Lung squamous cell carcinomas (SCC) account for 25-30% of all lung cancer cases, the leading cause of cancer-related mortality worldwide. One factor contributing to the high mortality rate is the preponderance of tumors discovered at advanced stage, demands the discovery and clinical validation of biomarkers for diagnosing early stage disease. Quantitative proteomics technologies are capable of identifying protein biomarkers with diagnostic, prognostic, and predictive value. Recent works have demonstrated the utility in using quantitative proteomics across normal, pre-cancerous, and cancerous lesions towards the discovery of biomarkers for early stage lung cancer, as well as discovering novel mechanisms of lung carcinogenesis.

Keywords: Lung squamous cell carcinoma; proteomics; isobaric tags for relative and absolute quantitation (iTRAQ); glutathione s-transferase pi 1 (GSTP1)


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optical imagining modalities (confocal endomicroscopy, optical coherence tomography) or immunohistochemical analysis of needle biopsies. Accordingly, the experimental design presented by Zeng et al., provides a paradigm for future studies targeting protein biomarkers for SCC and potentially other NSCLC subtypes.

Proteomic technologies have begun to elucidate the vast molecular complexity of tumors, including lung cancer, through the investigation of proteins expressed in pathologic contexts. The poor correlation of DNA/RNA expression with protein expression, the importance of post-translational protein modifications in protein activity, and the protein complement in minimally invasive settings such as blood and urine makes proteomic analysis attractive for tumor biomarker discovery (5). Isotope labeling strategies such as the isobaric tags for relative and absolute quantitation (iTRAQ) method coupled to mass spectrometry methods as employed by Zeng et al. (2) offer protein quantification in gel-free settings and multiplexed assays. The use of various proteomic technologies has elucidated potential diagnostic, prognostic, and predictive protein biomarkers in cancerous contexts. Several review articles have highlighted the detection of proteins, through gel-based or isotope labeling proteomics, in tissue samples or fluid samples, which might offer diagnostic, prognostic or predictive value in lung cancer (5-8). Though numerous studies have suggested protein biomarkers via proteomic technologies, clinical validation and biomarker reproducibility have proved immensely challenging towards development of novel diagnostic tests. Zeng and colleagues use of independent tissue specimens and traditional protein analysis techniques as validation serve as a useful template towards overcoming validation and reproducibility challenges.

Another area of need addressed by Zeng and colleagues is the use of quantitative proteomics in a specific histologic subtype of lung cancer, and the accounting for multiple pre-neoplastic stages. The heterogeneity of lung cancer has been cited as a potential hurdle towards sensitive/specific protein biomarkers (5). The specific investigation of biomarkers in lung SCC has been limited, and there are essentially no studies within SCC investigations accounting for pre-neoplastic progression. Shotgun proteomics revealed that elevated serum levels of C4b-binding protein alpha (C4BP), leucine-rich alpha-2-glycoprotein 1 (LRG1) and serum amyloid A protein (SAA) were associated with a shorter time to relapse in lung SCC samples (9). In the work by Zeng et al., the use of LCM to purify normal bronchial epithelium, pre-neoplastic lesions, and invasive SCC prior to proteomic analysis allows for meaningful investigations into early carcinogenic events in lung SCC and the potential for serum biomarkers towards detection, as well as insights into novel therapeutic targets.

Using an independent set of archival SCC tissue specimens, Zeng and colleagues verified a panel of three proteins [heat shock protein beta-1 (HSPB1), GSTP1, creatine kinase, brain (CKB)] as potential SCC biomarkers (2). Both GSTP1 (discussed below) and HSPB1 (HSPB1/HSP27) have considerable links to carcinogenesis (10) and in particular NSCLC (11,12). HSPB1 cytoprotective functions have been linked to therapeutic resistance, inhibition to apoptosis, increased cell proliferation and signal transduction in a variety of tumor types (10). Accordingly, increased HSPB1 expression is present in NSCLC tumors (11) and is predictive of poor patient survival (12). Unlike GSTP1 and HSPB1, the association between CKB with SCC is unclear. Although, the bulk of studies reveal that CKB activity increases in small cell lung cancer and other select solid tumors (13,14), there are no studies at present, other than the findings of Zeng and colleagues, that link CKB activity or expression to SCC. Regardless, based upon the observation of increased HSPB1 in the serum of NSCLC patients (15) and identification of GSTP1 gene alterations (16) and polymorphisms (17) from serum samples, it will be critical to assess in future studies the feasibility of serum-based assays for these genes to identify early SCC lesions.

GSTP1 was progressively decreased along the evolution of bronchial epithelial carcinogenesis as determined by quantitative proteomics and validated by both western blotting and immunohistochemistry in independent sets of tissues (2). The loss or deregulation of GSTP1, a detoxification enzyme and stress signaling protein, has been reported in lung cancer, and a number of studies have associated GSTP1 with chemosensitivity and patient prognosis, with a strong focus on polymorphisms and methylation status (18-20). Zeng and colleagues demonstrate that GSTP1 depletion in a human bronchial epithelial cell line (16HBE) enhanced transformation efficiency with repeated exposure to benzo[a]pyrene (B[a]P), a mutagenic component of tobacco smoke. Further, GSTP1 depletion in 16HBE cells significantly enhanced growth in low serum, promoted anchorage dependent and independent colony formation, and reduced apoptosis compared to control cells. As GSTP1 can catalyze the detoxification of B[a]P products, loss of GSTP1 protein
expression may enhance susceptibility to tobacco smoke-induced lung carcinogenesis, which is highly associated with lung SCC.

There is a critical unmet need for disease biomarkers that provide diagnostic, prognostic, and therapeutic value. The use of quantitative proteomics towards the discovery and validation of tumor biomarkers has yielded promising protein targets in both tumor tissues and fluids. The work of Zeng et al., showcases the value of collecting relatively pure cell populations representing the progression from normal tissue through pre-cancerous lesions to invasive disease as a means to discover novel protein biomarkers. Validation using independent tissue sets was also performed using more traditional protein assays. The challenge from this study is the reproducibility of the protein biomarkers by proteomic methods, the translation of these biomarkers into clinically viable blood/tumor tests, and the use of this information towards therapeutic decision-making and outcome prediction.

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