



The role of long noncoding RNA FOXF1-AS1 as a tumor suppressor in non-small cell lung cancer

Hao Zheng, Aswini R. Krishnan, Angela E. Zou, Weg M. Ongkeko

Division of Otolaryngology-Head and Neck Surgery, Department of Surgery, University of California, San Diego, La Jolla, California, USA

Correspondence to: Weg M. Ongkeko. VA San Diego Healthcare System, 3350 La Jolla Village Drive, Floor 6, Room 6029, San Diego, CA 92161, USA. Email: rongkeko@ucsd.edu.

Comment on: Miao L, Huang Z, Zengli Z, *et al.* Loss of long noncoding RNA FOXF1-AS1 regulates epithelial-mesenchymal transition, stemness and metastasis of non-small cell lung cancer cells. *Oncotarget* 2016. [Epub ahead of print].

Submitted Nov 04, 2016. Accepted for publication Nov 16, 2016.

doi: 10.21037/tcr.2016.12.46

View this article at: <http://dx.doi.org/10.21037/tcr.2016.12.46>

Non-small cell lung cancer (NSCLC) accounts for approximately 80% of lung cancer cases and is the leading cause of cancer-related death in the United States (1). A significant challenge associated with NSCLC treatment is the lack of early diagnostic tools, as more than 70% of NSCLC patients are diagnosed at advanced stages, rendering them ineligible for surgical resection (2). Advanced-stage NSCLCs are generally treated by platinum-based chemotherapy or radiation therapy, but both treatments are highly invasive with low specificity and high recurrence rate (3-5). It is therefore critical to better understand the molecular basis of NSCLC, to be able to define early genetic changes so as to diagnose early stage NSCLC and formulate treatment targeting these early changes. Since the groundbreaking discovery that long noncoding RNAs (lncRNAs) are not merely transcriptional noise but possess important functions, they have been implicated in various cancers (6). In lung cancer, lncRNAs such as *MALAT1*, *ANRIL*, and *HOTAIR* were reported to be significantly associated with NSCLC metastasis and highly correlated with patient survival (7). In particular, many lncRNAs have been found to act as direct and indirect regulators of the epigenome, mediating both epigenetic silencing and chromatin remodeling (8). Although it has become increasingly clear that understanding lncRNAs is critical to elucidating the molecular basis of lung cancer, the current knowledge of lncRNAs in NSCLC is limited.

In a recent study, Liyun Miao and colleagues screened paired NSCLC samples using GeneChip® Human Gene 2.0 ST Array (9). They identified lncRNA FOXF1-AS1 to exhibit significant downregulation in lung cancer *vs.* normal

tissue, and further validated this downregulation on 30 adenocarcinomas (AD) and 20 squamous cell carcinomas (SCC) tissues by qRT-PCR. Loss of FOXF1-AS1 was observed to occur consistently in both histologic subtypes (AD and SCC) and in all stages of NSCLC. Previous studies have identified FOXF1-AS1, also known as FENDRR, to be downregulated in gastric cancer, where decreased expression was associated with poor prognosis (10). In lung cancer, FOXF1-AS1 was also named in a panel of 111 dysregulated lncRNAs using RNA-seq data from The Cancer Genome Atlas (TCGA) (11). Another group reported that increased FOXF1-AS1 expression significantly reduced the number of metastatic lung nodules *in vivo* (10). These studies affirm the findings of Miao *et al.* that FOXF1-AS1 potentially functions as a tumor suppressor, underscoring the importance of further assessing this gene to better understand its role in carcinogenesis.

To explore the functional significance of FOXF1-AS1 in NSCLC, the authors stably overexpressed the lncRNA in two lung cancer cell lines (CALU1 and NCIH1975). They observed that overexpression of FOXF1-AS1 significantly inhibited cell migration, invasion, and tumor sphere formation, further corroborating the role of FOXF1-AS1 as a tumor suppressor. Moreover, the fact that lung cancer cells, which display numerous genetic and epigenetic alterations, were able to exhibit a less malignant phenotype with overexpression of FOXF1-AS1 suggests that this gene plays a relatively significant role in mediating NSCLC progression. Previously in gastric cancer, FOXF1-AS1 has also been shown to repress cell migration and invasion *in vitro* (10). Interestingly, FOXF1-AS1 was found to

not affect gastric cancer cell proliferation, a phenotype unexplored in the study by Miao *et al.* and one which would be valuable to evaluate in the context of lung cancer cells.

The authors also reported FOXF1-AS1 to be a regulator of epithelial-mesenchymal transition (EMT) in NSCLC cells. In cancer, EMT is known to lead to increased tumor cell motility and promotes metastasis by inducing self-renewal, the defining trait of cancer stem cells (12). When stably transfected with FOXF1-AS1 plasmids, lung cancer cell lines CALU1 and NCIH1975 displayed morphological change from a fibroblastoid appearance to cobblestone shape, a phenotype indicative of MET (mesenchymal-epithelial transition), a reverse process of EMT. Overexpression of FOXF1-AS1 also decreased expression of vimentin and induced expression of E-cadherin. The loss of E-cadherin, a metastatic suppressor of tumor progression, and upregulation of vimentin, an intermediate filament protein, are hallmarks of EMT occurrence (13). The data from Miao *et al.* suggest that FOXF1-AS1 expression reverses EMT and thereby may functionally inhibit NSCLC. To the best of our knowledge, Miao *et al.* are the first to investigate an EMT phenotype caused by FOXF1-AS1 in any malignancy.

The authors then mechanistically explored FOXF1-AS1 to identify potential pathways by which the gene executes its functionality. First, they investigated the likelihood of interactions between lncRNA FOXF1-AS1 and enhancer of zeste homolog 2 (EZH2) mRNA. EZH2 is the catalytic component of the polycomb repressive complex 2 (PRC2), which is responsible for the epigenetic maintenance of genes during embryonic development (14). EZH2 has been found to promote breast cancer motility and metastasis, prostate cancer metastasis via suppression of tumor suppressor DAB2IP, and melanoma progression (15,16). Recent research suggests that many lncRNAs, including HOTAIR and ANRIL, physically interact with EZH2 in targeting PRC2 to specific genomic sites (17). To evaluate the interaction between FOXF1-AS1 and EZH2, the authors verified that EZH2 was upregulated in lung tumor *vs.* normal tissues, and identified an inverse correlation between EZH2 and FOXF1-AS1 expression. An RNA immunoprecipitation assay confirmed a likely physical interaction between FOXF1-AS1 and EZH2. Next, the authors knocked down FOXF1-AS1 using shFOXF1-AS1, which significantly promotes cell migration and sphere formation ability. The effect of FOXF1-AS1 knockdown was negated when EZH2 was blocked using DZNep. Taken together, the results suggested that FOXF1-AS1 physically

interacts with EZH2, and EZH2 is required for cell migration and stem-like properties triggered by FOXF1-AS1 loss.

Finally, Miao *et al.* (9) explored FOXF1, a target gene of the p53 family and a regulator of migration and invasion, as a target of lncRNA FOXF1-AS1 (18). Existing literature reveals lncRNA FOXF1-AS1 to be transcribed bidirectionally with FOXF1, suggesting a likely interaction between the two (19). The authors found FOXF1 to be more highly expressed not only in cell lines with high expression of FOXF1-AS1, but also to be more lowly expressed in tumor *vs.* normal tissues which demonstrate lower expression of FOXF1-AS1. This was verified computationally in datasets from TCGA, with FOXF1 more lowly expressed in lung AD and SCC, in accord with FOXF1 dysregulation in other carcinomas (10,20). Future studies could investigate whether FOXF1-AS1 also correlates with FOXF1 expression in the TCGA lung cancer samples, as well as samples deposited in other cancer genomics databases. Binding assays involving FOXF1-AS1 and FOXF1 mRNA/protein could also be performed to further verify the physical interaction between the two.

While the findings provided by Miao *et al.* indicate promise for FOXF1-AS1 as a diagnostic or prognostic biomarker of NSCLC, deeper understanding of both FOXF1-AS1 and the large-scale mechanistic foundations of NSCLC pathogenesis remain to be explored. Studies suggest that FOXF1-AS1 targets FN1 and MMP2/MMP9 in gastric cancer, thereby posing the question of whether similar alterations are exhibited by FOXF1-AS1 dysregulation in lung cancer as well, as well as what other molecular interactions are induced by FOXF1-AS1 (10). Meanwhile, analysis of TCGA data on lung cancer revealed a panel of 111 dysregulated lncRNAs between tumor and normal tissues, most of which represent novel transcripts that remain to be evaluated (11). Beyond lncRNAs, other non-coding transcripts including microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), and small nucleolar RNAs (snoRNAs) remain relatively unexplored beyond reports of their dysregulation in lung cancer. Nevertheless, Miao *et al.* successfully present the significant potential of lncRNA FOXF1-AS1 to both genetically and phenotypically regulate NSCLC pathogenesis, and serve as a diagnostic or therapeutic candidate.

Acknowledgments

Funding: None.

Footnote

Provenance and Peer Review: This article was commissioned and reviewed by the Section Editor Long Chen (Department of PET-CT center at the Yunnan Tumor Hospital, The Third Affiliated Hospital of Kunming Medical University, Kunming, China; Department of Biochemistry and Molecular Biology of Kunming Medical University, Kunming, China).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/tcr.2016.12.46>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: <https://creativecommons.org/licenses/by-nc-nd/4.0/>.

References

- Chan BA, Hughes BG. Targeted therapy for non-small cell lung cancer: current standards and the promise of the future. *Transl Lung Cancer Res* 2015;4:36-54.
- Birring SS, Peake MD. Symptoms and the early diagnosis of lung cancer. *Thorax* 2005;60:268-9.
- Kuribayashi K, Funaguchi N, Nakano T. Chemotherapy for advanced non-small cell lung cancer with a focus on squamous cell carcinoma. *J Cancer Res Ther* 2016;12:528-34.
- Huisman C, Smit EF, Giaccone G, et al. Second-line chemotherapy in relapsing or refractory non-small-cell lung cancer: a review. *J Clin Oncol* 2000;18:3722-30.
- Haura EB. Treatment of advanced non-small-cell lung cancer: a review of current randomized clinical trials and an examination of emerging therapies. *Cancer Control* 2001;8:326-36.
- Huarte M. The emerging role of lncRNAs in cancer. *Nat Med* 2015;21:1253-61.
- Ricciuti B, Mencaroni C, Pagliarunga L, et al. Long noncoding RNAs: new insights into non-small cell lung cancer biology, diagnosis and therapy. *Med Oncol* 2016;33:18.
- Peschansky VJ, Wahlestedt C. Non-coding RNAs as direct and indirect modulators of epigenetic regulation. *Epigenetics* 2014;9:3-12.
- Miao L, Huang Z, Zengli Z, et al. Loss of long noncoding RNA FOXF1-AS1 regulates epithelial-mesenchymal transition, stemness and metastasis of non-small cell lung cancer cells. *Oncotarget* 2016. [Epub ahead of print].
- Xu TP, Huang MD, Xia R, et al. Decreased expression of the long non-coding RNA FENDRR is associated with poor prognosis in gastric cancer and FENDRR regulates gastric cancer cell metastasis by affecting fibronectin1 expression. *J Hematol Oncol* 2014;7:63.
- White NM, Cabanski CR, Silva-Fisher JM, et al. Transcriptome sequencing reveals altered long intergenic non-coding RNAs in lung cancer. *Genome Biol* 2014;15:429.
- Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* 2014;15:178-96.
- Kidd ME, Shumaker DK, Ridge KM. The role of vimentin intermediate filaments in the progression of lung cancer. *Am J Respir Cell Mol Biol* 2014;50:1-6.
- Yamaguchi H, Hung MC. Regulation and Role of EZH2 in Cancer. *Cancer Res Treat* 2014;46:209-22.
- Min J, Zaslavsky A, Fedele G, et al. An oncogene-tumor suppressor cascade drives metastatic prostate cancer by coordinately activating Ras and nuclear factor-kappaB. *Nat Med* 2010;16:286-94.
- Zingg D, Debbache J, Schaefer SM, et al. The epigenetic modifier EZH2 controls melanoma growth and metastasis through silencing of distinct tumour suppressors. *Nat Commun* 2015;6:6051.
- Martínez-Fernández M, Feber A, Dueñas M, et al. Analysis of the Polycomb-related lncRNAs HOTAIR and ANRIL in bladder cancer. *Clin Epigenetics* 2015;7:109.
- Tamura M, Sasaki Y, Koyama R, et al. Forkhead transcription factor FOXF1 is a novel target gene of the p53 family and regulates cancer cell migration and invasiveness. *Oncogene* 2014;33:4837-46.
- NCBI Resource Coordinators. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res* 2016;44:D7-19.
- Li T, Mo X, Fu L, et al. Molecular mechanisms of long noncoding RNAs on gastric cancer. *Oncotarget* 2016;7:8601-12.

Cite this article as: Zheng H, Krishnan AR, Zou AE, Ongkeko WM. The role of long noncoding RNA FOXF1-AS1 as a tumor suppressor in non-small cell lung cancer. *Transl Cancer Res* 2016;5(Suppl 7):S1440-S1442. doi: 10.21037/tcr.2016.12.46