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# Expression of miRNA-25 in young and old lung adenocarcinoma

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**Background:** An appropriate personalized molecular testing ensures the most efficacious treatment in lung cancer. It is still controversial whether younger lung adenocarcinoma (LUAD) patients have different molecular features compared with their older counterparts. MicroRNAs have been involved in lung cancer and their altered expression has been suggested as a potential biomarker in the pathogenesis, diagnosis, prognosis, and therapy of LUAD. **Materials and Methods:** To analyze putative differences in miR-25 expression between young (with age  $\leq 50$  years) and old adenocarcinoma patients, we quantified miR-25 levels with NanoString technology in 88 LUAD specimens. We further investigated a cohort of 309 LUAD patients from the cancer genome atlas (TCGA) database to test our hypothesis. **Results:** miR-25 expression was upregulated in young LUAD patients in comparison to the older ones ( $P = 0.03$ ) in our series. The analysis of public database TCGA confirmed our results, which miR-25 differentially expressed in the two aged groups ( $P = 0.0009$ ). Moreover, a consequential pairing of miR-25 with a target region in phosphatase and tensin homolog (*PTEN*) 3' untranslated region (UTR) and actually low *PTEN* expression seemed to be associated with high miR-25 ( $P = 0.001$ ) in young patients. **Conclusions:** The interaction of miR-25 and *PTEN* in young LUAD may define a subgroup of patients, highlighting the concept of molecular testing in different age subtypes.

**Key words:** Lung adenocarcinoma, MicroRNAs, miR-25, phosphatase and tensin homolog, young

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## INTRODUCTION

Despite lung cancer is one of the most common cancers and the leading cause of cancer-related death worldwide<sup>[1]</sup> the mechanisms controlling lung tumorigenesis remain unclear. An appropriate personalized molecular testing ensures the most efficacious treatment in lung cancer. It is still controversial whether younger lung adenocarcinoma (LUAD) patients have different molecular features compared with their older counterparts.<sup>[2]</sup>

MicroRNAs (miRNAs) are a class of small noncoding RNA acting in regulation of a multiplicity of target genes through mRNA degradation or translation repression.<sup>[3]</sup> MiRNAs have been involved in lung cancer, and their altered expression has been suggested as a potential biomarker in LUAD. However, few data are known

regarding the different expression level of these small RNAs in the young compared to old LUAD patients.

MiR-25 is a member of the miR-106b-25 family, with miR-106b and miR-93. The role of miR-25 on cancer can be oncogenic or tumor suppressive in different kinds of cancer.<sup>[4]</sup> In particular, the exact miR-25 involvement in LUAD is still understood as well as if miR-25 may have diverse effects depending on age.

In the current study, we assayed miR-25 expression level by NanoString platform in young and old LUAD patients surgically resected at the Unit of Thoracic Surgery in Pisa (Italy); furthermore, to better investigate the potential role of this miRNA in the two age subgroups on a larger population, we analyzed a cohort of 309 LUAD patients from the cancer genome atlas (TCGA) database.

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Phosphatase and tensin homolog (PTEN) is a negative regulator of phosphatidylinositol 3-kinase (PI3K) signaling pathway, one of the most important in cell growth regulation, with a crucial role in cancer.<sup>[5]</sup> Impaired PTEN function due to genetic and epigenetic alterations leads to uncontrolled activation of downstream signaling pathway, necessary in the cancer phenotype development.<sup>[6]</sup> In addition, recently, some miRNAs have been reported to regulate cell proliferation by targeting *PTEN*.<sup>[7,8]</sup> We used bioinformatics tools to verify if PTEN was a potential miR-25 predicted target. The interaction of miR-25 and PTEN in young LUAD may define a subgroup of patients, highlighting the concept of molecular testing in different age subtypes.

## MATERIALS AND METHODS

### Patients and tumor characteristics

We retrospectively selected 88-LUAD patients who underwent surgical resection at the Unit of Thoracic Surgery in the Department of Surgical, Medical, Molecular Pathology, and Critical Area at Pisa University. We used a cutoff value of 50 years to divide young (50 years or less), from old patients (range: 51–88 years). The diagnoses were formulated in formalin-fixed and paraffin-embedded (FFPE) histological samples, according to the World Health Organization classifications, describing identifiable predominant pattern present (lepidic, acinar, papillary, solid, and micropapillary), and invasive component (minimally invasive adenocarcinoma, adenocarcinoma *in situ*, or an invasive adenocarcinoma).<sup>[9,10]</sup> Molecular analysis (miRNA isolation and expression) was performed after the selection of the most representative paraffin blocks of the tumor tissues. The clinicopathological characteristics were available for all patients. The study was conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

### MicroRNA isolation

MiR-25 was isolated from FFPE tissues after deparaffinization and manual macrodissection of the neoplastic area, using the miRNeasy FFPE kit (QIAGEN Inc., Hilden, Germany), accordingly to the manufacturer's instructions. After quality and quantity evaluation with a NanoDrop ND-1000 (ThermoScientific, USA) spectrophotometer, the samples were stored at  $-80^{\circ}\text{C}$  until used.

### miR-25 expression by nanostring

MiR-25 expression was evaluated by the nCounter assay, with a reporter and capture probe specific for miR-25, in accordance with the manufacturer's instructions (NanoString Technologies, Seattle, WA, USA). Nanostring nSolver (version 2.5) software was used for the analysis of raw NanoString counts.

### The cancer genome atlas database

We extracted IlluminaHiSeq miR-25 expression plan together with the corresponding clinicopathological characteristics for 309 adenocarcinoma patients (LUAD) from the TCGA data portal (<http://tcga.cancer.gov/>; accessed October 2017).

### Statistical analysis

Chi-square and Student's unpaired *t*-test was applied using JMP10 software (SAS) to determine the association between miR-25 expression and clinicopathological parameters. A  $P < 0.05$  was considered statistically significant.

### MiR-25 target prediction and pathway analysis

We used different databases such as miRanda (<http://www.microrna.org/>) and TargetScan 6.2 (<http://www.microRNA.org/>), to predict putative miR-25 targets.

## RESULTS

### Patients and tumor characteristics

Eighty-eight patients with LUAD were enrolled in this study, 56 males and 32 females. The cutoff value of 50 years was used to identify younger LUAD patients and the older counterparts. The different subtypes of adenocarcinoma were characterized as follows: Lepidic (29/88, 33%), solid (26/88, 29.5%), acinar (22/88, 25%), and papillary (11/88, 12.5%) variants. Their stages were classified as I in 40 cases (17 IA, 23 IB), as II in 22 cases (13 IIA, 9 IIB), as III-IV in 26 cases (23 IIIA, 1 IIIB, and 2 IV).

### miR-25 expression and clinicopathological characteristics

miR-25 expression was evaluated using the nSolver Software version 2.5 (nanoString Technologies, Seattle, Washington) and compared with our patients' clinicopathological characteristics. The samples were divided into high and low expression groups based on the median fold-change value (6583 for miR-25). High miR-25 expression was significantly associated with younger ( $P = 0.01$ ) [Table 1]; *t*-test confirmed a higher miR-25 level ( $7531.54 \pm 563.39$ ) in younger than in older patients ( $5844.13 \pm 563.39$ ) ( $P = 0.03$ ) [Figure 1].

### The cancer genome atlas data analysis

A cohort of 309 LUAD patients from TCGA database was analyzed, to identify putative differences in miR-25 expression levels between young and old adenocarcinoma patients on a larger population. Twenty-three patients out of 309 TCGA cases showed an age of 50 years or less; in this younger group, miR-25 levels were higher ( $10901.9 \pm 967.38$ ) than in the older counterparts ( $7528.3 \pm 274.33$ ) ( $P = 0.0009$ ) [Figure 2].

### MicroRNA regulation prediction analysis

Bioinformatic tools showed that most of the predicted miR-25 targets are involved in critical pathways such as cell

**Table 1: MicroRNAs-25 expression level and clinicopathological characteristics in 88 lung adenocarcinoma patients**

| Variables            | Low | High | P    |
|----------------------|-----|------|------|
| Age (years)          |     |      |      |
| ≤50                  | 16  | 28   | 0.01 |
| >50                  | 28  | 16   |      |
| Gender               |     |      |      |
| Male                 | 31  | 25   | 0.18 |
| Female               | 13  | 19   |      |
| ADC previous pattern |     |      |      |
| Lepidic              | 15  | 14   | 0.05 |
| Solid                | 13  | 13   |      |
| Acinar               | 7   | 15   |      |
| Papillar             | 9   | 2    |      |
| T                    |     |      |      |
| T1                   | 10  | 12   | 0.79 |
| T2                   | 21  | 18   |      |
| T3-T4                | 13  | 16   |      |
| N                    |     |      |      |
| N0                   | 22  | 25   | 0.41 |
| N1                   | 6   | 9    |      |
| N2                   | 12  | 6    |      |
| Nx                   | 4   | 4    |      |
| Stage                |     |      |      |
| I                    | 20  | 20   | 1    |
| II                   | 11  | 11   |      |
| III-IV               | 13  | 13   |      |

ADC=Apparent diffusion coefficient

cycle regulation. Interestingly, we found a consequential pairing of miR-25 with a target region in *PTEN* 3' UTR [Figure 3].

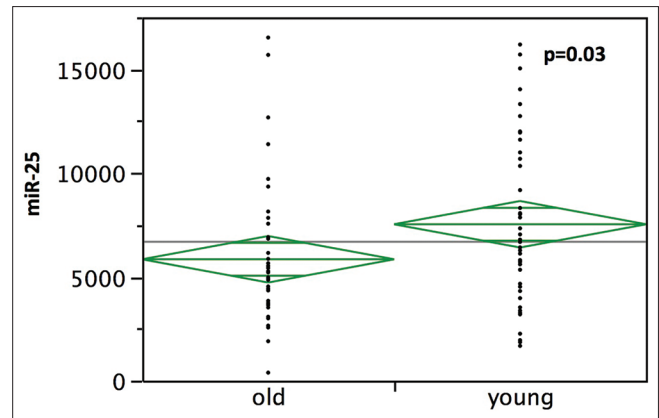
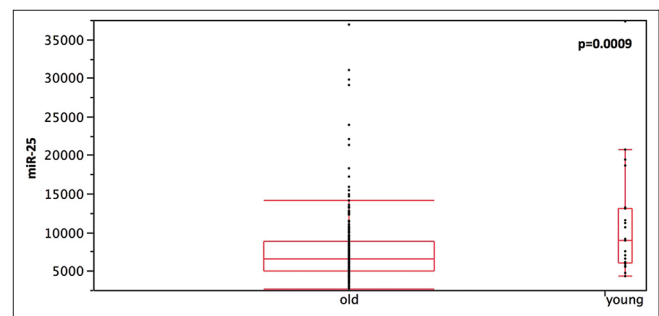
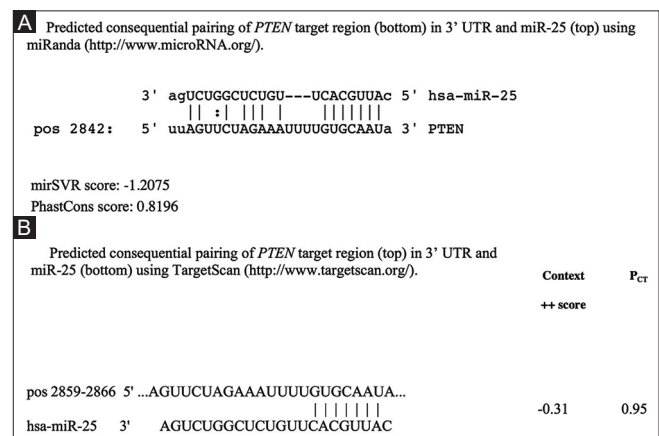
### Phosphatase and tensin homolog expression

The samples were divided into high and low *PTEN* expression groups based on the median fold-change value ( $1.53 \times 10^7$  for *PTEN*). Low *PTEN* levels were significantly associated with high miR-25 expression; samples with high *PTEN* showed lower miR-25 levels ( $6917 \pm 378$ ) than samples with low *PTEN* ( $8603 \pm 369$ ) (*t*-test,  $P = 0.001$ ) [Figure 4]. Moreover, most of the young patients (17/23) showed lower *PTEN* levels when compared with their older counterparts ( $P = 0.02$ ).

## DISCUSSION

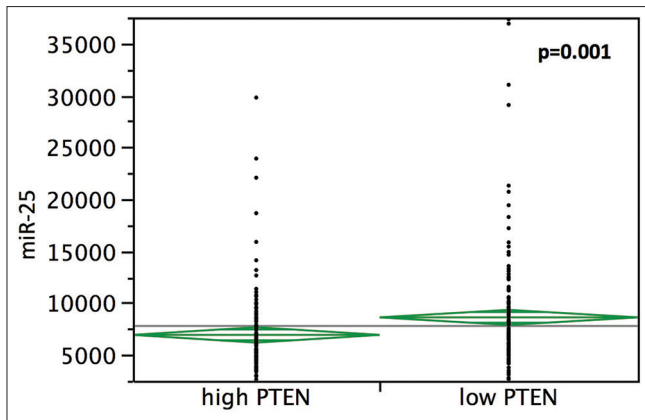
In the last decade, lung cancer incidence has been rising, and the age at diagnosis continues to decrease.<sup>[11]</sup> However, it remains controversial whether younger patients could represent a separate clinicopathological entity when compared to their old counterparts. We used the cutoff age of 50 years to define young lung cancer patients, which is commonly used to separate the two aged groups.<sup>[12-15]</sup>

Aberrant microRNA expression is involved in lung cancer regulation, affecting tumor suppression as well as


**Figure 1:** Comparison of miR-25 expression level in 44 young (with age ≤50 years) and 44 old lung adenocarcinoma patients

**Figure 2:** Comparison of miR-25 expression level in young (with age ≤ 50 years) and old lung adenocarcinoma patients extracted from the cancer genome atlas database

**Figure 3:** Predicted consequential pairing of phosphatase and tensin homolog target region (top) and miR-25-3p (bottom) using miRanda (<http://www.microRNA.org/>) in upper panel A, and TargetScan (<http://www.targetscan.org/>) in lower panel B

oncogenesis, indicating their importance in the regulation of lung cancer,<sup>[16]</sup> but few studies have been conducted on miRNAs expression profile in the two age groups.<sup>[17]</sup> MiR-25 may have different roles on the pathogenesis of several types of cancer, including small and nonsmall-cell lung cancer (NSCLC).<sup>[18,19]</sup> The present study aimed to better investigate the expression of miR-25 in young and old lung cancer patients. In detail, the miR-25 levels were compared





**Figure 4:** Relationship between miR-25-3p and phosphatase and tensin homolog expression level in 309 lung adenocarcinoma samples from the cancer genome atlas database

between ADC patients younger and older than 50 years, and we found that miR-25-3p was significantly upregulated in the young patients in our series of 88 patients. A cohort of 309 LUAD patients from the TCGA database confirmed our findings on a larger population and also using a different transcriptome-based technologies such as Illumina HiSeq quantification. Our study demonstrated higher miR-25 levels in young in comparison to old LUAD patients; however, Noren Hooten *et al.*<sup>[20]</sup> demonstrated changes in miRNA expression with human aging and Peña-Chilet *et al.*<sup>[21]</sup> suggested that miRNAs signature have the potential to be age-related in breast cancer.

The online prediction software's, such as miRanda, TargetScan, and others, identified different putative targets of miR-25, but few reports have investigated the downstream signaling pathway of miR-25. Wang *et al.*<sup>[22]</sup> demonstrated that miR-25 acts as oncogene in osteosarcoma cells by directly targeting the cell cycle inhibitor p27. Tissue miR-25 expression was also involved in female LUAD patients<sup>[23]</sup> and, recently, Ding *et al.* showed that miR-25 enhances cell migration and invasion in nonsmall cell lung cancer cells through extracellular signal-regulated kinase (ERK) signaling pathway by inhibiting Kruppel-like factor 4 (KLF4).<sup>[24]</sup>

PTEN is a main controller of PI3K signaling pathway with a central role in several tumors, including lung cancer.<sup>[25]</sup> The results concerning the prognostic value of PTEN downregulation seem to be contradictory, with association to unfavorable survival in NSCLC patients in some papers,<sup>[26-28]</sup> and the opposite conclusions in others.<sup>[29]</sup> However, Xiao *et al.*<sup>[30]</sup> suggested that PTEN expression levels might also add information on the effect of treatment for patients with NSCLC. In our work, we observed that lung cancer in young and old patients may be influenced by different regulatory mechanisms since we found *PTEN* downregulated in the younger group, probably due to

distinct age-related mi-R25 expression. Young LUAD patients with decreased expression of PTEN due to high miR-25 levels may represent a subtype of lung cancer and might benefit from individualized treatment plans.

## CONCLUSIONS

Our study provides new insights into the role of miR-25 in LUAD, in particular in young patients; the interaction of miR-25, and PTEN in young LUAD patients may define a subgroup of patients, highlighting the concept of molecular testing in different age subtypes. Further validations are needed to better define if an age-based genomic signature could be used as a tool in the personalized therapy of LUAD.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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