Comperative analysis of RNA methylation-related biomarkers in HPV-positive and HPV-negative head and neck cancers

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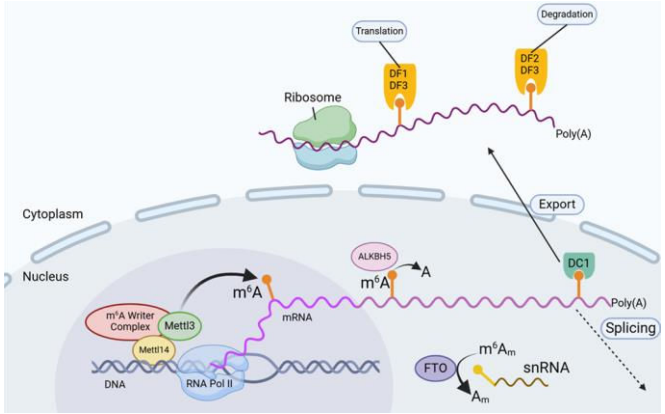
Research hypothesis:
m⁶A mRNA methylation affects molecular pathways in HNSCC

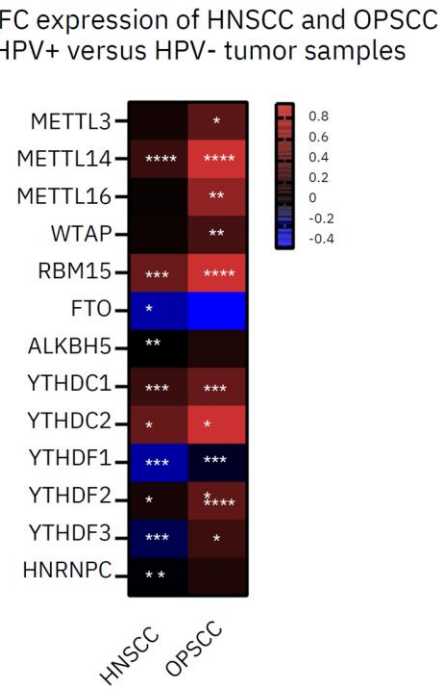


m6A RNA is associated in cancer proceses such as:

- Tumorigenesis
- Proliferation
- Invasion
- Metastasis

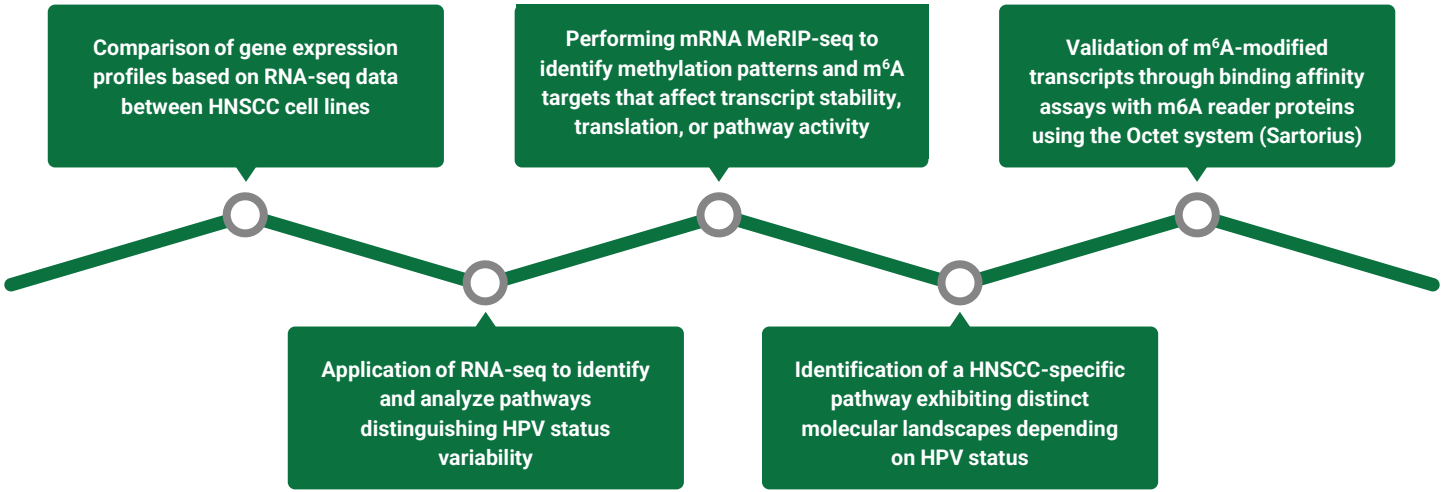
Mohr, et.al.
in preparation

- Differential expression analysis of m⁶A-related genes reveals significant variation between HPV-positive and HPV-negative tumor samples
- Genes such as METTL14, RBM15, and YTHDC1 exhibit strong upregulation in HPV-positive HNSCC, suggesting a potential role of m⁶A methylation in HPV-related oncogenic pathways



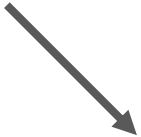
Log2FC expression values of HPV+ versus HPV- of m6A RNA methylation regulators in HNSCC and OPSCC tumor samples. Results are based on TCGA data using cBioportal and OncoDB databases.HNSCC HPV- n=415, HNSCC HPV+ n=72, OPSCC HPV- n=25, OPSCC HPV+ n=47. Differential expression data were calculated with Student t-test.

Project perspectives



Current research aims

Assessment of transcriptomic profile in HPV-positive and HPV-negative HNSCC cell lines



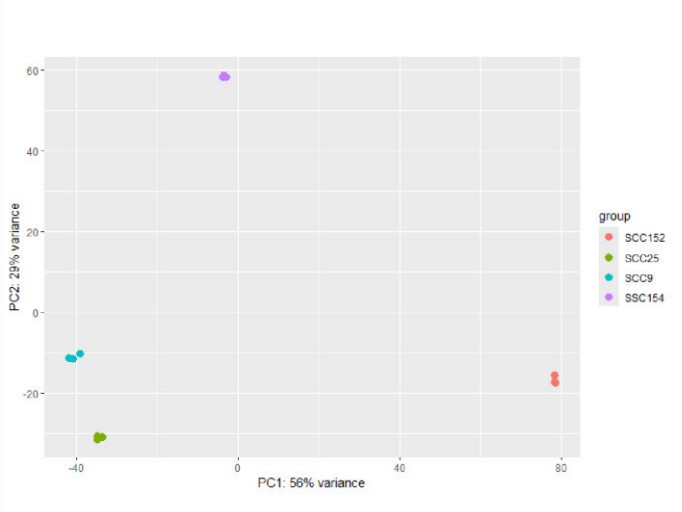
Comparison of gene expression profiles based on RNA-seq data, between HPV-positive and HPV-negative cell lines

Application of RNA-seq to identify and analyze pathways distinguishing HPV status variability

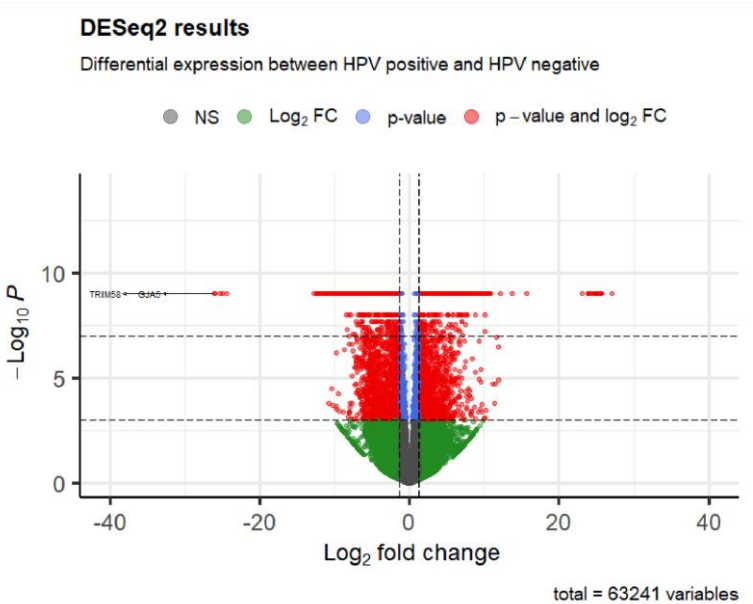
In vitro material characteristics

Cell line	HPV status	Localisation
SSC9	Negative	Tongue
SSC25	Negative	Tongue
FaDu	Negative	Hypopharynx
SSC152	Positive	Hypopharynx
SSC154	Positive	Tongue

RNA-seq data exhibits expression differences between HPV-positive and HPV-negative HNSCC cell lines



The PCA plot presents the results of RNA-seq analysis, distinguishing clusters of cell lines, indicating similar expression profiles for HPV-negative lines (SSC9, SSC25) and differing expression profiles for HPV-positive lines (SSC152, SSC154).



The volcano plot shows the differences in gene expression based on HPV status. Differentially expressed genes (DEGs) were selected based on a fold change greater than >1.5 . DEGs of statistically significant ($p<0.05$) differential expression are highlighted in red.

Identification of differentially expressed genes (DEGs)

- Determining genes with significantly altered expression levels between in cells with different HPV status
- Evaluation of RNA-seq results of cell lines material
- Typing 5 the most overexpressed and underexpressed clinically significant genes

Potentially selected HPV+ HNSCC **overexpressed** clinically significant genes

Gene name	HPV-	HPV+	fold change	p value HPV status	p value HPV-	p value HPV+
SPDYE6	0.10	0.85	8.52	9.64E-05	0.7	1
EZH2	12.47	48.94	3.92	0.0008	1	0.7
DBNDD2	13.18	45.48	3.44	5.74E-06	0.7	0.7
SCAI	0.83	2.84	3.39	0.011	0.7	1
CAAP1	7.13	18.21	2.55	0.0021	1	0.7

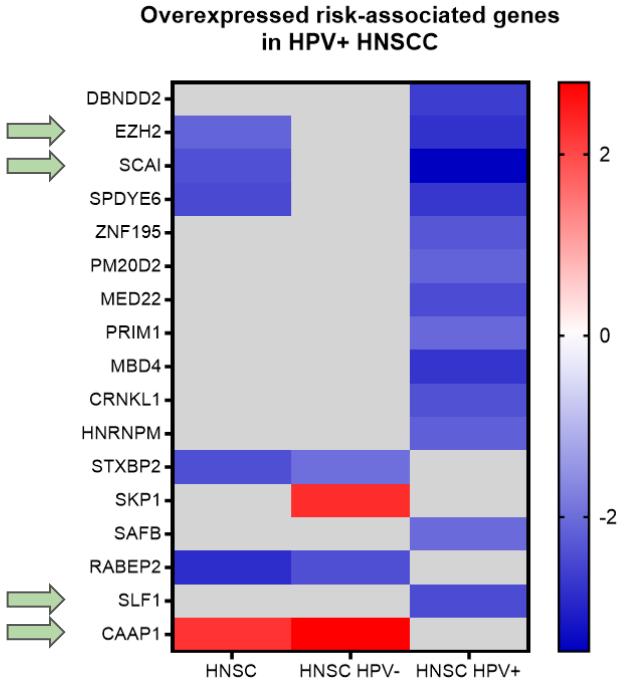
The table presents selected genes based on RNA-seq data normalized using TPM. The columns 'HPV-' and 'HPV+' show the average gene expression values in cell lines with similar HPV status. The 'fold change' column represents the HPV+/HPV- expression ratio (>1.5). The 'p value HPV status' indicates the p-value comparing samples with different HPV statuses. 'p value HPV-' and 'p value HPV+' describe the statistical significance of expression differences among cell lines within the same HPV status.

Potentially selected HPV+ HNSCC **underexpressed** clinically significant genes

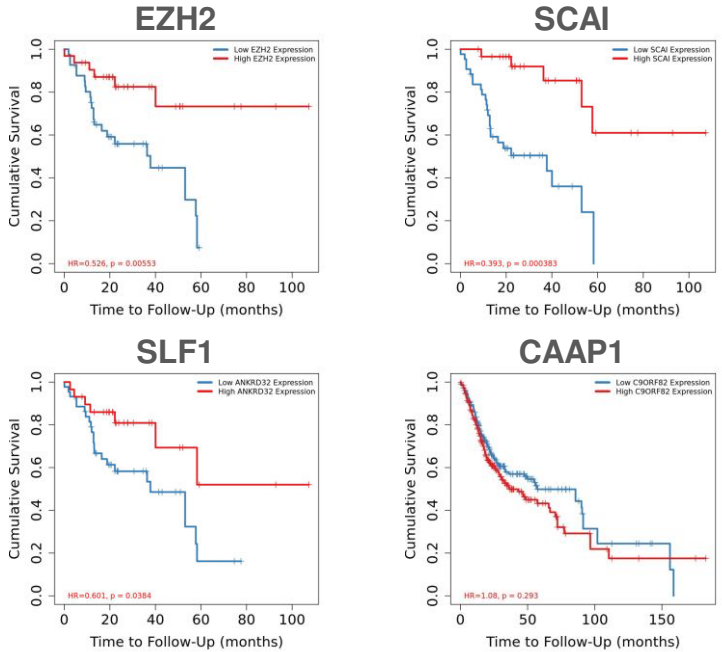
Gene name	HPV-	HPV+	fold change	p value HPV status	p value HPV-	p value HPV+
DLX1	4.65	0.24	19.13	1.27E-06	0.7	0.7
FADS3	102.62	18.69	5.48	4.27E-06	0.4	0.7
MEAK7	43.72	9.02	4.84	0.002	0.1	0.7
REXO2	152.12	41.06	3.70	8.27E-05	0.4	0.2
CDH3	198.79	56.72	3.50	8.05E-06	0.7	0.7

The table presents selected genes based on RNA-seq data normalized using TPM. The columns 'HPV-' and 'HPV+' show the average gene expression values in cell lines with similar HPV status. The 'fold change' column represents the HPV+/HPV- expression ratio (>1.5). The 'p value HPV status' indicates the p-value comparing samples with different HPV statuses. 'p value HPV-' and 'p value HPV+' describe the statistical significance of expression differences among cell lines within the same HPV status.

HPV-driven gene profiles predicting clinical outcomes in HNSCC

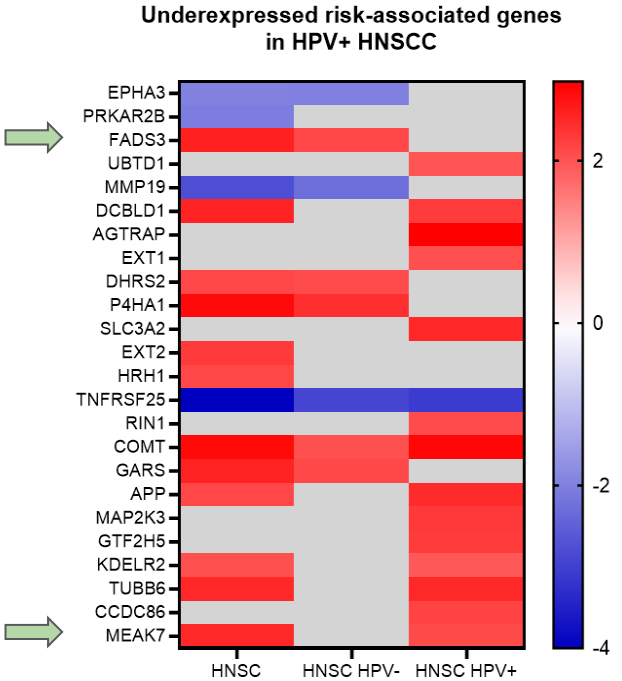


Heatmap graphs illustrating the association between upregulated gene expression and survival in HNSCC patients. Only statistically significant results ($p<0.05$) are shown. Data were sourced from TIMER 2.0 database, based on TCGA patient cohorts. Genes with clinical significance chosen for further analysis among HPV status–related DEGs in HNSCC cell lines, are indicated with green arrows.

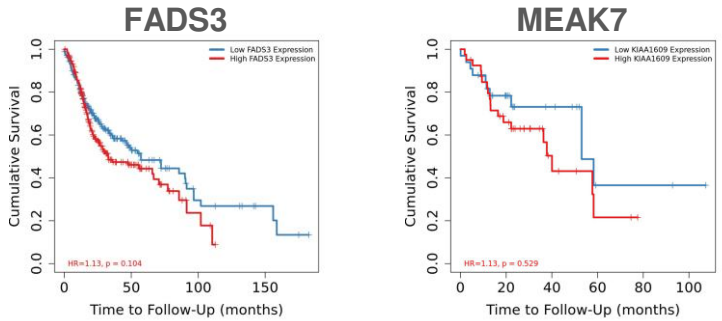


Kaplan-Meier survival curves of clinically significant genes showing overall survival for patients with **high (red)** and **low (blue)** expression levels of the listed genes.

HPV-driven gene profiles predicting clinical outcomes in HNSCC



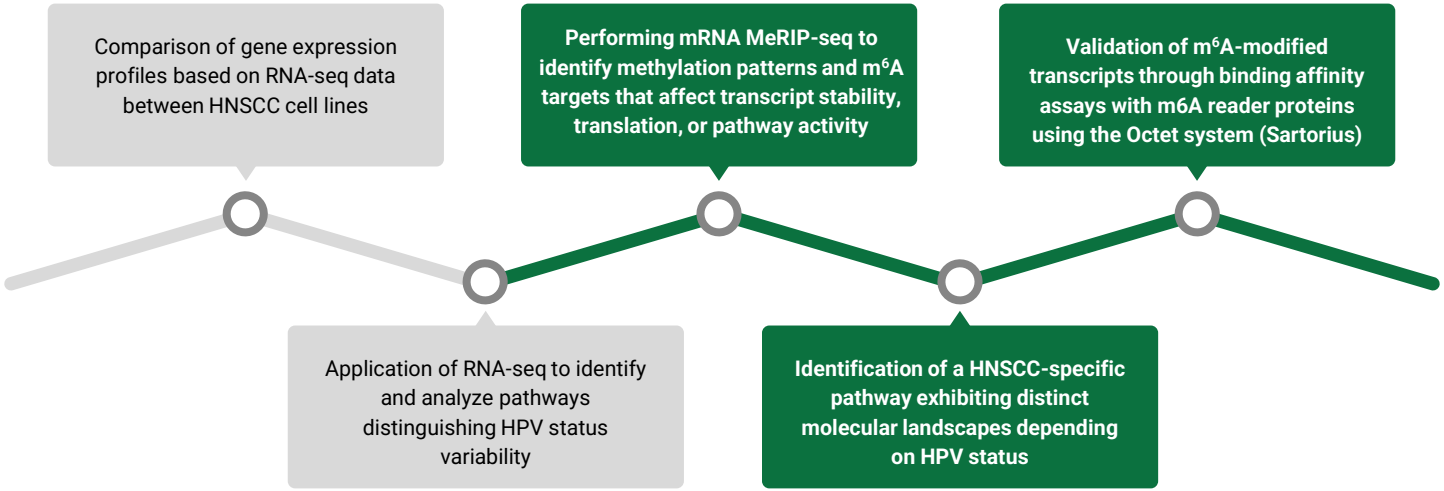
Heatmap graphs illustrating the association between downregulated gene expression and survival in HNSCC patients. Only statistically significant results ($p<0.05$) are shown. Data were sourced from TIMER 2.0 database, based on TCGA patient cohorts. Genes with clinical significance chosen for further analysis among HPV status–related DEGs in HNSCC cell lines, are indicated with green arrows.



Kaplan-Meier survival curves of clinically significant genes showing overall survival for patients with **high (red)** and **low (blue)** expression levels of the listed genes.

Conclusion: <u>There are significant changes related to the expression of selected genes and patient survival.</u>

Future perspectives



Publications Related to the Research Project

Manuscript in Final Preparation

Head and Neck Squamous Cell Carcinomas: Hypoxia, RNA Methylation, and HPV as Drivers of Oncogenesis and Therapy Resistance

Marcel Mohr, Julia Kozikowska, Zuzanna Petryszyn, Kamila Ostrowska, Wiktoria Suchorska, Katarzyna Kulcenty

Other activities associated with the PhD studies

- Participation in the 16th INTERNATIONAL CONFERENCE OF CONTEMPORARY ONCOLOGY
- Submitting a research proposal for the PRELUDIUM grant, NCN
- Enrolled in a training course on RNA-seq data analysis

Thank you

for your attention