nisms from the shape of the dose-response curve and that presence of a resistance factor (e.g., the ERCC1 mentioned by Brunetto et al.) would give a shoulder on the dose-response curve when log cell survival was plotted against linear doses (analogous to competitive inhibition of drug effect). Mutation of an obligate target or activating system, would give a reduced slope on the curve, analogous to noncompetitive inhibition of drug effect.³

On the basis of our hypothesis and on our observation of the flattening of the dose-response curve in NSCLC, we postulated that our inability to cure metastatic NSCLC with chemotherapy is ultimately due to deficiency or saturation of something required for drug efficacy. Furthermore, as no metastatic epithelial cancer can be cured even by high-dose chemotherapy, we hypothesized that all epithelial cancers (e.g., breast, colorectal, and lung cancers) may share a common reason for this incurability.² For different epithelial cancers or for different patients with the same type of cancer, a higher or lower maximum cell kill may be achievable, and it may take fewer or more drugs or higher or lower doses to achieve this maximum cell kill, but the outcome is ultimately the same. Resistance factors such as ERCC1 could affect degree of palliation in individual patients but would not in themselves be responsible for incurability.

A corollary of this is that, even if all epithelial cancers are incurable when metastatic, higher drug doses, or addition of more agents may be useful for relatively sensitive tumors such as breast and small cell lung cancer (SCLC) but would be much less useful for more resistant tumors such as NSCLC and pancreatic cancer. In keeping with this, when we did the same type of analysis in SCLC as in NSCLC, the maximum achievable cell kill was greater in SCLC, and cell kill seemed to keep increasing over a wider dose range, but a maximum possible cell kill was nevertheless ultimately reached.⁴

A second corollary is that if we are correct that a common mechanism is responsible for the incurability of all epithelial malignancies, then a single new approach targeting this mechanism could similarly have a dramatic impact on outcome of a broad spectrum of epithelial malignancies.

David J. Stewart, MD, FRCP
Department of Thoracic/Head and Neck Medical Oncology
The University of Texas MD Anderson Cancer Center
Houston, Texas

REFERENCES


Clinical Significance of Serum Vascular Endothelial Growth Factor in Malignant Pleural Mesothelioma

To the Editor:

We read with great interest the article by Yasumitsu et al.,¹ who reported the clinical significance of serum vascular endothelial growth factor (VEGF) in malignant pleural mesothelioma (MPM). VEGF is an important regulator of angiogenesis and might have critical roles in MPM progression. Demirag et al.² demonstrated a significant correlation between

Disclosure: The authors declare no conflicts of interest.
Address for correspondence: Nobukazu Fujimoto, MD, PhD, Department of Respiratory Medicine, Okayama Rosai Hospital, 1-10-25 Chikkomidorigamachi, Minamiku, Okayama 7028055, Japan. E-mail: tfujii@okayama=h.oro.fuku.go.jp
Copyright © 2011 by the International Association for the Study of Lung Cancer
ISSN: 1556-0864/11/0605-0971

Copyright © 2011 by the International Association for the Study of Lung Cancer

971
the VEGF expression on immunohistochemistry and short survival of MPM.

Yasumitsu et al. reported in their article that patients with MPM demonstrated higher levels of serum VEGF than those of normal subjects. In addition, they also mentioned that patients group of MPM with higher serum levels of VEGF had shorter overall survival than those with lower level; however, in their report, serum VEGF levels showed significant tendencies to increase as the clinical stage went up. Based on these findings, it is supposed that patients with higher serum levels of VEGF contained more patients with advanced stage of MPM. These findings indicate that serum levels of VEGF reflect the tumor burden or disease progression of MPM. In such situation, the prognostic significance should be examined based on multivariate analysis as Cox regression model, as in the report of Demirag et al. At this point, we suppose we had better be more cautious about the prognostic significance of serum VEGF in MPM.

Nobukazu Fujimoto, MD, PhD
Kenichi Gemba, MD, PhD
Takumi Kishimoto, MD, PhD
Department of Respiratory Medicine
Okayama Rosai Hospital
Okayama, Japan

REFERENCES

How Do We Do It?

Another Optimal Methodology for Endobronchial Ultrasound Sample Handling

To the Editor:

We read with great interest the article by Nakajima and Yasufuku1 in the January issue of the Journal of Thoracic Oncology. These authors reported a methodology for analysis of samples obtained by endobronchial ultrasound (EBUS), not only initially developed for mediastinal staging of non-small cell lung carcinoma (NSCLC) but also widely used for the initial diagnosis of mediastinal metastatic lymph nodes. In this article, they report a multidirectional analysis to obtain precise diagnosis and molecular testing, particularly emphasizing the critical use of freshly stored material.

We would like to report our own experience of a cell aspirate freezing method, allowing multiple cell analyses. This method, although it presents certain limitations, has a number of notable differences.

Our sampling method has already been described.2 The entire procedure is performed by a cytopathologist for rapid on-site examination and specimen handling. One to three smears are performed for each aspirate, and in the case of abundant material, the extra aspirate is flushed into a tube containing Roswell Park Memorial Institute cell culture medium. After the last aspirate, the needle is rinsed with culture medium to obtain a cell suspension. Clots or tissue fragments, when observed are removed, fixed in 10% formalin, and paraffin embedded for cell blocks. Representativeness of cell suspensions is checked by a stained cytospin. Cell suspensions are centrifuged, and cell pellets are frozen at −80°C in 20% dimethylsulfoxide (Sigma, France) as usually performed for cell lines: one to four frozen aliquots are stored per specimen.

From March 16, 2007 to December 9, 2010, 450 patients underwent conventional transbronchial needle aspiration (TBNA; N = 160) or EBUS-guided TBNA (N = 280). As systematic storage at −80°C was started in May 2007, 425 cell aspirates have now been frozen and stored (96% of all TBNA performed over the last 38 months). Two hundred sixteen of these stored samples have been thawed for complementary techniques. The other samples have been kept frozen for possible subsequent analysis, if necessary.

When complementary techniques are required, the samples are thawed, and cytospin is performed to check the quality of the cells. It must be emphasized that this method of cell freezing is usually associated with very well-preserved cell morphology.

Depending on the suspected diagnosis, frozen and fresh cells can be investigated by various methods. Flow cytometry is used for the diagnosis of lymphoma (lymphocyte immunotyping), small cell lung carcinoma (CD56 expression), or DNA ploidy. Tumor cell differentiation markers (TTF1, P63, CK7/CK20, estrogen receptors) are studied in NSCLC or in patients with a history of extrathoracic cancer, either on smears, cytospins from frozen cells or cell blocks. Molecular analysis for EGFR and Ras mutations is performed on thawed cell pellets in NSCLC, and fluorescence in situ hybridization analysis is performed on cytospins from frozen cells.

In conclusion, we agree with Nakajima and Yasufuku that an optimal methodology is essential for the management of specimens obtained by EBUS-TBNA, particularly when rapid on-site examination is not possible. Fresh cell aspirates in cell culture medium stored at −80°C in dimethylsulfoxide, which is a very simple method, ensure optimal cell preservation for morphology, allowing a wide range of complementary techniques (flow cytometry, molecular genotyping, etc.).

Valérie Gounant, MD
Service de Pneumologie
Service de Chirurgie Thoracique
Hôpital Tenon, AP-HP
Paris, France

Disclosure: The authors declare no conflicts of interest.

Address for correspondence: Valérie Gounant, MD, Service de Pneumologie, Hôpital Tenon, 4, rue de la Chine, 75020 Paris, France. E-mail: Valerie.gounant@tnn.aphp.fr

Copyright © 2011 by the International Association for the Study of Lung Cancer

ISSN: 1556-0864/11/0605-0972