The Expression Of Signal Transduction Proteins And Their Relationship To Clinical Findings In Patients With Nonsmall Cell Lung Cancer

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Citation

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Abstract

In nonsmall cell lung cancer (NSCLC), prognostic factors have been established including tumor stage and performance status, however, survival within the same stage is variable and additional prognostic factors are needed. We investigated a microarray of paraffin-embedded sections from 108 cases of NSCLC which were immunostained with monoclonal antibodies against Ki-67, FRAP, HER2, PTEN, VEGF, COX-2, CD117, and PDGF, using an enhanced sensitivity avidin-biotin peroxidase technique. Staining was graded as a percentage of positive cells, and results correlated with survival by log-rank test and Kaplan Meier survival plots. Improved survival was correlated with female gender, squamous cell histology, low Ki67 expression, and lack of FRAP expression. VEGF and CD117 expression was correlated with stage, while COX2 expression showed significant differences by ethnicity. There was no statistical difference between the staining patterns of HER2, PTEN, and PDGF by pathologic factor, patient demographics, or survival outcome.

INTRODUCTION

Lung cancer is the second most common form of cancer, and the leading cause of cancer death, in both men and women in the United States. Data from the American Cancer Society for 2006 projects that nearly 174,000 cases of lung cancer will have been diagnosed, with approximately 160,000 deaths.[1] The incidence in men has begun to decrease, but may be reaching a plateau in women. While prostate and breast cancers are more common in men and women, respectively, lung cancer is still a far more lethal malignancy in either sex, with a five-year survival rate that is less than 15%. In men 32% of all deaths from cancer are going to be due to lung cancer, while in women, 25% of all cancer-related deaths are due to lung cancer.

The philosophy of therapy for nonsmall cell lung carcinoma (NSCLC) has recently changed considerably to include active front-line and salvage therapies, as well as adjuvant therapy for completed resected lesions. Approximately 35% to 40% of these patients have metastatic disease at the time of diagnosis. Chemotherapy for stage IV non-small cell lung cancer is still suboptimal, and for all intents and purposes, metastatic lung cancer remains incurable. Front-line therapy in advanced NSCLC provides a median survival of 6 to 8 months and a 1-year survival of 10-20% in older series; in more recent series, one-year survival rates of around 35% are now commonly noted. Adjuvant platinum-based therapy affords a modest survival benefit in a majority of recent trials. With the development of newer regimens and agents, such as pemetrexed and epidermal growth factor receptor inhibitors, as well as additional agents in development, greater degree of clinical benefit may be delivered.

In NSCLC, several prognostic factors have been established including tumor stage and performance status, but survival within the same TNM category shows a great variability. As in other malignancies, factors in addition to Tumor-Nodes-Metastases staging are proving to be potent indicators of the prognosis of patient subsets. Thus, further definition of independent prognostic variables is needed. We report the result of an investigation of the immunohistochemical expression of a panel of biologically relevant markers in patients with NSCLC to determine if their expression correlates with prognosis in a cohort of patients.

METHODS

STUDY SUBJECTS

Patients were identified retrospectively from a tumor tissue

bank maintained by the Department of Pathology of the University of Texas Medical Branch (UTMB). All tissues were obtained from paraffin-fixed blocks of tissues created from the years 1997-2002, and were obtained either from a diagnostic core needle biopsy, or at the time of surgical resection by either a standard lobectomy, or pneumonectomy, as dictated by the clinical situation. There was insufficient material from routine fine needle aspiration to perform the study-related investigations. Approval for the study was obtained from the institutional review board (IRB). Criteria for inclusion in the study required that (1) for the patients with resectable disease, they must not have received radiation or chemotherapy before surgery, (2) patients who underwent uncomplicated complete surgical resection (lobectomy or pneumonectomy) for clinical stage I or II NSCLC by the same surgeon (J.B.Z.), (3) the patients have follow-up data and treatment information, and (4) glass slides and paraffin blocks of the tumor were available for analysis. Demographic information was obtained from the medical chart, and the tumor information was obtained from the pathology report. All subjects' identifying information was coded to preserve confidentiality. The histologic type and grade of the tumor was reconfirmed by one pathologist (A.H.). Follow-up and cause of death was retrieved from files of the tumor registry of the hospital.

All patients were evaluated and cared for by the UTMB Thoracic Oncology Group. Front-line chemotherapy, when indicated, consisted of carboplatin and either a taxane (paclitaxel or docetaxel) or gemcitabine. External beam radiotherapy was delivered using anteroposterior and off-cord fields to 60 Gray in 30 fractions for definitive therapy, or 30 Gray in 10 fractions for palliation; patients with Stage IIIa or IIIb without effusion disease received combined weekly taxane and carboplatin with external beam radiotherapy. No patient received prophylactic cranial irradiation.

LUNG CANCER TISSUE ARRAY AND IMMUNOHISTOCHEMISTRY

Sections from a representative nonnecrotic area of the tumor were selected for the tissue microarray construction utilizing a Tissue Arrayer-I® (Beecher Instruments, Silver Spring, MD), in accordance with the manufacturer's instructions. Tumor tissue was assembled to produce the tissue array, sectioned into 1.5 mm diameter by 4 μm thick slices, and then the sections were transferred to adhesive-coated slides. One section from each tissue array block was stained with hematoxylin–eosin. The remaining sections were evaluated by immunohistochemistry.

Microarrays of paraffin-embedded sections from cases of NSCLC were immunostained with monoclonal antibodies directed against Ki-67 (H-300, directed against amino acids 2641-2940), HER-2-neu (HercepTest), PTEN (phosphatase and tensin homolog deleted from chromosome 10; clone A2B1, directed against amino acids 388-400), vascular endothelial growth factor B (VEGF; clone N-19, directed agains the N-terminus), cyclo-oxygenase isoenzyme 2 (COX-2; clone H62), CD117 (c-KIT; stem cell factor receptor), and platelet-derived growth factor B (PDGF; clone H-55, directed against amino acids 136-190), using an enhanced sensitivity avidin-biotin peroxidase technique, as described previously by Haque and colleagues.[2] The same microarray was immunostained with H-266, a polyclonal antibody against a recombinant protein corresponding to amino acid 1920-2185 mapping an internal region of FKBP-rapamycin associated protein (FRAP), also using an enhanced sensitivity avidin-biotin peroxidase technique. All antibodies were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA), except HercepTest and anti-CD117, which were obtained from DAKO Cytomation A/S (Carpenteria, CA). The immunostained slides were evaluated and graded by the same pathologist (A.H.) without knowledge of the patient's clinical history. Staining was graded on the basis of the percentage of positively stained cells: 0=0-5%, 1=6-25%, 2=25-50%, 3=50-75%, 4=>75%.

STATISTICAL ANALYSIS

The Kaplan-Meier method was used to estimate the survival of patients. The log-rank test was used to compare cumulative survival among these patients stratified by various factors. The Fisher exact test was used to examine the association of the target proteins of interest and with multiple demographic and clinical factors. P values less than 0.05 were considered significant.

RESULTS

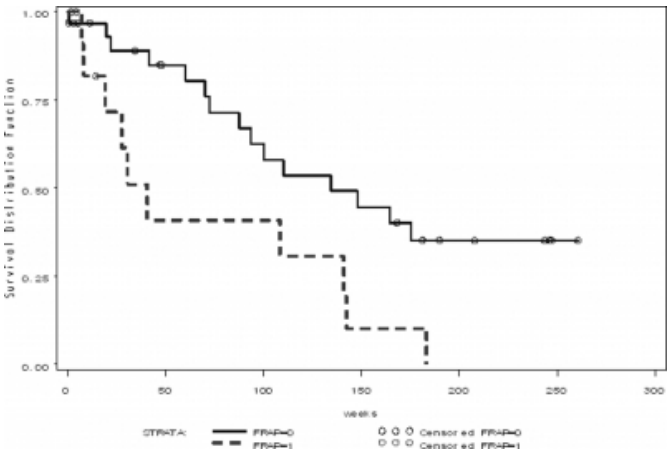
A total of 108 patients qualified for inclusion in the study based on the previously mentioned criteria. Mean age of group was 60.3 years, and the median age was 61 years (range 37 – 90 years); 41% were females (mean age: 59 years) and 59% were males (mean age: 61years). There were 65 Caucasian, 30 African American, and five Hispanic patients; 8 were of unknown ethnicity. Twenty-four of 44 female patients had adenocarcinoma (55%), 14 had

squamous cell carcinoma (32%), and 6 had undifferentiated carcinoma (13%). Twenty-five of 64 males had adenocarcinoma (39%), 33 had squamous cell carcinoma (52%) and 6 had undifferentiated carcinoma (9%). Fifty-two percent had Stage I disease, 23% Stage II, 20% Stage III, and 5% Stage IV disease. Due to the requirement for sufficient pathologic material, many potential patients with higher clinically-staged disease were excluded, as the diagnosis was usually made on the basis of a fine needle aspiration. Mean survival of females was 118 weeks, and of males was 107 weeks; survival with adenocarcinoma was 102 weeks and with squamous cell carcinoma was 155 weeks.

Immunoexpression of FRAP was seen in 20% of tumors. Fifty-one percent of patients with no expression of FRAP were alive compared to 23% with FRAP expression (p=<0.010, Figure 1).

Figure 1

Figure 1: Impact of FRAP expression upon survival

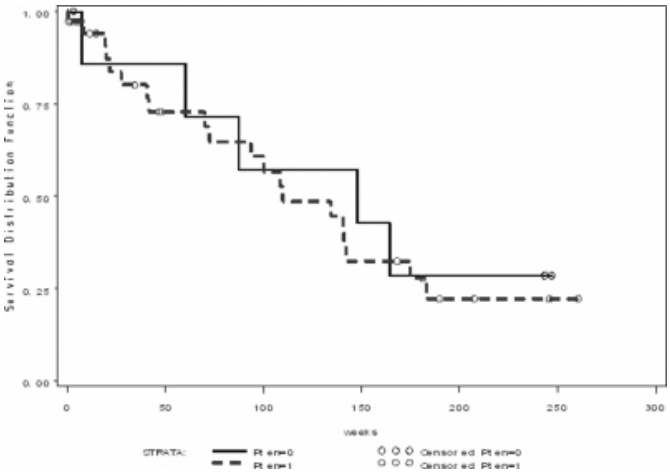


FRAP is a serine/threonine kinase which is highly conserved in organisms ranging from yeast to mammals, and homologues have been identified in plants, C. elegans, and D. melanogaster.[3,4,5,6,7] Preclinical data indicates that FRAP plays a critical role in the regulation of cell size and proliferation. Interference with embryonic FRAP function is lethal. FRAP acts as a cell sensor of nutrient levels, energy, and the presence of mitogens. FRAP is activated after binding of growth factors such as insulin, the insulin-like growth factors, and epidermal growth factor, to their respective cell surface receptors. Upon receptor activation, there is activation of phosphoinositol-3-kinase, which subsequently phosphorylates inositol. Phosphoinositol leads to activation of AKT, which in turn activates FRAP both directly and by phosphorylation via the tuberin/hamartin/RHEB complex. This subsequently initiates the translation of key mRNAs for proteins required for cell cycle progression from G1 to S phase. Blocking FRAP affects the activity of the 40S ribosomal protein S6 kinase (p70s6k) and the function of the eukaryotic initiation factor 4E-binding protein-1 (4E-BP1), leading to growth arrest in the G1 phase of the cell cycle. (reviewed in Hay and Sonenberg, and Bjornsti and Houghton)[8,9]

In contrast to the results observed with FRAP, there was no correlation observed between any of the clinical outcomes assessed and the expression of PTEN (Figure 2).

Figure 2

Figure 2: Impact of PTEN expression upon survival



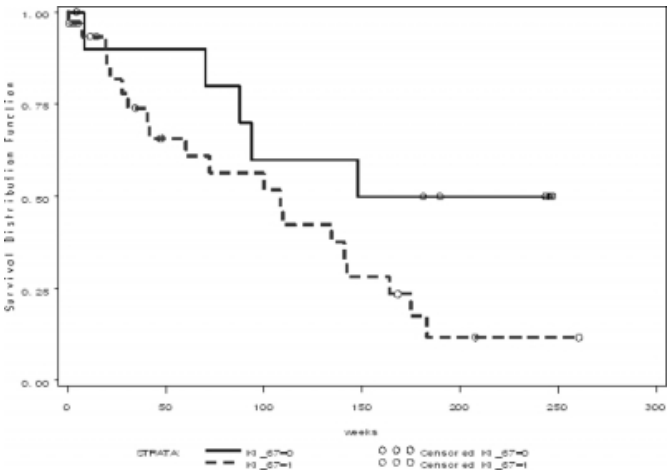
PTEN is one of the most frequent targets for mutation in human cancer. Located on chromosome 10 (10q23), it has been identified as a tumor suppressor gene; somatic deletions or homozygous mutations of PTEN have been detected in a large proportion of human cancers.[10,11,12] PTEN is a lipid phosphatase which counteracts the activity of PI3K by removing the phosphate groups from phosphoinositol. This, in turn, leads to a potential decrease in activation of AKT, and then downstream of that, FRAP. However, it is unclear as to why there appears to be no correlation between these two factors in this patient population.

Seventy percent of the tumors showed high proliferation index (>30% Ki-67-positivity), which was associated with poorer survival (Figure 3), although it did not reach significance (p=0.06); squamous cell carcinoma had a stronger association with Ki-67 expression compared to adenocarcinoma (p=0.04). This is similar to other tumor histologies such as breast cancer, where a greater fraction of

cells that are actively in mitosis is correlated with a worse prognosis. It is possible that the differences observed between the two major NSCLC histologic subtypes in our sampling may either reflect differences in underlying biology, or relatively small numbers within our sample. To further characterize these findings will require a prospective trial with sufficient statistical power.

Figure 3

Figure 3: Impact of Ki67 expression upon survival



VEGF and CD117 correlated with stage; 83% and 35% of stage II or higher tumors expressed VEGF (P=0.025) and CD117 (P=0.010) respectively. COX2 expression showed significant difference between Caucasian, African American and Hispanics (p=0.005). There was no statistical difference between the staining pattern of the other two cell surface tyrosine kinase receptors analyzed here, HER2-neu and PDGF, and cell type, tumor stage or survival

DISCUSSION

Many recent studies have been reported which have characterized a growing number of molecular aberrations which describe the neoplastic state. In many cases, attempts have been made to determine any potential prognostic significance of these changes. These studies have covered a wide range of tumor types and stages of disease, including NSCLC.

Currently, the accepted staging system for NSCLC takes into account only histopathologic features, such as tumor size, location, degree of local invasion, and intrathoracic lymph node status, in addition to the absence or presence of metastases. However, this information only provides a crude approximation of a prognosis for a population of patients with similar characteristics; it cannot be used to predict whose disease is more likely to progress, require adjuvant or more aggressive therapy, to customize therapy for a specific lesion.

Eight potential biomarkers were chosen for this study as they have either demonstrated prognostic significance in other tumor types, even to the point of altering clinical practice, such as HER2-neu expression in breast cancer, or they have demonstrated a potential impact upon prognosis in NSCLC. Furthermore, the biological behavior of six of the proteins can be manipulated pharmacologically by approved agents which, in turn, may affect prognosis.

In our study there was no significant correlation between the cell type, tumor stage, or overall survival and the expression patterns of HER2-neu, PTEN, or PDGF. HER-2 is a homodimeric cell-surface tyrosine kinase of the epidermal growth factor receptor (EGFR) family. It has no known ligand, but does retain competent kinase activity. Dimerization, either with another subunit of HER2 itself or another member of the EGFR family member after ligand binding may lead to activation of the kinase function. Increased HER-family kinase activity is associated with an increased tumor cell growth fraction, resistance to the cytocidal effects of chemotherapy and ionizing radiation, tumor neoangiogenesis, metastatic potential, and decreased apoptosis. All of these are associated with a more aggressive tumor biology, which has been demonstrated most clearly in breast cancer, where an increasing degree of HER2-neu expression is associated with a progressive decrement in survival. This tendency is partially abrogated by the targeted monoclonal antibody trastuzumab. This agent, which is approved for use in women with HER2-neu-overexpressing breast cancer, improves the rate of tumor response from 29% to 45%, and the median survival from 20.3 to 25.1 months.[13] While HER2-neu expression can also be demonstrated in a minority of tumor specimens from other lesions, such as carcinomas of the prostate, colorectum, or even lung, it is unclear whether it has any biologic activity or impact upon prognosis in these other lesions.[14,15,16,17,18,19] An evaluation of the potential activation of downstream targets of HER2 has not been reported in these lesions. Thus, it is difficult to determine if HER2 expression has any biologic significance in human neoplasia outside of breast cancer.

That VEGF expression correlates with stage is not surprising. As tumors enlarge and progress, they produce proangiogenic factors in order to stimulate the production

and maintenance of their neovasculature. VEGF is possibly the most potent and widely expressed of these cytokines. While some of the available antineoplastic agents possess some antiangiogenic activity, such as thalidomide and alpha interferon, the anti-VEGF monoclonal antibody bevacizumab is the only agent with specific anti-angiogenic activity associated with clinical benefit. However, specific attempts to interfere with VEGF-induced angiogenesis have been met with mixed results. Bevacizumab has been approved for use in the United States for the therapy of colorectal cancer. Trials in breast cancer failed to show sufficient clinical activity, however early studies in NSCLC have demonstrated potential clinical benefit.[20,21,22] Further evaluation of bevacizumab and similar agents may delineate a role for antiangiogenic therapy in NSCLC.

The finding of CD117 expression trending with tumor stage is unexpected. CD117, the stem cell factor receptor (also known as c-KIT) has been found by immunohistochemistry on a proportion of solid tumors, predominately gastrointestinal stromal tumors (GISTs), where the vast majority is CD117 positive.[23] CD117 is also a membrane receptor tyrosine kinase whose activity is inhibited by the agent imatinib mesylate, the agent of choice for GISTs. In most reports, CD117 expression can also be demonstrated on a significant fraction of SCLC (40%-87.7%).[24,25] Likewise, 61%-77% of the closely-related entity large cell neuroendocrine carcinoma (LCNEC) also expresses CD117.[26,27] In one previous report, Butnor and associates examined 96 pulmonary and pleural tumors for CD117 and found positive staining in only a minority of the NSCLC: 2 of 22 squamous cell carcinomas, and 4 of 17 adenocarcinomas.[28] This closely matches reports of NSCLC staining from other investigators except Arber and associates, who noted CD117 staining in 71% and 76% of squamous cell carcinomas and adenocarcinomas, respectively.[29,30,31,32,33,34] It is possible that these latter values are due to differences in either techniques and/or scoring systems. In any event, it is unclear whether or not CD117 expression has any impact upon prognosis, with some groups associating a negative effect on prognosis with expression in SCLC and LCNEC, while others have failed to make such a correlation. In a similar manner, we could find no link between outcome and CD117 expression. The finding of increased expression with increasing tumor bulk and stage could be a reflection of the small number of such patients, or an epiphenomenon.

Less attention has been devoted to PDGF in NSCLC. Older studies from one group indicate that in adenocarcinomas, those tumors which express PDGF along with Insulin-like Growth Factor II, basic Fibroblast Growth Factor, and Transforming Growth Factor-beta-1, had an inferior survival than those whose lesions lacked expression of any of the above.[35] In a separate report evaluating the expression of PDGF alone, 56% of adenocarcinomas (49 of 88) expressed PDGF. The patients with PDGF-negative lesions had a far superior five-year overall survival of 53% versus 17%.[36] Vignaud and coworkers have suggested that at least some of the PDGF is actually secreted by macrophages within the tumor stroma of NSCLC, while half of the tumor cells and the tumor-associated endothelial cells express the PDGF receptor, implying a potential autocrine loop.[37] We did not assess for the presence or cellular origin of PDGF or its receptor, and in contrast to the findings of Takanami and colleagues, we were unable to demonstrate any correlation with patient outcomes. While PDGF secretion cannot be directly affected by currently available drugs, the activation of the PDGF receptor tyrosine kinase can be inhibited by imatinib mesylate. It remains to be demonstrated whether this will produce any clinical benefit in patients with solid tumors.

COX is the key enzyme in the conversion of arachidonic acid to prostanoids, which are bioactive lipid derivatives which participate in both normal tissue homeostasis as well as tumorigenesis. COX-1 is thought to play a role in normal tissue upkeep; conversely, COX-2 is an isoform which is inducible by cytokines which are associated with inflammations and neoplasia, such as lipopolysaccharide, interleukin 1, tumor necrosis factor alpha, EGF, and PDGF.[38,39,40,41,42,43,44] COX-2 expression is greater in a variety of malignant epithelial lesions when compared to the matched normal tissues.[42] Elevated COX-2 mRNA and protein levels have been found in both preinvasive lung cancer lesions as well as invasive and metastatic tumors.[38,39,40,41, 42] Prior work has demonstrated a correlation between COX-2 expression and worse survival in NSCLC.[45,46,47] The exact mechanisms of action remain unclear, though COX-2 expression is associated with an inhibition of apoptosis through BCL-2-dependent and independent mechanisms,[48,49] and a promotion of tumor angiogenesis though increased VEGF production.[50, 51] It may also function as a local immunosuppressant, defeating any host anti-tumor immune surveillance.[52] Our data is the first indication of potential ethnic differences in COX-2

expression as none of the Hispanic patients produced COX-2, and only 20% of the African American patients did so, compared to 43.5% of Caucasian patients. However, our numbers are small enough to caution against drawing conclusions at this point. Clearly further investigation is warranted to determine if this pattern holds in a larger population of patients, and if so, as well as potential environment and genetic causes. The COX isoenzymes can be inhibited by acetylsalicylic acid and nonsteroidal anti-inflammatory drugs; antitumor activity has been demonstrated with COX-2 inhibition in both preclinical and clinical studies with several tumor types, including NSCLC, indicating potential clinical benefit.

Ki67 is a well-established proliferation marker of unknown function, similar to proliferating cell nuclear antigen (PCNA). However, Ki67 is expressed only in cycling cells in the G1-S-G2/M phases of mitosis, but is not found in G0 cells. By contrast, PCNA has a longer half life, and thus can be found in cells which are no longer cycling, having entered G0. Proliferative activity has been correlated with prognosis in lung cancer in multiple studies. Ki67 activity also appears to increase as preinvasive lung lesions progress from mild dysplasia to carcinoma in situ.[53,54,55,56,57,58,59,60] Further, Soomro and Whimster noted that Ki67 staining could be correlated with the histologic subtype of the tumor: SCLC exhibited the greatest staining (consistent with the higher growth rate and more rapid clinical progression), followed closely by LCNEC, then squamous cell carcinoma and then adenocarcinomas.[54] These results closely parallel our, though SCLC/LCNEC were excluded at the outset. While we were not able to demonstrate statistical significance in a correlation between high Ki67 expression and survival, there was a strong trend observed. Furthermore, it may be that the determination of the separation point between high-growth and low-growth fractions may determine the degree of significance. We adopted a cut-off of Ki67 staining >30% of cells; by comparison, Scagliotti and colleagues adopted a cut-off of Ki67 > 25% positive cells, and showed a statistically significant impact on survival, though no correlation with histologic subtype.[55]

The apparent disconnection in the results between PTEN and FRAP expression is, at first, difficult to explain. PTEN is a tumor suppressor; loss of expression is correlated with neoplastic progression, decreased apoptosis, and resistance to chemotherapy. PTEN is a phosphatase which blocks signals from RAS and cell surface receptor tyrosine kinases through dephosphorylation of the lipid products of phosphoinositol-3-kinase (PI3K). PI3K phosphorylates the third position in the inositol ring, and these phosphorylated lipids act as second messengers to recruit and activate downstream targets, including the serine/threonine kinase Akt/PKB (protein kinase B). Among its multiple targets, AKT phosphorylates and activates FRAP. The impact of PTEN loss upon prognosis in NSCLC is still unclear. In agreement with our findings, Olaussen and colleagues found that PTEN loss was seen in a fraction of patients with resectable NSCLC, but that it had no prognostic impact.[61] However, Goncharuk and coworkers, and Bepler and associates noted that PTEN loss was associated with an increase rate of lymph node metastases and cancer-related death.[62, 63] Phosphorylated AKT is associated with resistance to chemotherapy and radiotherapy in vitro in NSCLC, and with lymph node involvement and a poor prognosis in patients. Likewise, David and coworkers noted a correlation between the presence of phospho-AKT and a poor outcome.[64] Of note, Capuzzo and associates were able to show that patients whose tumors were positive for phospho-AKT had a statistically superior rate of response and disease control, and median time to disease progression after therapy with the EGF receptor tyrosine kinase inhibitor gefitinib, when compared to patients with lesions that were negative for phospho-AKT.[65] There was also a trend towards superior median overall survival as well (15 months versus 8.3 months). There has been little work evaluating the possible prognostic significance of FRAP expression.

In light of the data indicating that activation of signal transduction activity upstream of FRAP is correlated with more aggressive disease and a worse outcome, it is not surprising to find similar data for FRAP. To date, there has been little information reported which specifically links FRAP expression with prognosis, and none of it in NSCLC. Both Zhou et. al., and Pangrahi and coworkers note the potential importance of signaling through FRAP as having a negative upon patient outcome in breast cancer, but FRAP expression was not analyzed separately from other aspects of the signaling pathway.[66,67] While understanding the importance of the activated pathway, we assessed only the expression level of FRAP, but not its potential activation by evaluating for either phospho-FRAP, or its immediate downstream signaling element, phospho-p70S6K. Inhibition of FRAP activity by the immunosuppressant macrolide rapamycin blocks p70S6K and CDC2 phsophorylation, and induces cell cycle arrest. Although it is difficult to explain

any lack of correlation of PTEN expression and outcome in our cohort, it has been demonstrated that many NSCLC lines may have phosphorylation of AKT and other elements of the pathway, yet maintain a wild-type PTEN gene, indicating activation of AKT by cross-talk with other signal transduction pathways.[8,9] Additionally, there is considerable crosstalk among the various signaling pathways, such that there is more a network rather than any defined point-to-point pathway. As such, there is evidence that AKT may be phosphorylated and possibly activated by factors other than PI3K; for example, Persad and associates that AKT function may also be regulated by phosphorylation on serine 473 by integrin-linked kinase (ILK).[7,8] There may also be mechanisms to activate FRAP without involvement of AKT; there is data which indicates that phosphatidic acid binds directly to FRAP, and may competitively inhibit the binding of the rapamycin-FK506 binding protein complex to FRAP.[9] Phosphatidic acid may also activate FRAP via activation of phospholipase D. However, phosphatidic acid also stimulates AKT phosphorylation, thus it is unclear if either mechanism, or both, accounts for the stimulation of FRAP after phosphatidic acid exposure.

Our exploratory evaluation has demonstrated a significant association between Ki67 and survival, and FRAP and survival. Furthermore, there is a correlation between VEGF and CD117 expression and tumor stage, though not survival; it is not clear from this cohort if either of these factors might have had an effect on outcomes such as tumor response to therapy. The observation that there may be a correlation between COX-2 expression and ethnicity deserves further study. While there are observations of ethnic differences in the incidence and outcomes of certain tumors, such as prostate cancer, adenocarcinoma of the gastroesophageal junction, and multiple myeloma, the etiology of such differences is unknown.

While the findings above are of interest, our study is limited by its retrospective nature and relatively small numbers, especially of patients with higher stages, large cell carcinoma, Hispanic patients, and common treatments (such as types and doses of chemotherapy and radiation therapy). It is possible that these findings could be altered by a larger patient cohort; our data requires validation in a prospectively-acquired patient cohort.

In summary, our analysis has demonstrated that at least two molecular markers, Ki67 and FRAP, are significantly associated with prognosis in NSCLC. This adds to previous data from others about the prognostic significance of Ki67, indicating that the size of the proliferative fraction can determine the overall outcome. Additionally, this study contributes further to the importance of the kinase that is immediately upstream of FRAP, AKT, in that the activation of this signal transduction pathway also adversely affects patient survival. However, it is possible to take advantage of potential manipulation of this pathway to positive effect by tyrosine kinase inhibitors (such as gefitinib, erlotinib, or rapamycin).

Of the other markers evaluated, VEGF, CD117, PDGF, and COX-2 may also be targeted for pharmacologic intervention. However, based upon our results, it is unclear if such therapies will have any impact upon the biology of the tumor and patient outcomes. In some settings, such as interfering with VEGF function in colorectal cancer, or CD117 in GISTs, positive outcomes can be demonstrated, while in other setting no effect has been observed. Clearly more effort must be devoted not only to the assessment of the increasing number of potential molecular markers and their prognostic significance, but also to provide a better understanding of the roles that these markers may play in order to develop better therapies to optimize clinical benefit.

CONFLICT OF INTEREST STATEMENT

The authors report that they have no conflicts of interest with regards to products and companies noted in this manuscript.

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