

Impact of piR_004530 Reactivation in Lung Cancer: Implications for Recurrence and Survival of Lung Squamous Cell Carcinoma Patients

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BACKGROUND

Lung squamous cell carcinoma (LUSC) accounts for a large proportion of non-small-cell lung cancer (NSCLC) but lacks reliable biomarkers for recurrence and survival. piRNAs are emerging regulators in cancer biology, yet their prognostic role in LUSC remains unclear. We investigated piR_004530 expression, function, and clinical impact in resected NSCLC, focusing on LUSC.

METHODS

Patients: Tumor and matched normal tissues from 243 NSCLC patients; piR_004530 quantified by qRT-PCR.

Cell lines: LUSC lines (HCC-15, SK-MES-1) transfected with piR_004530; assays for migration, invasion, proliferation, apoptosis, and clonogenicity.

Organoids: LUSC organoids generated from resected tumors; piR_004530 delivered via nucleofection; viability assessed with luminescence-based 3D assays.

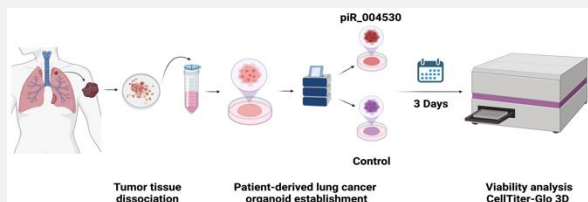


Figure 1. Study workflow. For derivation of patient-derived lung cancer organoids from NSCLC resected patients and nucleofection procedure used to analyze the role of piR_004530 overexpression and its impact on organoid viability (Created with BioRender.com)

RESULTS

• Expression of piR_004530 in NSCLC tissues

Upregulated in LUSC compared to paired normal tissues ($p < 0.001$).

High expression → shorter RFS and OS.

• Prognostic value of piR-004530

SCC patients: Prognostic impact was stronger. High expression associated with higher relapse rate ($p=0.007$), shorter DFS (35 vs 90.5m, $p<0.001$), and OS (13.5m vs NR, $p=0.004$). Multivariate Cox validated independence (DFS HR 2.53; OS HR 2.15).

• Interaction with adjuvant therapy

In LUSC patients without adjuvant therapy, high piR-004530 = worse DFS, OS, TTR.

piR-004530 helps identify high-risk subgroup who may benefit from adjuvant treatment.

DCA showed piR-004530 model > stage alone.

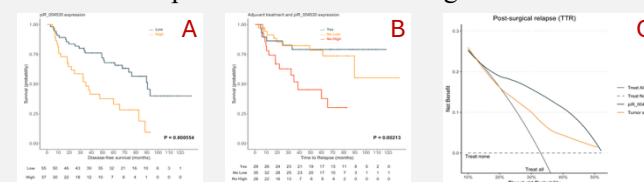


Figure 2. High piR_004530 predicts poor prognosis in LUSC patients. A. OS by piR-004530 expression; B. RFS by piR-004530 expression; C. ROC curve showing predictive performance for time-to-relapse.

• Functional effects in LUSC cell lines

-Overexpression enhanced migration and invasion

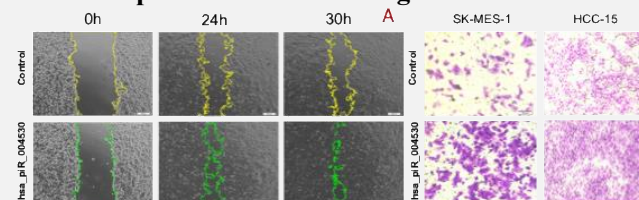


Figure 3. In vitro analysis in LUSC cell lines to determine the functional role of piR_004530. A. Wound-healing assays showing enhanced migration; B. Transwell invasion assays showing enhanced invasion.

-Colony formation increased in HCC-15, apoptosis reduced in SK-MES-1, and proliferation enhanced at 96–120h.

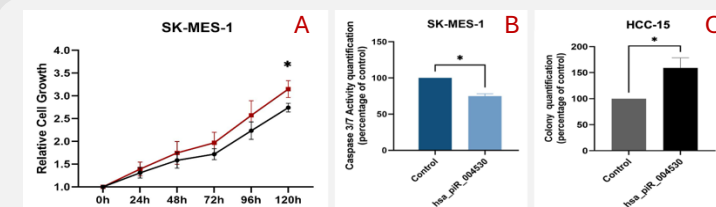


Figure 4. Proliferation, apoptosis, and colony formation. A. Proliferation assays showing enhanced growth; B. Caspase 3/7 assays showing reduced apoptosis; C. Colony formation assays showing increased colonies.

• Patient-derived organoids

Overexpression of piR_004530 increased viability in LUSC organoids ($p < 0.05$).

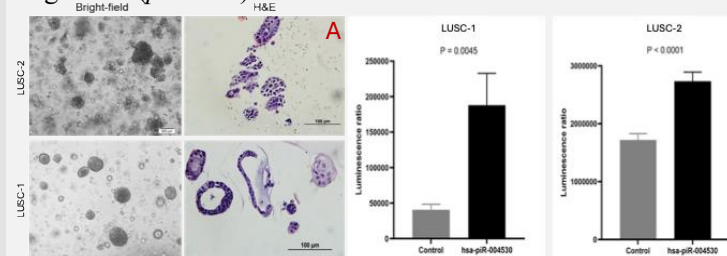


Figure 5. piR_004530 overexpression boosted cell viability of patient-derived LUSC organoids. A. Representative organoid images and histology confirming LUSC origin; B. Viability assays showing increased growth in piR_004530-overexpressing organoids.

DISCUSSION & CONCLUSIONS

piR_004530 was tumor-specific, largely absent in normal tissue, and its high expression predicted poor survival, especially in LUSC. Functional assays showed that piR_004530 promoted migration, invasion, colony formation, proliferation, and stemness, while reducing apoptosis. Validation in patient-derived organoids confirmed its role in sustaining tumor growth. Overall, piR_004530 acts as an oncogenic piRNA and potential biomarker for relapse risk stratification and adjuvant therapy guidance in LUSC patients.

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