

# The tRNA-derived fragment signature in lung cancer

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A new class of regulatory small non-coding RNAs called tRNA-derived fragments (tRDFs) has distinct biological roles in various types of cancer. In this issue of *Molecular Therapy – Oncolytics*, Zitong Gao et al. performed a thorough study of tRDFs in 1,550 patient samples of non-small cell lung cancer (NSCLC) and discovered 52 tRDFs with four subtypes. Based on expression profiles, six tRDFs were selected as diagnostic biomarkers. Two of these biomarkers were successfully validated in plasma samples, and six biomarkers confirmed the consistency of distinguished expression in NSCLC cell lines. Furthermore, 5a tRF-Ile-AAT/GAT may serve as an early-stage prognosis biomarker. Overall, the results of this study represent the first systematic analysis of tRDFs in lung cancer, and they identified four distinct tRDF subtypes and investigated their potential diagnostic, prognostic, transcriptional, and immunological implications in the disease.

Non-coding RNAs, including long and small non-coding RNAs, play critical roles in tumorigenesis. With the development of high-throughput sequencing technology and bioinformatics analysis, scientists have found that small RNA fragments (tRFs) called tRDFs are dysregulated in many cancers and that their expression changes during the development and staging of the cancer.<sup>1</sup> tRFs are generated from either pre-tRNAs or mature tRNAs, are classified into several groups based on their incision loci, including i-tRFs, 5-tRFs, 3-tRFs, and 1-tRFs, and are involved in a variety of biological processes. They have also been found to be abnormally expressed in major diseases like cancer, viral infectious diseases, and neurodegenerative diseases, which could lead to new biomarkers for the identification of organ damage.<sup>2</sup> Recent research by several groups indicates that tRDFs have a role in tumor initiation and development by showing that tRDF

expression is dysregulated in lung cancer and chronic lymphocytic leukemia.<sup>3</sup> Consistent evidence indicates that tRDFs have the potential to be biomarkers in cancer diagnosis. However, no study has yet to investigate the full spectrum of tRDFs in terms of their expression levels and biological roles in NSCLC.

In this study, Zitong Gao et al.<sup>4</sup> integrated four tRDF data resources by combining different sections of tRNA fragments and examining the expression pattern of tRDFs in 1,550 samples from TCGA, GEO, and plasma sequencing data (Figure 1). Within these three datasets, 152 tRDFs were found to be common. Differential expression analysis was performed in order to understand the expression patterns in tumor and normal samples from two GEO datasets. It shows that 11 DE tRDFs are strongly linked to adenocarcinoma and can be used to diagnose both tumors and normal cells. When DE tRDFs from different data platforms were compared, it was found that 6 tRDFs in TCGA-lung adenocarcinoma (LUAD) overlapped with 11 differentially expressed (DE) tRDF candidates, which was referred to as a signature to be diagnostic biomarkers. In order to validate the potential diagnostic value of 11 tRDF candidates, small RNA sequencing (RNA-seq) was done on 50 patients with LUAD and 60 healthy controls. The results showed that there were significant differences between normal and tumor samples for two tRDFs: 3P tRNA-Arg-TCG-1-1 and 5P tRNA-Asn-GTT-2-3. Then, they used qRT-PCR for further validation to find the 11 tRDF candidates in NSCLC cell lines, and these results showed that four tRDF signatures went up and two went down between NSCLC cell lines compared with normal cells. This qPCR analysis shows that the diagnostic value of tRDFs found through bioinformatics analysis was consistent.

There is evidence from several studies suggesting that tRDFs can be identified in blood samples and have the potential to serve as diagnostic biomarkers for a variety of disorders, including cancers. Due to the limitations of clinical and pathological approaches, it is critical to make a precise diagnosis of a patient's disease at an early stage. The combination of plasma and tissue samples might also help researchers learn more about tRDFs in cancer research by making the conclusion stronger and more solid. Also, survival-related tRDFs are an important part of predicting clinical outcomes in NSCLC, and the 5-year survival rates of early-stage NSCLC showed that the outcomes were better than those of advanced-stage NSCLC. Survival analysis was performed based on specific endpoints of follow-up time ranging from 6 to 60 months. 5a\_tRF-Ile-AAT/GAT showed excellent prognostic value in both the early and late stages, and 3P\_tRNA-Arg-TCT-4-1 was identified to be sensitive in the early following days, which was within 1 year.

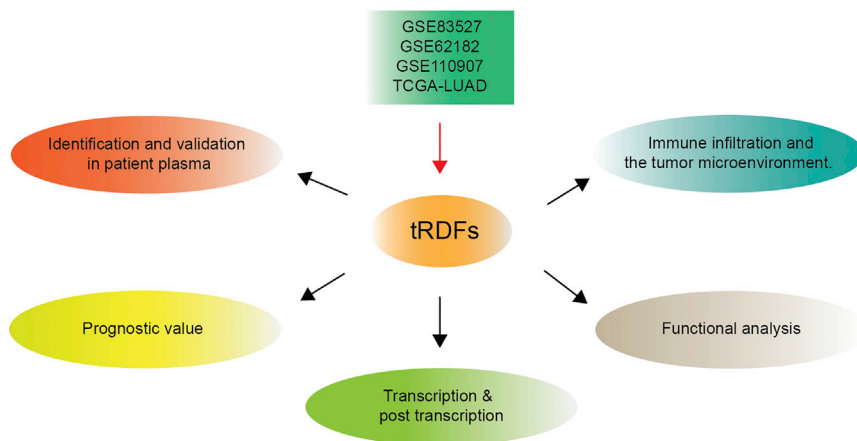
The TCGA-LUAD tumor data and the expression of 52 tRDFs were used in the further investigation to find tRDFs' involvement in transcriptional and post-transcriptional events. The results indicated that 69 microRNAs (miRNAs) correlated with 5-tRDFs, including 63 miRNAs that were positively correlated with 15 5-tRDFs and 23 miRNAs that were negatively correlated with six 5-tRDFs. There was a strong link between the miRNA-targeted genes and the signaling pathways of phosphatidylinositol 3-kinase (PI3K)-Akt, mitogen-activated protein kinase (MAPK), endocytosis, and Ras. Many studies have shown that hsa-miR-145-5p is a suppressor that targets tumor-specific genes and proteins. One of the

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**Figure 1. A comprehensive analysis of tRNA-derived fragments in lung cancer**

signatures, 5a tRF-Asp-GTC, was found to have a strong correlation with this suppressor, hsa-miR-145-5p. Sun et al. demonstrated in *in vitro* studies that 5a tRF-Ile-AAT/GAT is abundant in lung cancer and can control the cell cycle.<sup>5</sup> Also, in this present study, too, 5a tRF-Ile-AAT/GAT was found to be highly expressed in tumor tissue and was one of the tRDFs in the signatures.

The association between tRDFs and mRNA was analyzed to determine the implicational biological role of tRDFs in LUAD. There was a strong relationship between 1,806 genes and tRDFs. This included 1,560 genes that were upregulated and 246 genes that were downregulated. tRDFs are found in hematopoietic and lymphoid tissue, as well as the

blood circulatory system, and in recent years, researchers have become interested in how the immune system controls tRDFs. Based on the integrative analysis, it was found that tRDFs from both the non-coding transcriptome and the immunogenomics profiles were strongly correlated with the infiltrating levels of 14 immune cell types. Overall, this study describes how tRDF expression affects immune-related biological processes and signaling pathways in the tumor immune microenvironment (TIME). In addition, the fact that tRDF-mediated cancer immunity in NSCLC could be a treatment target and have therapeutic value was also mentioned.

However, this study has limitations, as the authors comprised five datasets and only

TCGA-LUAD/lung squamous cell carcinoma (LUSC) contained follow-up time so the prognosis analysis was limited to those two cohorts. In this analysis, they only analyzed mRNA expressions from TCGA-LUAD, as there were no more expression profiles associated with small RNA-seq from GEO. In addition, some of the subtypes of 5'-tiRNA and 3'-tiRNA were left out due to their length differences from the other four subtypes. This study lays important groundwork for future research on non-coding tRNAs in cancer diagnosis and other major diseases. It also helps develop new datasets for databases.

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