array and most of the procedures were performed by an automated method and not by the manual method. We initially tried to include a needle aspiration biopsy specimen, yet it was difficult to mount the small amount of tissue on the tissue array, so IHC was performed by the manual method; however, the quality of IHC staining was unstable. So, we discarded the data drawn from the manual method. It is our opinion that the method used in this study was quite reliable and reproducible.

Dr. Toffalorio and coworkers also questioned about clone 8F1 antibody. However, another research group in our institute and other researchers have already reported studies that were performed using the same antibody clone.[1–3] We think that this antibody is one of the reliable antibodies that can be used for IHC of ERCC1.

In terms of the correction of multiple variables, we did not perform multivariate analysis. Because we did not have a large number of cases in each stage, we thought it would be impractical to conduct multivariate analysis on such a small number of cases. Furthermore, the most important point that should be addressed was not the difference in survival but the difference of protein expression between the primary tumors and the metastatic lymph nodes, so we thought that multiple comparison correction was not a critical prerequisite for this study. However, we have a plan to include the results of multivariate analysis in a future study with a larger number of cases.

We had 30 N1 and 52 N2 patients in the study population. Because we selected non-small cell lung cancer patients with nodal metastasis, those patients are homogenous in terms of nodal metastasis in our opinion. We thought that the T stage was not an important factor because the purpose of this study was comparing between the primary tumor and the metastatic nodes, and the survival in this group of patients is usually determined by nodal metastasis rather than the status of the primary tumor. For the same reason, the treatment method (such as the extent of surgical resection) would not be an important factor for the difference in the protein expression levels. We thought that the treatment in this study was relatively homogenous in that all the patients received complete surgical resection and platinum-based chemotherapy.

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Serum Concentrations of Erlotinib at a Dose of 25 mg Daily

To the Editor:

In this journal, our group recently reported a retrospective review of the clinical efficacy of erlotinib at a dose of 25 mg/d for patients with metastatic non-small cell lung cancers (NSCLCs) with somatic mutations in the epidermal growth factor receptor (*EGFR*) gene.[1] The seven patients included in that study attained a response rate of 71.5% and a median progression-free survival of 17 months (95% CI, 6–35 months). We speculated that the serum concentrations achieved with erlotinib 25 mg/d were similar to the serum concentrations observed with gefitinib 250 mg/d. Based on the published phase I trials for these EGFR tyrosine kinase inhibitors, the mean serum trough concentration attained with gefitinib 250 mg/d was between 0.16 and 0.24 μg/mL or 0.35 and 0.53 μM,[2] whereas the mean serum concentration measured with erlotinib 25 mg/d was approximately 0.22 μg/mL or

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