Deep proteogenomic investigations elucidate the NRF2 antioxidant mechanism as a major driving mechanism of lung adenocarcinoma in Asia

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Abstract

Background: Lung adenocarcinoma is a global leading cause of death. Despite modern therapeutic interventions, undesirable outcomes such as drug resistances and disease recurrence still occur. Therefore, continued investigations of disease driving mechanisms and counteracting strategies are urgently needed.

Methods: We re-visited two deep-proteogenomic resources of lung adenocarcinoma published recently. These resources were derived from patient cohorts with decent sizes in Taiwan and China. The gene set enrichment analysis (GSEA) was performed. A heatmap was produced by the generalized association plot (GAP).

Results: Among 189 common oncogenic pathways investigated, the nuclear factor erythroid 2-related factor 2 (NRF2) downstream antioxidant mechanism was uncovered for the first time the leading oncogenic mechanism of lung adenocarcinoma in Taiwan. The gene levels of NRF2 (also known as NFE2L2) is negatively correlated with those of KEAP1 (Pearson's correlation = –0.275, $p = 0.009$) in patients' tumor tissues. Furthermore, the protein levels of EIF2S2 and PGD are higher in patients with more advanced stages in the Taiwan cohort ($p = 0.001$ and 0.05, respectively), and are indicative of poorer progression-free survival (PFS) and overall survival (OS) in the China cohort (all Cox-regression $p < 0.05$). On the other hand, EPHX1 is higher in patients with earlier stages in Taiwan ($p = 0.003$), and are indicative of better PFS and OS in China (both Cox-regression $p < 0.05$). When the patients were stratified using the median protein abundances for Kaplan–Meier visualizations, patient strata with higher EIF2S2, PGD, and EPHX1 have significantly poorer PFS (log-rank $p = 0.041$); poorer OS ($p = 0.006$), and better PFS and OS ($p = 0.001$ and 0.030), respectively.

Conclusion: The NRF2 downstream antioxidant mechanism is one major driving mechanism of lung adenocarcinoma in Asia, and represents important directions for future therapeutic interventions. Major downstream proteins such as EIF2S2, PGD, and EPHX1 are indicative of cancer stages and prognosis.

Keywords: Ferrotopsis; Inflammation; Metabolic reprogramming; Systems biology

1. INTRODUCTION

Lung cancers are aggressive malignancies and leading causes of death globally.[1,2] These diseases can be broadly categorized as small cell lung cancer and nonsmall cell lung cancer (NSCLC); the latter comprises >80% of lung cancers and can be further categorized as adenocarcinoma, squamous cell carcinoma, large cell carcinoma, etc. Lung adenocarcinoma (LUAD) is the most prevalent type of lung cancer.[1] Tobacco smoking is a well-established etiology of lung cancer. However, a large proportion of lung cancer patients are nonsmokers, and the etiology of their disease remains largely elusive.

At the early stages, LUAD often lacks overt disease-specific symptoms, making early detection difficult. People diagnosed at the advanced stages often have metastases, requiring systematic treatments.[3] Extensive prior research has uncovered several oncogenic mechanisms, such as epidermal growth factor receptor (EGFR) activation, which motivated researchers to develop EGFR antagonists. Early EGFR antagonists such as gefitinib and erlotinib interfere with overactive EGFR. However, drug-resistant mutations often occur after treatment. Next-generation agents such as osimertinib, rociletinib, and olmutinib were subsequently developed with the goal of antagonizing drug-resistant mutations in tumors expressing EGFR. In patients without overt EGFR activation, independent oncogenic mechanisms of ALK and ROS-1 have been discovered. EML4-ALK fusion has been found to be prevalent in Asian female nonsmokers.[4] Therapeutic agents targeting EGFR,[5] ALK,[6] and ROS-1,[7] alongside companion diagnostics, have been incorporated into treatment roadmaps, guiding precision medicine tailored to patients' specific

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diseases. Additionally, significant progress has been made in cancer immunology involving the immune checkpoints PD-1 and PD-L1.[3] These studies have fostered the clinical development of immunological checkpoint inhibitors.

In the UK 2020 treatment guidelines, LUAD patients are treated according to the following algorithm. First, patients' EGFR, ALK, and ROS-1 activation are checked. If activation is found in any of these oncogenic mechanisms, then the corresponding therapeutic agents can be used as first-line treatments. Additionally, the criterion for anti-PD-L1 treatment, a PD-L1 expression level >50%, is checked. If the criterion is met, anti-PD-L1 agents can be used. Otherwise, "all-negative" patients (ie, negative for EGFR, ALK, ROS-1 expression, and PD-L1 expression < 50%) can be treated with untargeted chemotherapy agents. Although therapeutic agents have been developed for antagonizing major oncogenic pathways such as EGFR, ALK, and ROS-1, undesirable outcomes such as drug resistance and disease progression still occur in the majority of patients. Additionally, chemotherapy strategies are complicated by tumor resistance, as cancer cells may activate metabolic reprogramming mechanisms under chemotherapeutic stress. Therefore, continued investigations of LUAD-driving mechanisms are warranted.

The above studies and treatment guidelines are mainly based on clinical evidence in Western countries. In Taiwan, a distinct demographic distribution of LUAD patients has been observed, characterized by relatively higher proportions of nonsmokers and female patients than in Western countries.[8] Thus, the molecular mechanisms and optimum treatment strategies may also differ. Recently, two high-quality, quantitative deep proteogenomic datasets derived from tumor tissues of LUAD patients in Taiwan[9] and China,[10] each with ~100 patients, were released into the public domain. These datasets represent invaluable resources for deciphering the driving mechanisms of the disease in the Asian population. Thus, we were motivated to revisit these datasets, with the purpose of identifying Asian-prevalent oncogenic mechanisms of LUAD and new strategies for therapeutic interventions.

2. METHODS

2.1. Data source

The Taiwan quantitative proteomics data were obtained from Supplementary Table S1E, http://links.lww.com/JCMA/A89 of Chen et al.[9] and downloaded from the following website: https://ars.els-cdn.com/content/image/1-s2.0-S0092867420307431-mmc1.xlsx.

These quantitative proteomics data comprise 7605 nonredundant, missing-value-free proteins identified in the tumor tissues and paired normal adjacent tissues (NATs) of a cohort of 89 LUAD patients. The values are log2-transformed ratios of protein abundance in tumors (X_{tumor}) and NATs (X_{NAT}), referred to as the T/N ratios, which are effectively the difference in log2-transformed abundances in tumors and NATs.

$$\log\left(\frac{X_{tumor}}{X_{NAT}}\right) = \log(X_{tumor}) - \log(X_{NAT}) \quad (1)$$

The demographic and clinical information about the Taiwanese cohort was obtained from the National Cancer Institute (NCI) Proteomics Data Commons (PDC000219); 103 lung cancer patients, including LUAD, squamous cell carcinoma and other lung cancer patients were included. We focused on 89 patients with available proteogenomic data (Supplementary Table S1E, (http://links.lww.com/JCMA/A89). All of these patients were LUAD patients. Additionally, the messenger RNA data were obtained from Supplementary Table S1E, http://links.lww.com/JCMA/A89 of Chen et al.[9]

The Chinese cohort comprised 103 naive LUAD patients who were treated by surgical resection.[10] The clinical and normalized intensity-based absolute quantification (iBAQ) proteomic data from these patients were obtained from Supplementary Tables S1 and S4A, http://links.lww.com/JCMA/A89 of Xu et al,[10] respectively.

2.2. Gene set enrichment analysis

GSEA is a nonparametric statistical method based on ranks.[11,12] The analysis evaluates the entire set of proteins, rather than individual proteins in the set, to determine their relative rank positions in the proteome. GSEA software is intimately integrated with the Molecular Signatures Database (MSigDB). This analysis was performed on July 31, 2020 using GSEA software. A wide spectrum of 189 common oncogenic gene sets in MSigDB (version c6, v7.1) was evaluated sequentially using the relative positions of the gene-set members in the rank of the expected value of log-transformed ratios of protein abundance, ie

$$E\left(\log\left(\frac{X_{tumor}}{X_{NAT}}\right)\right) = \mathrm{E}\left(\log(X_{tumor})\right) - E\left(\log(X_{NAT})\right) \quad (2)$$

2.3. Statistics and Visualization

Cox regression and Kaplan–Meier plots were generated using SPSS software (IBM, Armonk, NY). The heatmap was produced using a generalized association plot (GAP).[13]

3. RESULTS

3.1. NRF2 antioxidant mechanisms underlie LUAD in patients with or without smoking histories in Taiwan

We first investigated the Taiwanese cohort comprising 89 lung adenocarcinoma patients with deep and comprehensive proteogenomic assessments of tumors and NATs.[9] Among them, 77 patients were nonsmokers, and 12 patients had smoking histories. The demographic and clinical information of these patients is summarized in the Table 1. The distinct etiologies of smoking histories also manifest as differences in the clinical characteristics of age at diagnosis (p = 0.027, nonsmokers are younger) and gender (nonsmokers are mostly female: 70.13%). The majority of the patients had early-stage LUAD (IA/IB percentage for nonsmokers: 79.22%; for smokers: 83.33%). The majority of the tumors were at nodal stage 0 (83.12% and 83.33% for nonsmokers and smokers, respectively).

We then conducted the GSEA for these patients. For nonsmokers, the nuclear factor erythoid–2–related factor 2 (NRF2; also known as NFE2L2) antioxidant gene set displayed the highest normalized enrichment score (1.913) among all 189 cancer-related gene sets in MSigDB version c6, v7.1 (Fig. 1A, C). The NRF2 antioxidant gene set comprises NRF2 itself and 466 downstream genes, including NQO1,[14] GSTA2,[15] TXNRD1,[14,16,17] GCLC,[17,18] HMOX1,[16,18] EIF2S2,[19,20] PGD,[21] EPHX1,[22] UGT1A6,[23] GSR, and IL17A, which are together responsive to oxidative and electrophilic stress induced by xenobiotic carcinogens or chemotherapeutic agents.[15,21] The NRF2 antioxidant gene set is shown in Supplementary Table 1, http://links.lww.com/JCMA/A89. According to the original literature where this gene set was derived, genes were evaluated by the contrasting gene expression levels of Kelch-like ECH-associated protein 1 (KEAP1)-deleted cells, NRF2-deleted cells and wild-type controls, in conjunction with chromatin immunoprecipitation and sequencing assays (ChIP-Seq).[14]

Table 1

Clinical characteristics of adenocarcinoma patients in Taiwan

Characteristics		Non smoker		Smoker		*p*
		Number	%	Number	%	
Patient no.		77		12		
Age	mean ± sd	62.88 ± 10.26		68.67 ± 7.22		0.027
Gender						<0.001
	Male	23	29.87	12	100.00	
	Female	54	70.13	0	0.00	
Overall stage						0.787
	IA	34	44.16	6	50.00	
	IB	27	35.06	4	33.33	
	IIA	5	6.49	0	0.00	
	IIB	1	1.30	0	0.00	
	IIIA	5	6.49	2	16.67	
	IIIB	1	1.30	0	0.00	
	IV	4	5.19	0	0.00	
Nodal stage						0.565
	N0	64	83.12	10	83.33	
	N1	5	6.49	0	0.00	
	N2	8	10.39	2	16.67	
EGFR mutation status						0.062
	Wild type	8	10.39	5	41.67	
	Exon19del	27	35.06	4	33.33	
	L858R	33	42.86	2	16.67	
	Exon19del/ L858R	1	1.30	0	0.00	
	Others	8	10.39	1	8.33	

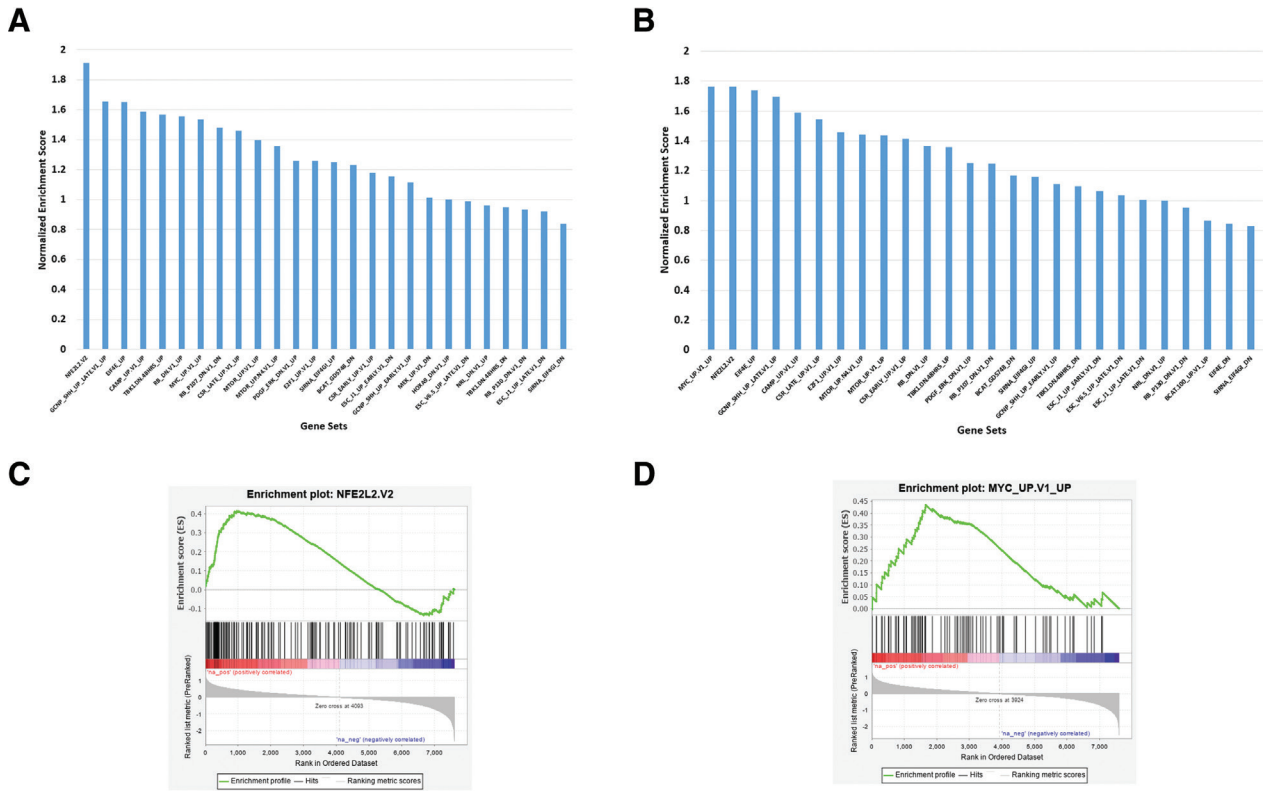

Fig. 1 The gene set enrichment analysis (GSEA) of driver mechanisms of LUAD in patients with or without histories of smoking. A, The 26 leading oncogenic gene sets identified by the GSEA in nonsmoking adenocarcinoma patients in Taiwan. B, The 26 leading oncogenic gene sets in adenocarcinoma patients with histories of smoking. C, The enrichment plot of the NRF2 downstream antioxidant gene set, the top gene set in nonsmoking adenocarcinoma patients. D, The enrichment plot of the Myc-related gene set, one of the top two gene sets in adenocarcinoma patients with histories of smoking.

On the other hand, for patients with a smoking history, the Myc-related gene set and the NRF2 antioxidant gene set achieved the highest normalized enrichment score (1.763) among all 189 gene sets evaluated (Fig. 1B). The Myc-related gene set comprises 179 genes (Fig. 1D). Hence, the NRF2 antioxidant mechanism is the leading oncogenic mechanism of lung adenocarcinoma in patients with or without smoking histories in Taiwan

3.2. NRF2-downstream antioxidant proteins of LUAD patients in Taiwan and China

NRF2 is negatively regulated by KEAP1.[15,24] We therefore extracted the gene expression information and found that the messenger RNA levels of the two genes were negatively correlated (Pearson's correlation = –0.275, p = 0.009, Fig. 2A), consistent with prior knowledge. The log2-transformed T/N ratios of NRF2-downstream proteins in the Taiwan cohort are visualized as a heat map (Fig. 2B). In this visualization, many proteins manifest different T/N ratios in different disease stages (Fig. 2B). For example, the T/N ratios of eukaryotic translation initiation factor 2 subunit beta (EIF2S2)[19,20] and 6-phosphogluconate dehydrogenase (PGD)[21] were higher in patients with stages II–IV tumors than in those with stage I tumors (p = 0.001 and 0.05, respectively). In contrast, the levels of Epoxide hydrolase 1 (EPHX1) were lower in patients with stages II–IV disease than in those with stage I disease (p = 0.003). The ratio distributions are also shown in boxplots in Fig. 3A–C.

We then investigated the proteomic and clinical data of LUAD patients in China. The abundance of NRF2-downstream proteins in tumors was evaluated. By the end of follow-up, a total of 58 patients experienced tumor recurrence, and 26 patients succumbed to the disease. The abundance of EIF2S2 was negatively associated with progression-free survival (PFS) and overall survival (OS) rates (Cox regression p = 0.005 and 0.041, respectively). When the patients were stratified using the median values of EIF2S2 expression (Fig. 3D, G), patients with higher abundance of EIF2S2 had significantly poorer PFS rates (log-rank p = 0.041).

The 6-phosphogluconate dehydrogenase (PGD)[21] abundance was also negatively associated with PFS and OS rates (Cox regression p = 0.042 and 0.004, respectively). When the patients were stratified using the median values of PGD expression (Fig. 3E, H), patients with higher abundance had significantly poorer overall survival rates (log-rank p = 0.006).

In contrast, EPHX1 abundance was positively associated with PFS and OS rates (Cox regression p = 0.006 and 0.008, respectively). When the patients were stratified using the median values of EPHX1 expression (**Fig. 3F and 3I**), patients with higher abundance had significantly better PFS and OS rates (log-rank p = 0.001 and 0.030).

4. DISCUSSION

NRF2 is a leucine zipper transcription factor, the binding of which to genomic transcription elements, collectively known as antioxidant response elements (AREs), can activate cellular machinery to cope with xenobiotic electrophiles and reactive oxygen species.[16,18,22] NRF2 is under the regulation of KEAP1, a binding partner and negative regulator of NRF2.[15,24] The binding of NRF2 and KEAP1 proteins can keep the NRF2 downstream antioxidant machinery at bay.[24] Aberrant activation of the NRF2 antioxidant machinery may cause oncogenic metabolic reprogramming.[25] This aberrant expression may be due to somatic mutations disrupting the binding of the two proteins.[24] However, in the Taiwanese cohort investigated in this study, the somatic point mutation rates of NRF2 (≦4%) and KEAP1 (≦5%) are low,[9] suggesting that alternative oncogenic mechanisms may be involved. The data in Fig. 2A suggest that KEAP1 and NRF2 are still mutually regulated in the Taiwanese cohort, consistent with prior knowledge. Recently, a sequestosome 1 gene (SQSTM1, also known as p62) has been shown to be involved in activating the NRF2 system through the SQSTM1-KEAP1-NRF2 oncogenic axis.[26] In the presence of stress, NRF2 and SQSTM1 can form a positive feedback regulation loop to stimulate each other.[27,28]

Under normal physical conditions, NRF2 downstream cascades are responsible for removing reactive oxygen species (ROS),[29] counteracting lipid peroxidation,[16] and cross-talking with the glutathione and thioredoxin antioxidant machinery and the AhR-XRE xenobiotic metabolism system.[15,23] However, the same machinery may enhance drug resistance and cancer progression. For example, recent studies have shown that the glutathione and thioredoxin antioxidant machinery[30] can drive carcinogenesis.[31] NRF2 has also been suggested to facilitate oncogenesis by increasing the Warburg effect.[32]

Our analysis unveiled that the NRF2 antioxidant gene set members rank outstandingly high in the list of T/N protein ratios in the Taiwanese cohort. This may suggest that the tissues are under oxidative stress, possibly through environmental stimuli. On the other hand, this may indicate a prevalent oncogenic mechanism of LUAD in the Taiwanese population. We scrutinized the NRF2 downstream genes and found that EIF2S2, PGD and EPHX1 are

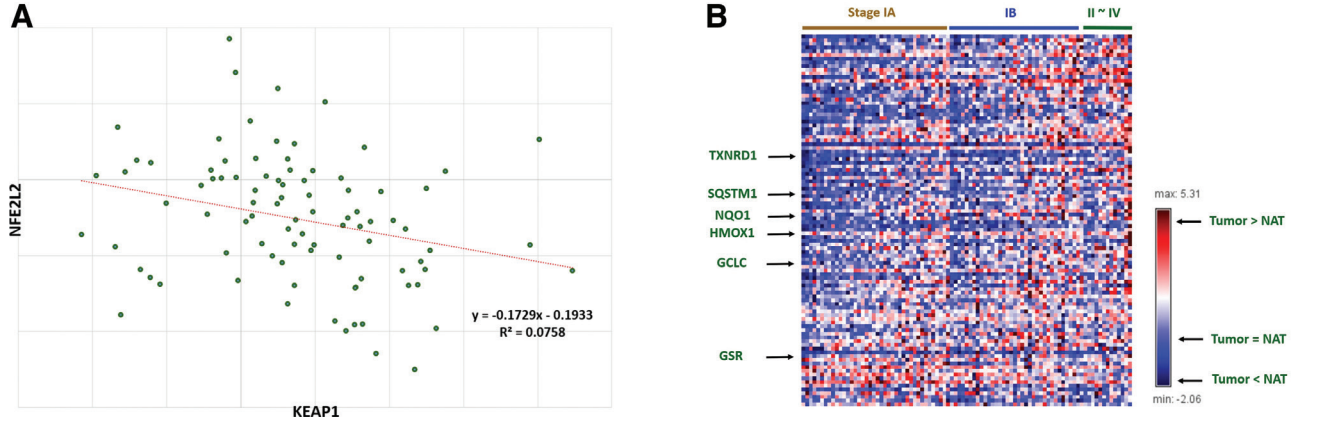

Fig. 2 Expression levels of major nuclear factor erythroid 2-related factor 2 (NRF2) upstream and downstream genes. A, The scatter plot of log2-transformed T/N mRNA ratios of KEAP1 and NFE2L2, also known as NRF2 (Pearson's correlation = –0.275, p = 0.009). B, Heatmap of log2-transformed T/N ratios of antioxidant genes. A zero log2-transformed T/N ratio indicates that the abundances in tumors and NATs are equal.

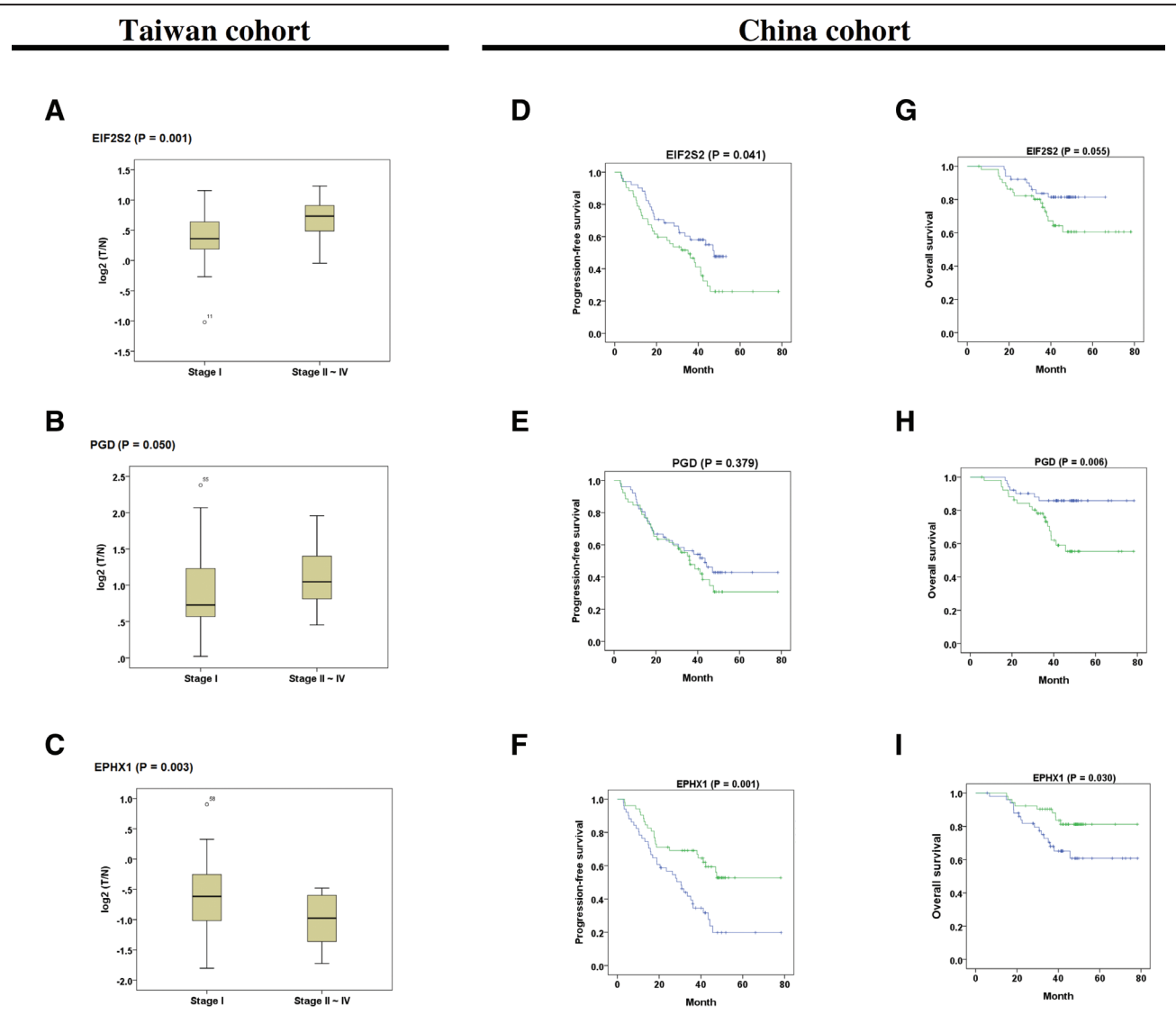

Fig. 3 Major NRD2 downstream proteins in relationship with tumor stages and clinical outcomes. A–C, The log2-transformed T/N ratios of the NRF2 downstream proteins EIF2S2, PGD, and EPHX1 in the Taiwan cohort. D–I, The tumor progression-free survival and overall survival rates of patients stratified by the protein abundance of EIF2S2, PGD, and EPHX1 in the Chinese cohort. Blue line: protein abundance < median (n = 51). Green line: protein abundance ≥ median (n = 52).

significantly associated with LUAD stages in Taiwanese patients. We then evaluated one additional high-quality proteomics dataset, the Chinese cohort, and found that the protein abundance of EIF2S2, PGD, and EPHX1 could also significantly indicate patient progression in this population. EIF2S2 is an RNA binding protein that facilitates protein translation initiation.[19,20] This gene has been suggested as a therapeutic target for NSCLC, based partly on the analysis of The Cancer Genome Atlas (TCGA) database. PGD encodes 6-phosphogluconate dehydrogenase. It is known to enhance cisplatin resistance in lung and ovarian cancers[33] and prevent ferrotopsis of cancer cells.[34] Genetic variants of EPHX1 are known to be associated with lung cancer.[35]

The NRF2 antioxidant mechanism is under the regulation of long noncoding RNAs, microRNAs,[29] and circular RNAs.[36] For example, it is regulated by miR-155 in lung cancer.[37] Downstream genes, for example, PGD, are regulated by microRNAs such as miR-206 and miR-613.[33] The detailed regulation of the NRF2 antioxidant system by these noncoding RNAs will be investigated in our future research.

In conclusion, the antioxidant mechanism downstream of NRF2 was identified as the driving mechanism of lung adenocarcinoma. The downstream proteins EIF2S2, PGD, and EPHX1 are indicative of cancer stage and posttreatment prognosis. The elucidation of such an important mechanism demonstrates the excellent qualities of the deep proteogenomic datasets.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://doi.org/10.1097/JCMA.0000000000000264.

REFERENCES

1. Cheng TY, Cramb SM, Baade PD, Youlden DR, Nwogu C, Reid ME. The International Epidemiology of Lung Cancer: latest trends, disparities, and tumor characteristics. *J Thorac Oncol* 2016;**11**:1653–71.
2. Tyczyński JE, Parkin DM. Textbook of Prevention and Detection of Early Lung Cancer. Oxfordshire: Taylor & Francis, pp 1–18.
3. Yuan M, Huang LL, Chen JH, Wu J, Xu Q. The emerging treatment landscape of targeted therapy in non-small-cell lung cancer. *Signal Transduct Target Ther* 2019;**4**:61.
4. Li Y, Li Y, Yang T, Wei S, Wang J, Wang M, et al. Clinical significance of EML4-ALK fusion gene and association with EGFR and KRAS gene mutations in 208 Chinese patients with non-small cell lung cancer. *PLoS One* 2013;**8**:e52093.
5. Paez JG, Jänne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004;**304**:1497–500.
6. Kwak EL, Bang YJ, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non–small-cell lung cancer. *New Engl J Med* 2011;**364**:588–9.
7. Shaw AT, Ou SH I, Bang YJ, Camidge DR, Solomon BJ, Salgia R, et al. Crizotinib in ROS1-rearranged non–small-cell lung cancer. *New Engl J Med* 2014;**371**:1963–71.
8. Lin HT, Liu FC, Wu CY, Kuo CF, Lan WC, Yu HP. Epidemiology and survival outcomes of lung cancer: a population-based study. *Biomed Res Int* 2019;**2019**:8148156.
9. Chen YJ, Roumeliotis TI, Chang YH, Chen CT, Han CL, Lin MH, et al. Proteogenomics of non-smoking lung cancer in East Asia delineates molecular signatures of pathogenesis and progression. *Cell* 2020;**182**:226–44.e17.
10. Xu JY, Zhang C, Wang X, Zhai L, Ma Y, Mao Y, et al. Integrative proteomic characterization of human lung adenocarcinoma. *Cell* 2020;**182**:245–61.e17.
11. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* 2005;**102**:15545–50.
12. Shi J, Walker M. Gene Set Enrichment Analysis (GSEA) for interpreting gene expression profiles. *Curr Bioinformatics*. 2007;**2**:133–7.
13. Chang SC, Chen CH, Chi YY, Ouyoung CW. Relativity and resolution for high dimensional information visualization with Generalized Association Plots (GAP). In: Härdle W, Rönz B, editors. *Compstat*. Heidelberg: Physica, 2002:55–66.
14. Malhotra D, Portales-Casamar E, Singh A, Srivastava S, Arenillas D, Happel C, et al. Global mapping of binding sites for Nrf2 identifies novel targets in cell survival response through ChIP-Seq profiling and network analysis. *Nucleic Acids Res* 2010;**38**:5718–34.
15. Nguyen T, Nioi P, Pickett CB. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *J Biol Chem* 2009;**284**:13291–5.
16. Dodson M, Castro-Portuguez R, Zhang DD. NRF2 plays a critical role in mitigating lipid peroxidation and ferroptosis. *Redox Biol* 2019;**23**:101107.
17. Yan X, Zhang X, Wang L, Zhang R, Pu X, Wu S, et al. Inhibition of Thioredoxin/Thioredoxin reductase induces synthetic lethality in lung cancers with compromised glutathione homeostasis. *Cancer Res* 2019;**79**:125–32.
18. Lacher SE, Lee JS, Wang X, Campbell MR, Bell DA, Slattery M. Beyond antioxidant genes in the ancient Nrf2 regulatory network. *Free Radic Biol Med* 2015;**88**(Pt B):452–65.
19. Tanaka I, Sato M, Kato T, Goto D, Kakumu T, Miyazawa A, et al. eIF2β, a subunit of translation-initiation factor EIF2, is a potential therapeutic target for non-small cell lung cancer. *Cancer Sci* 2018;**109**:1843–52.
20. van Galen P, Mbong N, Kreso A, Schoof EM, Wagenblast E, Ng SWK, et al. Integrated stress response activity marks stem cells in normal hematopoiesis and leukemia. *Cell Rep* 2018;**25**:1109-17.e5.
21. Tonelli C, Chio IIC, Tuveson DA. Transcriptional regulation by Nrf2. *Antioxid Redox Signal* 2018;**29**:1727–45.
22. Yi YW, Oh S. Comparative analysis of NRF2-responsive gene expression in AcPC-1 pancreatic cancer cell line. *Genes Genomics* 2015;**37**:97–109.
23. Dietrich C. Antioxidant functions of the aryl hydrocarbon receptor. *Stem Cells Int* 2016;**2016**:7943495.
24. Best SA, Sutherland KD. "Keaping" a lid on lung cancer: the Keap1-Nrf2 pathway. *Cell Cycle* 2018;**17**:1696–707.
25. Rojo de la Vega M, Chapman E, Zhang DD. NRF2 and the hallmarks of cancer. *Cancer Cell* 2018;**34**:21–43.
26. Chi HC, Tsai CY, Tsai MM, Yeh CT, Lin KH. Molecular functions and clinical impact of thyroid hormone-triggered autophagy in liver-related diseases. *J Biomed Sci* 2019;**26**:24.
27. Moscat J, Karin M, Diaz-Meco MT. p62 in cancer: signaling adaptor beyond autophagy. *Cell* 2016;**167**:606–9.
28. Frias DP, Gomes RLN, Yoshizaki K, Carvalho-Oliveira R, Matsuda M, Junqueira MS, et al. Nrf2 positively regulates autophagy antioxidant response in human bronchial epithelial cells exposed to diesel exhaust particles. *Sci Rep* 2020;**10**:3704.
29. Espinosa-Diez C, Miguel V, Mennerich D, Kietzmann T, Sánchez-Pérez P, Cadenas S, et al. Antioxidant responses and cellular adjustments to oxidative stress. *Redox Biol* 2015;**6**:183–97.
30. Telorack M, Meyer M, Ingold I, Conrad M, Bloch W, Werner S. A glutathione-Nrf2-thioredoxin cross-talk ensures keratinocyte survival and efficient wound repair. *Plos Genet* 2016;**12**:e1005800.
31. Harris Isaac S, Treloar Aislinn E, Inoue S, Sasaki M, Gorrini C, Lee Kim C, et al. Glutathione and thioredoxin antioxidant pathways synergize to drive cancer initiation and progression. *Cancer Cell* 2015;**27**:314–5.
32. Chang CW, Chen YS, Tsay YG, Han CL, Chen YJ, Yang CC, et al. ROS-independent ER stress-mediated NRF2 activation promotes warburg effect to maintain stemness-associated properties of cancer-initiating cells. *Cell Death Dis* 2018;**9**:194.
33. Zheng W, Feng Q, Liu J, Guo Y, Gao L, Li R, et al. Inhibition of 6-phosphogluconate dehydrogenase reverses cisplatin resistance in ovarian and Lung cancer. *Front Pharmacol* 2017;**8**:421.
34. Dixon Scott J, Lemberg Kathryn M, Lamprecht Michael R, Skouta R, Zaitsev Eleina M, Gleason Caroline E, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. *Cell* 2012;**149**:1060–72.
35. Kiyohara C, Yoshimasu K, Takayama K, Nakanishi Y. EPHX1 polymorphisms and the risk of lung cancer: a HuGE review. *Epidemiology* 2006;**17**:89–99.
36. Zhang X, Wang L, Li H, Zhang L, Zheng X, Cheng W. Crosstalk between noncoding RNAs and ferroptosis: new dawn for overcoming cancer progression. *Cell Death Dis* 2020;**11**:580.
37. Gu S, Lai Y, Chen H, Liu Y, Zhang Z. miR-155 mediates arsenic trioxide resistance by activating Nrf2 and suppressing apoptosis in lung cancer cells. *Sci Rep* 2017;**7**:12155.