Investigating PAD-mediated citrullination in HPV-driven head and neck squamous cell carcinoma

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BACKGROUND

Human papillomaviruses (HPVs) are small non-enveloped viruses.

Based on their oncogenic potential, mucosal HPVs are classified as low-risk and high-risk HPVs.

High-risk HPVs can induce the neoplastic transformation of infected cells, due to viral genome integration into the host chromosomes and overexpression of the two main viral oncoproteins, E6 and E7.

Different malignancies have been associated with HPV, including head and neck squamous cell carcinoma (HNSCC).

HNSCC comprises a group of cancers that affect different anatomic sites of the upper aerodigestive tract.

The classical major risk factors for HNSCC development are tobacco smoking, alcohol abuse, and HPV infection, especially HPV-16, the highest-risk strain accounting for more than 90% of HPV-associated HNSCC.

AIM

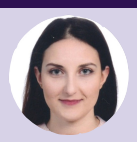
Our previous findings on cervical cancer revealed a novel mechanism by which high-risk HPVs manipulate host regulatory pathways involved in the cell cycle and survival to enhance viral fitness. This implies that PADs may be promising targets for developing new host-targeting antivirals to prevent cervical cancer progression.

In this study, we further explore the impact of PAD-mediated protein citrullination on HPV-induced transformation, in the context of head and neck squamous cell carcinoma.

CONCLUSION

In conclusion, in the context of HNSCC, we have demonstrated that PAD1, PAD2 and PAD4 are the only PADs isozymes expressed and modulated, although HPV infection does not appear to significantly influence this regulation, both in vivo and in vitro. Furthermore, preliminary results from immunohistochemistry analysis on PAD1 revealed that PAD1 expression is associated with tumour differentiation.

CONTACT



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RESULTS

PADI genes alterations in HNSCC are rare event

Figure 1. TCGA-curated clinical data set of head and neck squamous cell carcinoma (n=515) was assessed for samples harbouring different genomic alterations in PADI genes. *Analysis revealed that PADI genes are rarely altered in HNSCC.*

Only PAD1, PAD2, and PAD4 are expressed in HNSCC…

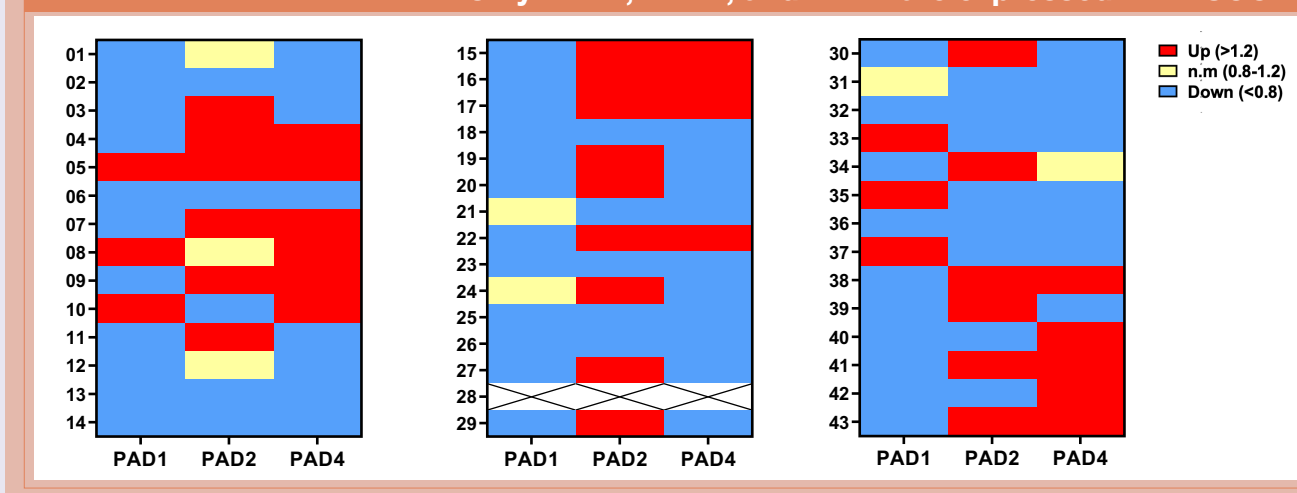

Figure 2. Heat-map of PADs mRNA expression in head and neck squamous cell carcinoma (HNSCC). Total RNA was extracted from cancerous and normal mucosal biopsies collected from 43 HNSCC patients. PADs expression was analysed via RT-qPCR, normalized to the housekeeping gene GAPDH and expressed as mean fold change over normal mucosa. PAD1, PAD2 and PAD4 expression levels from were considered upregulated for fold of induction > 1.2, not modulated for 0.8-1.2, and downregulated if < 0.8 *PAD1, PAD2, and PAD4 are the only PAD enzymes expressed and modulated in HNSCC samples.*

… but it is not influenced by HPV status

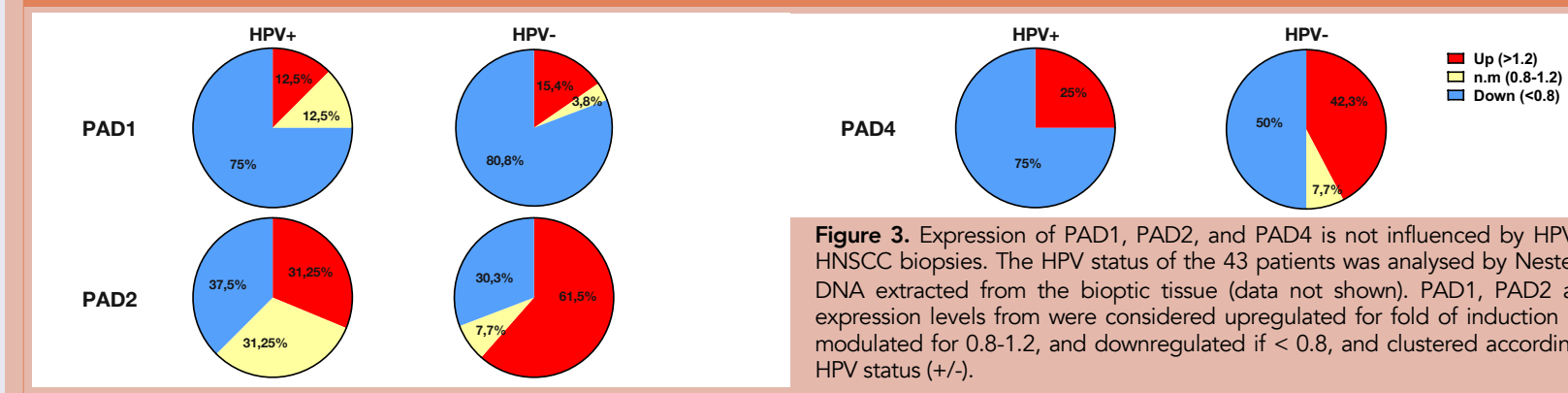

Figure 3. Expression of PAD1, PAD2, and PAD4 is not influenced by HPV status in HNSCC biopsies. The HPV status of the 43 patients was analysed by Nested-PCR on DNA extracted from the bioptic tissue (data not shown). PAD1, PAD2 and PAD4 expression levels from were considered upregulated for fold of induction > 1.2, not modulated for 0.8-1.2, and downregulated if < 0.8, and clustered accordingly to the HPV status (+/-).

PAD1 expression and association with HNSCC differentiation

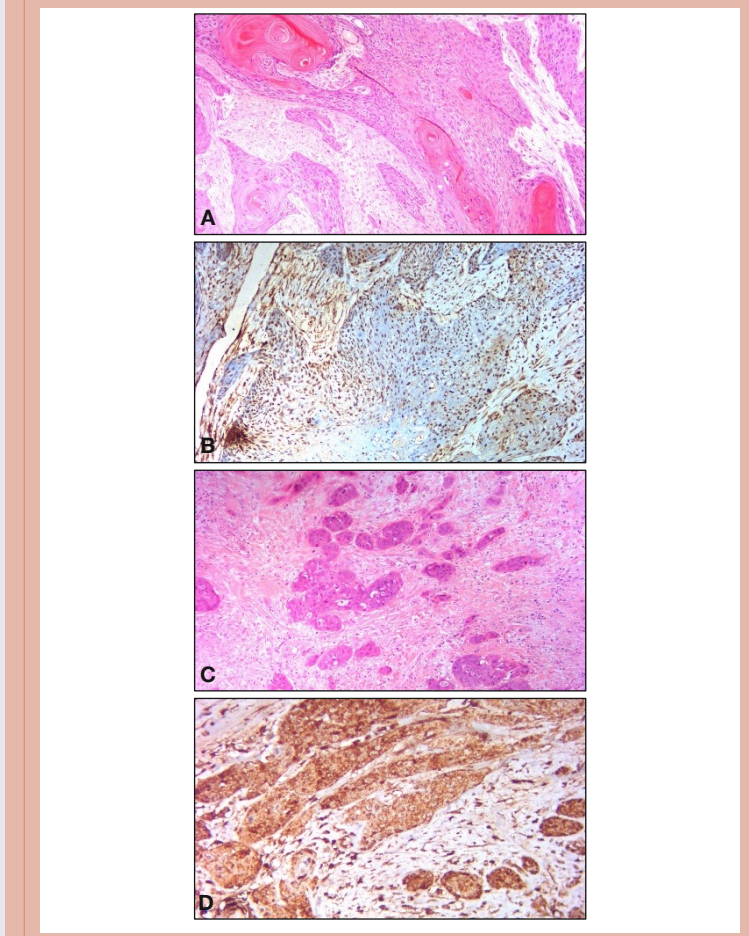

Figure 4. PAD1 immunohistochemistry. Formalin-fixed paraffin-embedded HNSCC tissues from the same cohort of patients were subjected to immunohistochemistry analysis for PAD1. A-B) Well-differentiated SCC with a weak expression of PAD1 (A: H&E; B: IHC). C-D) Poorly differentiated SCC with a strong and diffuse expression of PAD1 (C: H&E; D: IHC). Original magnification: 20X.

PADs expression and citrullination is not significantly modulated by HPV oncoproteins

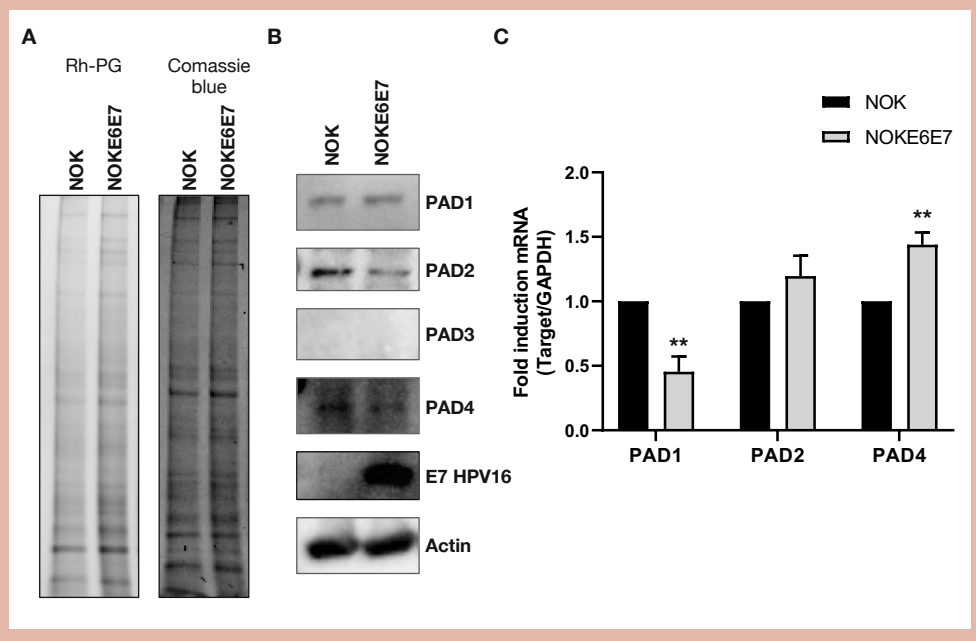

Figure 5. Citrullination profile and PADs expression analysis on immortalised keratinocytes. A) Protein lysates from immortalised keratinocytes (NOKs) and NOKs expressing HPV-16 viral oncoproteins E6 and E7 (NOKE6E7) were exposed to a rhodamine phenylglyoxal (Rh-PG) citrulline-specific probe and subjected to gel electrophoresis to detect total proteins (left panel). Equal loading was assessed by Coomassie blue staining (right panel). *A similar pattern of citrullinated proteins was noticed in keratinocytes expressing E6 and E7 compared to NOK control cells.* B) The same protein lysates were used to perform a Western blot analysis using antibodies against PAD1, PAD2, PAD3, PAD4, E7 of HPV-16, and actin. *Western blot analysis revealed that PAD1, PAD2, and PAD4 are the only PAD enzymes expressed both in NOK and NOKE6E7, but not significantly modulated at protein level.* C) mRNA expression levels of PADI isoforms by RT-qPCR of NOK and NOKE6E7 were normalized to the housekeeping gene GAPDH and expressed as mean fold change over NOK. Values are expressed as mean fold change ± SEM. Differences were considered statistically significant for $P<0.05$ (*, $P<0.05$; **, $P<0.01$, unpaired t-test). *At the mRNA level PAD1 and PAD4 expressions are significantly slightly altered by the presence of E6 and E7, however this modulation does not seem to affect total citrullination or PAD1 and PAD4 proteins in NOKE6E7 compared to NOK.*