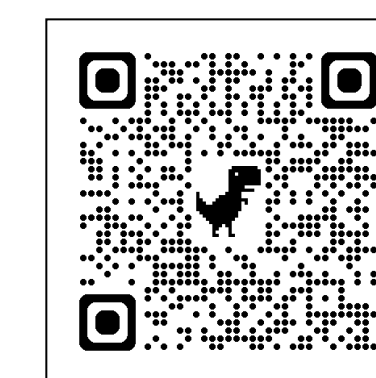


KLF5/miRNA regulatory network modulates tumorigenesis by regulating EMT, DNA damage and apoptosis in TSCC

Subham Kumar Behera¹, Priyajit Biswal, Anthony Lalruatfela, Sruti Biswal, and Bibekanand Mallick*

RNAi and Functional Genomics Lab., Dept. of Life Science, National Institute of Technology Rourkela, Rourkela-769008, Odisha, India

¹Presenting author: subhamkumar.phd96@gmail.com *Corresponding author : vivek.iitian@gmail.com



Abstract

Introduction

Transcription factors (TFs) play pivotal role in regulation of gene expression and their altered expression leads to cancer development. Along with protein coding genes, TFs modulate expression of microRNAs (miRNAs). Dysregulations in these TFs/miRNAs regulatory network is crucial in understanding oncogenesis; however, it is poorly understood in tongue squamous cell carcinoma (TSCC).

Objective

1. Identification of key TFs/miRNAs regulating oncogenesis in TSCC.
2. Investigating the role of TFs/miRNAs and their targets to decode their mechanisms of action in TSCC.

Materials and methods

We analyzed and obtained differentially expressed genes and miRNAs in TSCC. We selected TF, KLF5 based on its role in multiple cancer-related events. We validated the role of KLF5 in TSCC upon its overexpression by adopting different molecular assays, such as qRT-PCR, western blotting, confocal and fluorescence imaging.

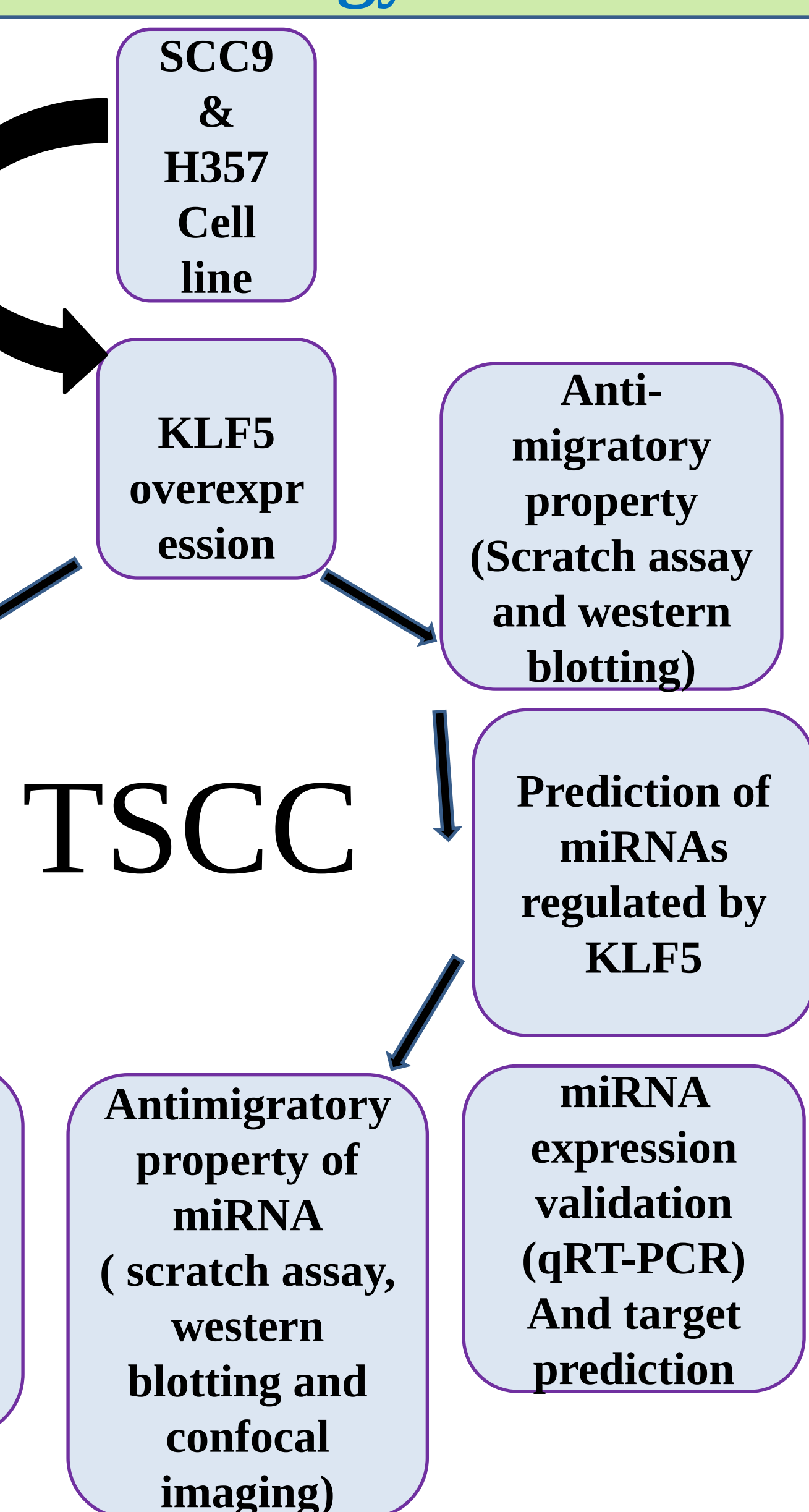
Results

KLF5 expression is significantly downregulated in H357 and SCC9 TSCC cells. We predicted miRNAs regulated by KLF5, among which miR-203a is one of them, downregulated in TSCC. Further, overexpression of KLF5 reduces cell migration, and reduces the expression of mesenchymal markers like N-Cadherin, while increasing the expression of E-Cadherin in TSCC. Further, there was increased γH2AX, suggesting increased DNA damage upon KLF5 transfection. AO/EtBr staining, annexin V/PI apoptosis assay and western blot of apoptosis related genes- BAX and BCL2 confirmed KLF5 overexpression induces apoptosis in TSCC.

Conclusion

Upregulation of KLF5 inhibits cell migration and induces apoptosis. KLF5 induces expression of miR-203a, which in turn may exert its regulatory effects.

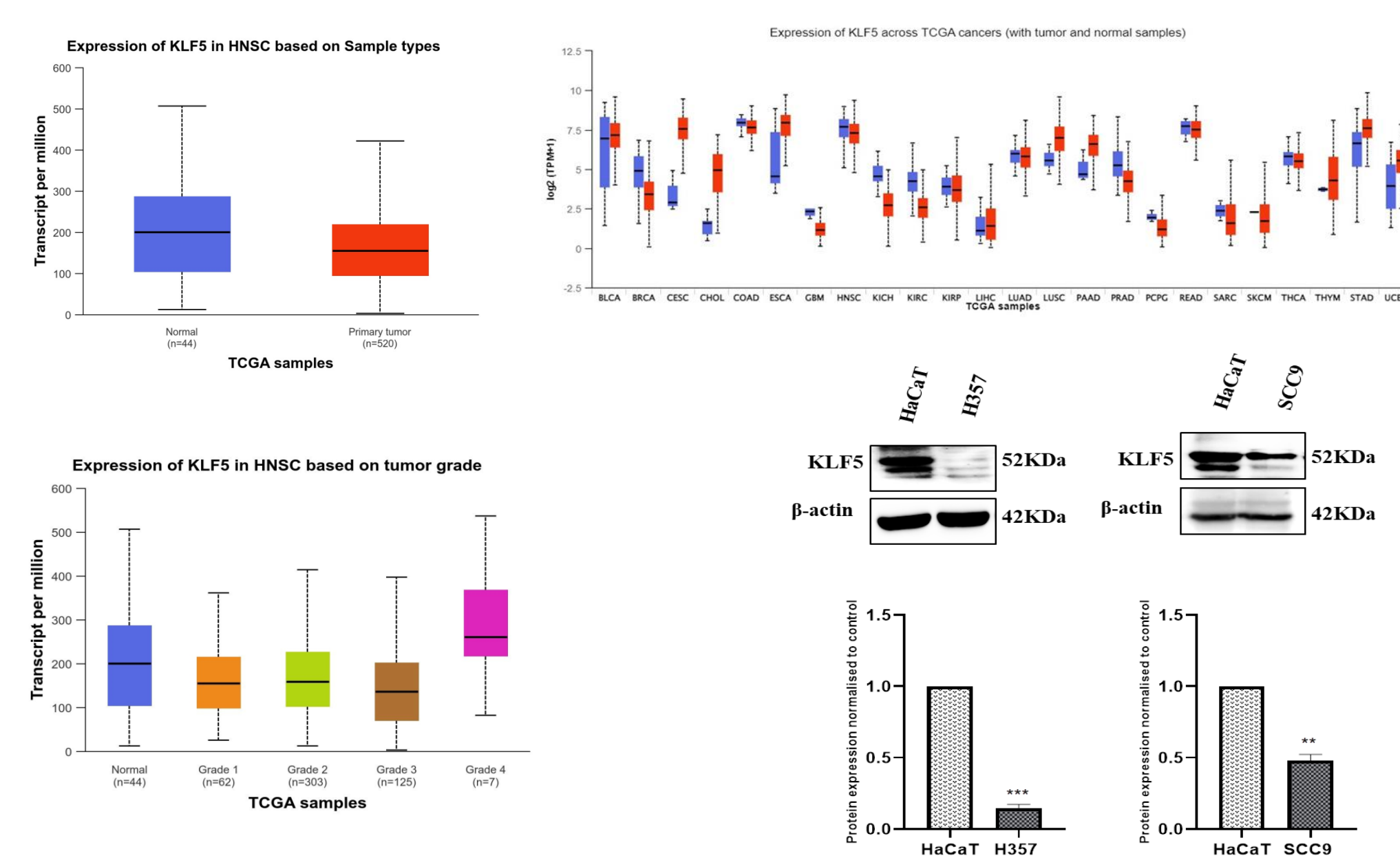
Methodology



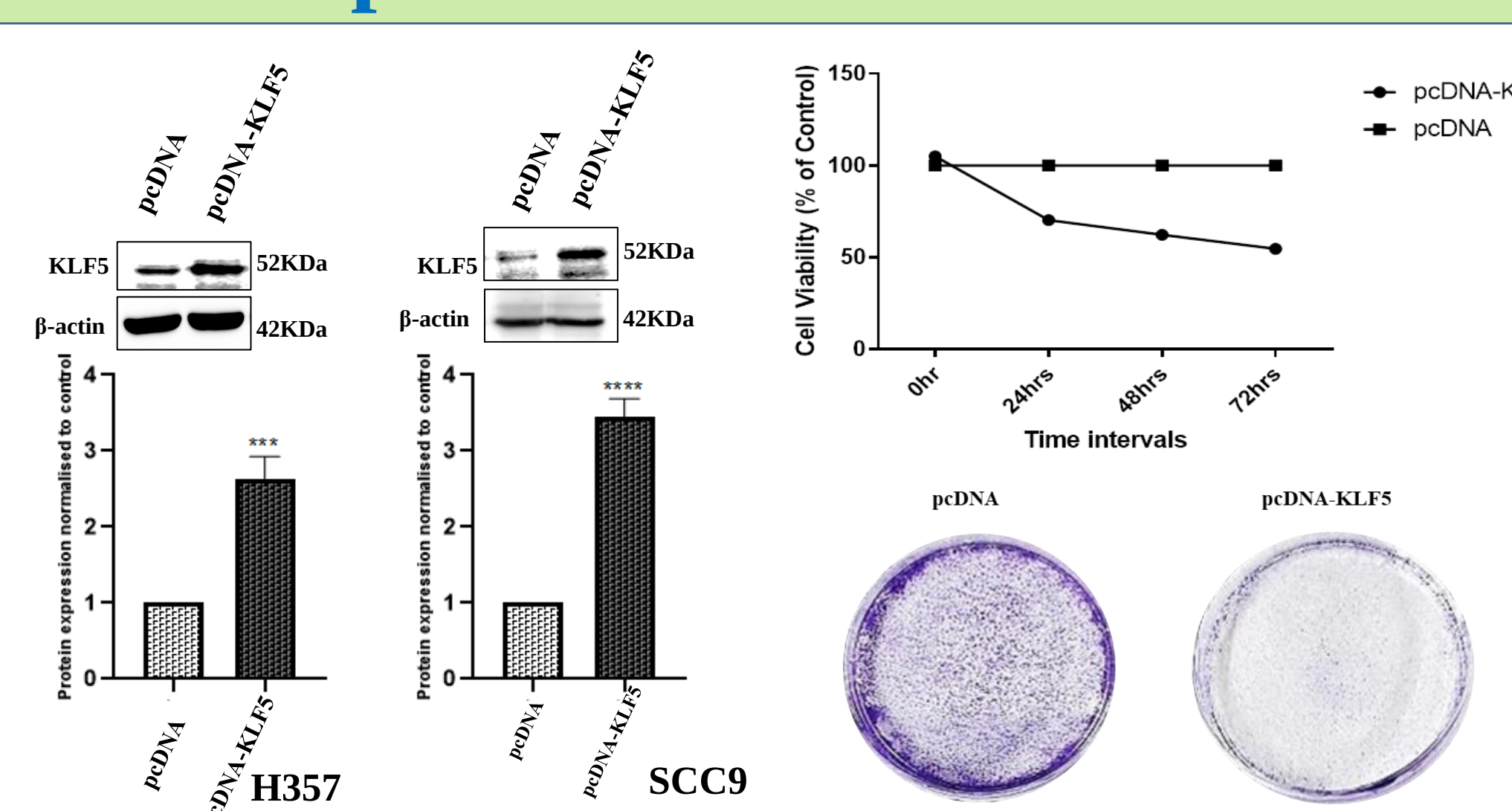
Conclusion

- This study suggested that KLF5 is significantly downregulated in SCC9 and H357 TSCC cell lines and overexpression of KLF5 reduces cell viability, proliferation, migration and induces DNA damage and apoptosis.
- KLF5 transcriptionally activate miR-203a by binding to the promoter of 203a gene. After activation, mature miR-203a target and inhibit SNAI2 by binding at 3'UTR region thereby inhibiting cell migration.

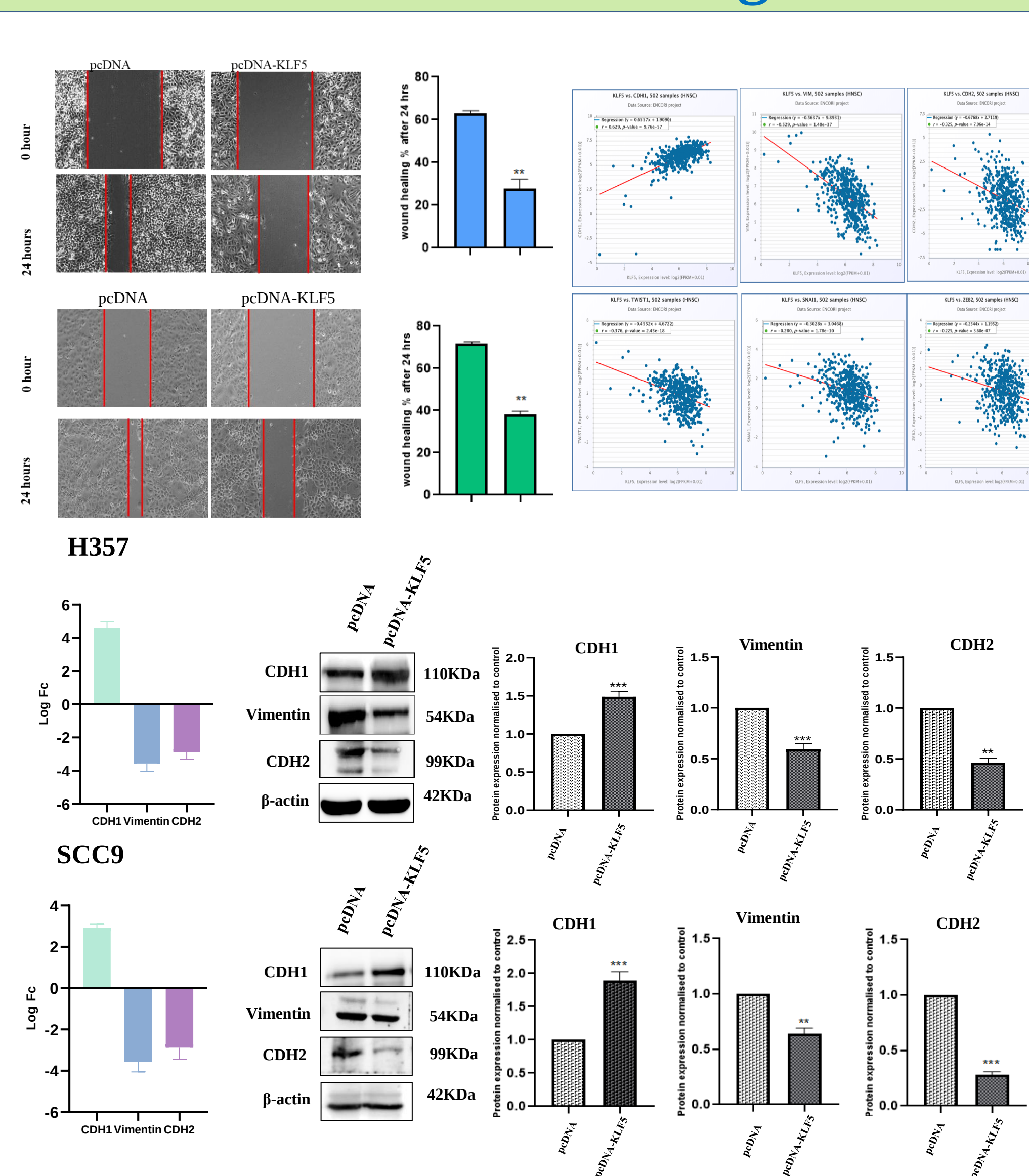
KLF5 is downregulated in TSCC



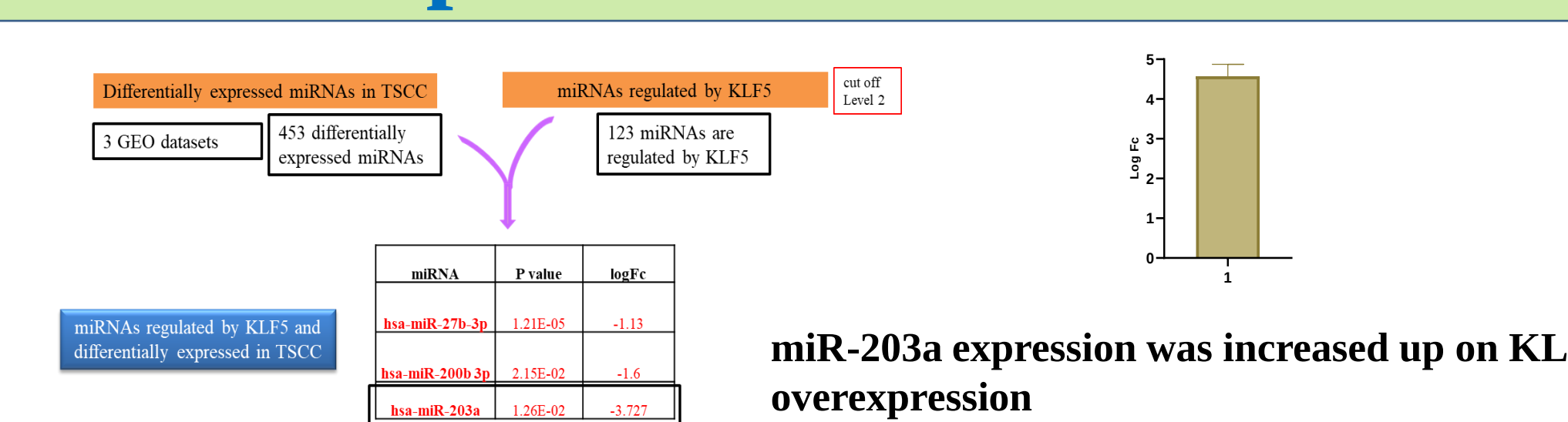
KLF5 reduces cell viability and proliferation in TSCC



KLF5 inhibits cell migration



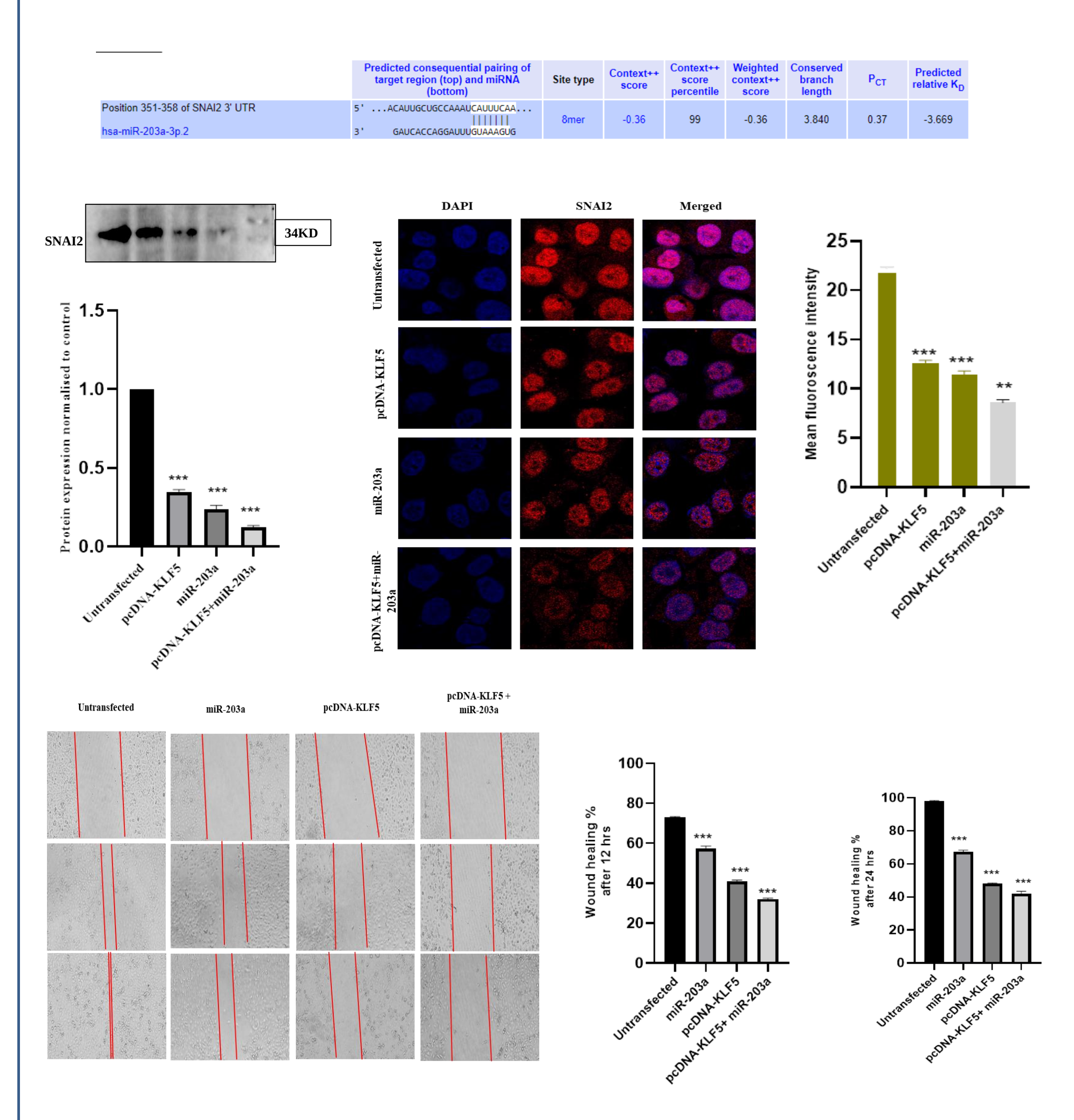
KLF5 positively regulates the expression of miR-203a



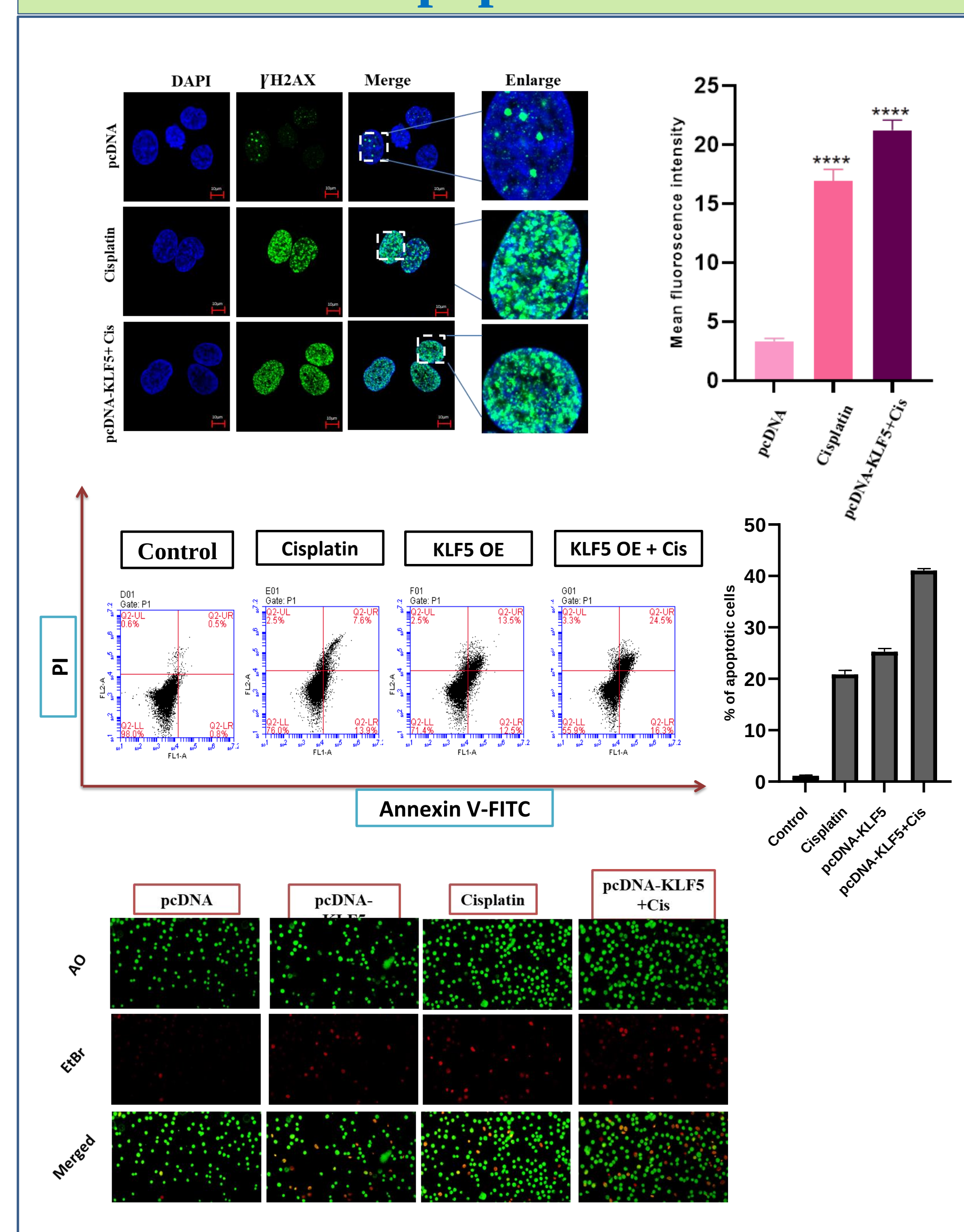
References

- Y. Luo, C. Chen, The roles and regulation of the KLF5 transcription factor in cancers, *Cancer science*, 112 (2021) 2097-2117.
- R.S. Tarapore, Y. Yang, J.P. Katz, Restoring KLF5 in esophageal squamous cell cancer cells activates the JNK pathway leading to apoptosis and reduced cell survival, *Neoplasia (New York, N.Y.)*, 15 (2013) 472-480.
- J. Liang, T. Sun, G. Wang, H. Zhang, Clinical significance and functions of miR-203a-3p/AVL9 axis in human non-small-cell lung cancer, *Personalized medicine*, 17 (2020) 271-282.

KLF5/miR-203a activation targets SNAI2 and inhibits cell migration



KLF5 induces DNA damage and apoptosis



Acknowledgement and Contact

- Authors acknowledges NIT, Rourkela for providing laboratory and all other facilities to carry out this research.
- University Grants Commission (UGC) Bahadur Shah Zafar Marg, New Delhi for funding.

Contact : Prof. Bibekanand Mallick,

RNAi, and Functional Genomics Lab., Department of Life Science, National Institute of Technology Rourkela, PIN-769008, Odisha, India. Emails: vivek.iitian@gmail.com, mallickb@nitrrkl.ac.in; Tel: +91-661-2462685; Fax: +91-661-2472926