China Lung Cancer Research Highlight

The use of quantitative proteomics towards biomarker discovery in lung squamous cell carcinoma

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Abstract: The high mortality rate in advanced lung cancer, due to a 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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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 initial detection of SCC at advanced tumor stage. The five-year survival rates of lung cancers detected in stage 1A compared to stage 4 drop from 50% to 2% (1), necessitating the discovery of early stage biomarkers. The identification of biomarkers towards early detection of SCC is under intense investigation, aided by proteomic technologies. Such biomarkers for early SCC would not only improve diagnosis, but also shed light on possible mechanisms of early oncogenic mechanisms, leading to rational therapeutic strategies aimed to combat SCC in its earliest stages. The work of Zeng and colleagues highlights the use of quantitative proteomics to identify biomarkers that distinguish early SCC, and describes the role of glutathione S-transferase pi 1 (GSTP1) in SCC carcinogenesis (2).

Invasive SCC, like other malignancies, is the consequence of the accumulation of molecular events, characterized by pathological changes (pre-neoplastic lesions) to the normal respiratory mucosa (3). SCC follows a multi-step progression with the normal squamous epithelium of bronchus transitioning to hyperplasia, followed by squamous metaplasia and carcinoma *in situ* (CIS) and finally invasive carcinoma. Interrogations of pre-neoplastic lesions have substantial value, revealing oncogenic mechanisms critical to tumor development, as well as providing information towards early intervention. Despite advances in imaging modalities, identification of pre-neoplastic SCC lesions is difficult and biomarker studies of pre-neoplastic SCC lesions have been limited to identification of genomic alterations, specifically, loss of heterozygosity to regions of chromosome 3 and allelic loss of *TP53* (4). Thus the work by Zeng *et al.*, is a novel approach towards identifying biomarkers for early SCC lesions. Using quantitative proteomics techniques on pathologically identified samples of neoplastic and pre-neoplastic SCC lesions by laser captured microdissection (LCM), Zeng and colleagues have defined a panel of protein biomarkers associated with SCC carcinogenesis. This work is particularly attractive as protein biomarkers are more amenable towards application for early detection of SCC via a blood-based test for early SCC, integration of protein biomarkers into bronchoscopic

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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References

1. Heist RS, Engelman JA. SnapShot: non-small cell lung cancer. Cancer Cell 2012;21:448.e2.
2. Zeng GQ, Zhang PF, Deng X, et al. Identification of candidate biomarkers for early detection of human lung squamous cell cancer by quantitative proteomics. Mol Cell Proteomics 2012;11:M111.013946.
3. Wistuba II, Gazdar AF. Lung cancer preneoplasia. Annu Rev Pathol 2006;1:331-48.
4. Wistuba II, Behrens C, Milchgrub S, et al. Sequential molecular abnormalities are involved in the multistage development of squamous cell lung carcinoma. Oncogene 1999;18:643-50.
5. Pastor MD, Nogal A, Molina-Pinelo S, et al. Proteomic biomarkers in lung cancer. Clin Transl Oncol 2013;15:671-82.
6. Hassanein M, Callison JC, Callaway-Lane C, et al. The state of molecular biomarkers for the early detection of lung cancer. Cancer Prev Res (Phila) 2012;5:992-1006.
7. Hassanein M, Rahman JS, Chaurand P, et al. Advances in proteomic strategies toward the early detection of lung cancer. Proc Am Thorac Soc 2011;8:183-8.
8. Pastor MD, Nogal A, Molina-Pinelo S, et al. Identification of proteomic signatures associated with lung cancer and COPD. J Proteomics 2013;89:227-37.
9. Liu YS, Luo XY, Li QR, et al. Shotgun and targeted proteomics reveal that pre-surgery serum levels of LRG1, SAA, and C4BP may refine prognosis of resected squamous cell lung cancer. J Mol Cell Biol 2012;4:344-7.
10. Ciocca DR, Oesterreich S, Chamness GC, et al. Biological and clinical implications of heat shock protein 27,000 (Hsp27): a review. J Natl Cancer Inst 1993;85:1558-70.
11. Małusecka E, Zborek A, Krzyzowska-Gruca S, et al. Expression of heat shock proteins HSP70 and HSP27 in primary non-small cell lung carcinomas. An immunohistochemical study. Anticancer Res 2001;21:1015-21.
12. Malusecka E, Krzyzowska-Gruca S, Gawrychowski J, et al. Stress proteins HSP27 and HSP70i predict survival in non-small cell lung carcinoma. Anticancer Res 2008;28:501-6.
13. Rubery ED, Doran JF, Thompson RJ. Brain-type creatine kinase BB as a potential tumour marker--serum levels measured by radioimmunoassay in 1015 patients with histologically confirmed malignancies. Eur J Cancer Clin Oncol 1982;18:951-6.
14. Nikliński J, Furman M, Laudański J, et al. Evaluation of carcinoembryonic antigen (CEA) and brain-type creatine kinase (CK-BB) in serum from patients with carcinoma of the lung. Neoplasma 1991;38:129-35.
15. Zimmermann M, Nickl S, Lambers C, et al. Discrimination of clinical stages in non-small cell lung cancer patients by serum HSP27 and HSP70: a multi-institutional case-control study. Clin Chim Acta 2012;413:1115-20.
16. Ellinger J, Haan K, Heukamp LC, et al. CpG island hypermethylation in cell-free serum DNA identifies patients with localized prostate cancer. Prostate 2008;68:42-9.
17. Ozerkan K, Atalay MA, Yakut T, et al. Polymorphisms of glutathione-s-transferase M1, T1, and P1 genes in endometrial carcinoma. Eur J Gynaecol Oncol 2013;34:42-7.
18. Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene

2003;22:7369-75.
19. Sun N, Sun X, Chen B, et al. MRP2 and GSTP1 polymorphisms and chemotherapy response in advanced non-small cell lung cancer. Cancer Chemother Pharmacol 2010;65:437-46.
20. Gao P, Yang X, Xue YW, et al. Promoter methylation of glutathione S-transferase pi1 and multidrug resistance gene 1 in bronchioloalveolar carcinoma and its correlation with DNA methyltransferase 1 expression. Cancer 2009;115:3222-32.

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