Targeting PAD-mediated citrullination to treat human papillomavirus infections

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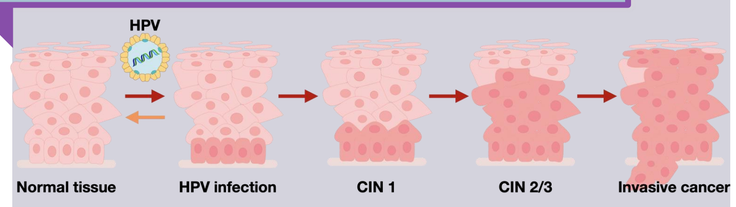
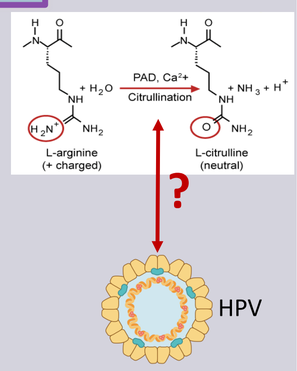
BACKGROUND



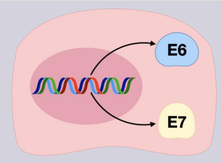
- HPVs are able to evade the innate immune response and persist in the host.
- Escape from innate immune surveillance appears to be the hallmark of HPV infections.

AIM

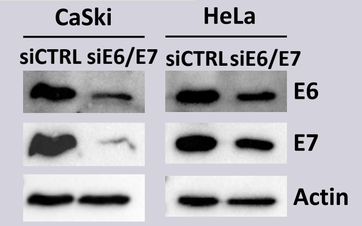
- Citrullination is an arginine PTM, catalyzed by calcium-dependent enzymes, named peptidyl arginine deaiminases (PADs).
- There are five PAD isozymes (PADs 1-4 and 6) that are uniquely distributed in various tissues.
- Citrullination dysregulation has been correlated with inflammation-dependent processes, including autoimmune diseases, cancer and neurodegenerative disorders.
- **The main goal of this study is to characterize the citrullination process in high-risk (hr) HPV positive cells.**



EXPERIMENTAL MODEL



HPV E6 and E7 silencing



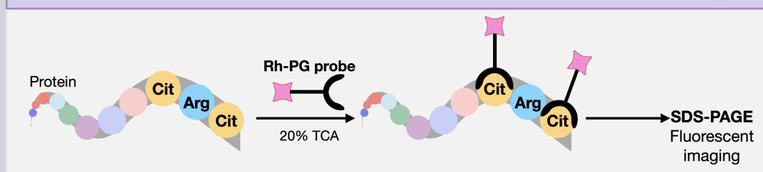
HPV-transformed cell lines **CaSki (HPV16)** & **HeLa (HPV18)**

Cellular models of HPV persistent infection

Total extracts from HeLa or CaSki cells transfected with siRNA E6/E7, or siRNA CTRL for 72 h were subjected to immunoblot analysis with anti-E6, anti-E7, or anti-actin.

RESULTS

HPV citrullination profile



The rhodamine-phenylglyoxal (Rh-PG) probe chemoselectively labels citrulline over arginine at acidic pH. Subsequent separation by SDS-PAGE and fluorescent analysis reveals citrullinated proteins.

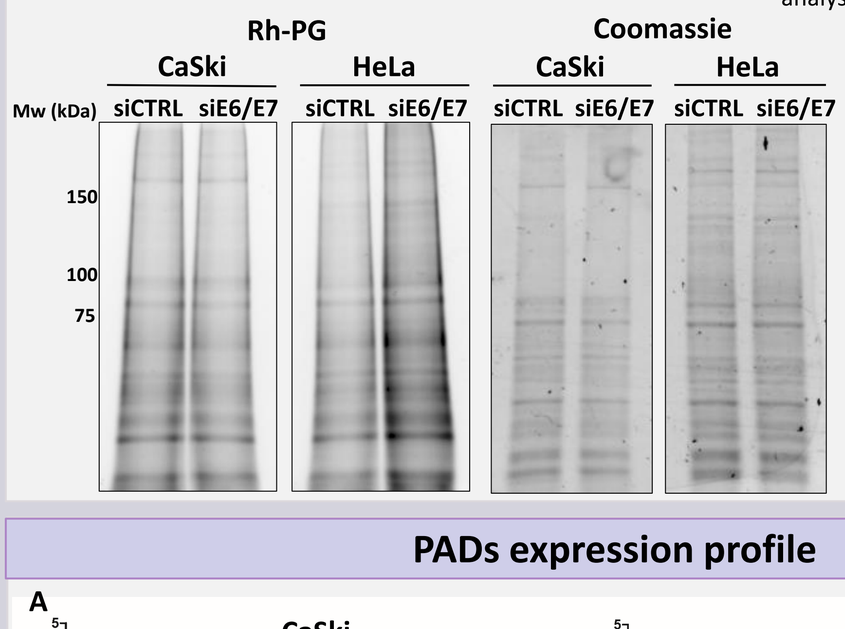


Fig. 1 Protein lysates from CaSki and HeLa transfected with siRNA E6/E7 or siRNA CTRL were exposed to a Rh-PG citrulline-specific probe and subjected to gel electrophoresis to detect total proteins. Equal loading was assessed by Coomassie blue staining.
A similar pattern of citrullinated proteins was noticed in cells silenced for E6 and E7 compared to CTRL.

Antiviral activity of PADs inhibitors against HPV infection

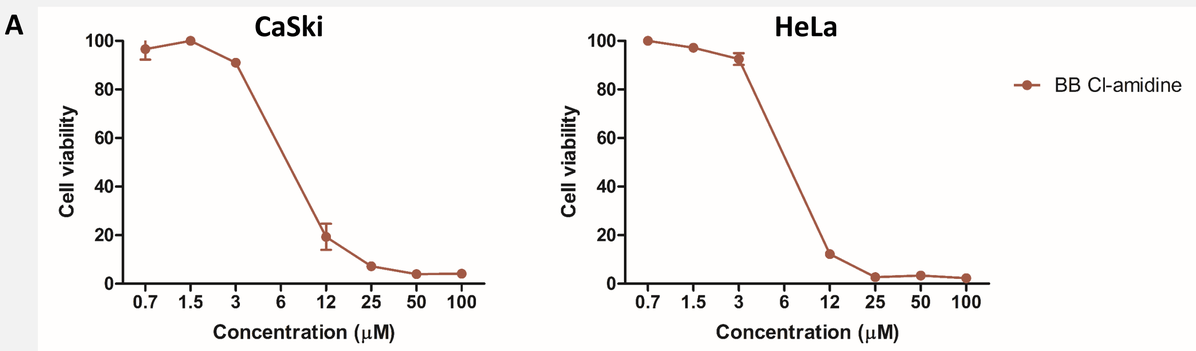
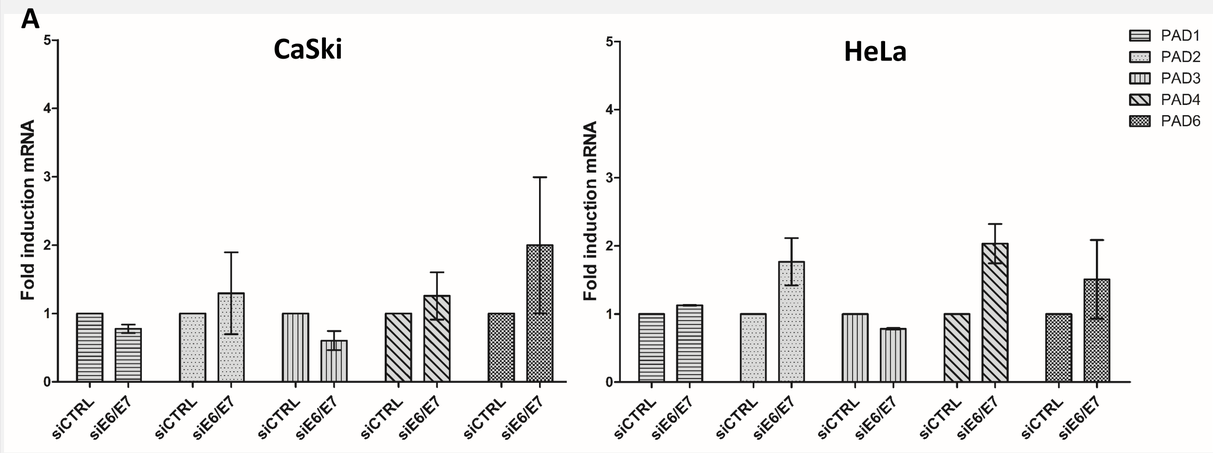


Fig. 3 (A) CaSki and HeLa were treated with increasing concentrations of the cell-permeable pan-PAD inhibitor BB Cl-Amidine. At 48 hpt, the number of viable cells was determined for each BB-Cl-A concentration using the MTT method. Values are expressed as means ± SEM (error bars) of three independent experiments (***P < 0.001, one-way ANOVA followed by Bonferroni's post test for comparison of Cl-A treated *vs.* untreated cells). **(B)** Protein lysates from CaSki and HeLa treated with non-toxic concentration of BB-Cl-A (3 μM) were subjected to immunoblotting using the anti-E6, E7, p53, p21 antibodies or anti-actin to show equal loading.
BB-Cl-Amidine treatment decreases the expression of E6 and E7, and restores p53 and p21 expression levels.

PADs expression profile



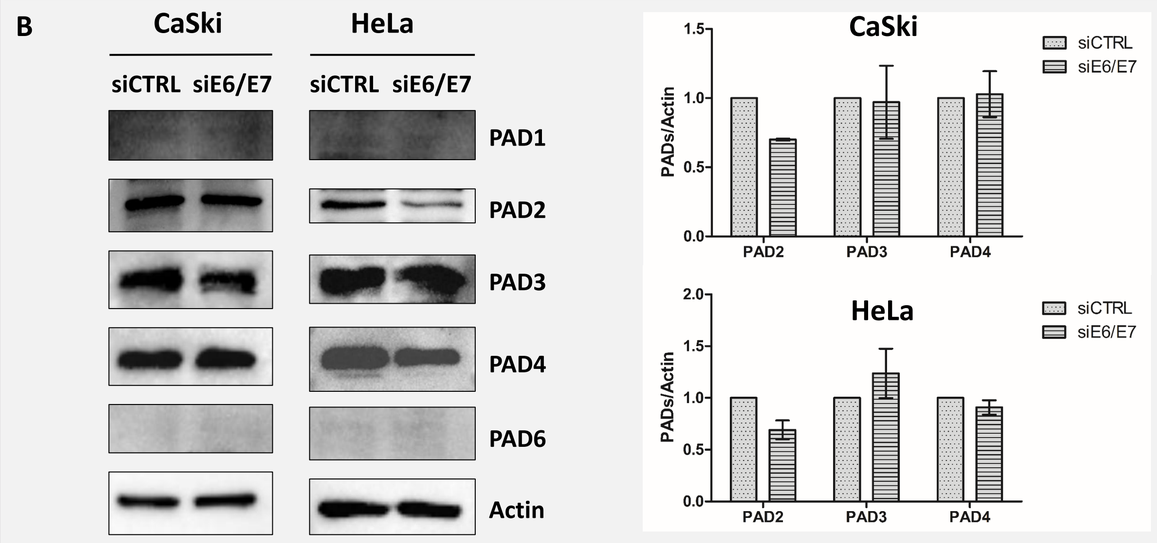


Fig. 2 (A) mRNA expression levels of PADI isoforms by RT-qPCR of CaSki and HeLa treated with siRNA E6/E7 or siRNA CTRL were normalized to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase GAPDH and expressed as mean fold change ± SEM over siRNA CTRL. **(B)** Western blot analysis of protein lysates from CaSki and HeLa treated with siRNA E6/E7 or siRNA CTRL using antibodies against PAD1, PAD2, PAD3, PAD4, PAD6 or actin. One representative blot and predictive densitometric analysis is shown of three independent experiments. Values are expressed as fold change in PAD expression normalized to actin.

PAD4 expression in cervical intraepithelial neoplasia (CIN) and invasive squamous cell carcinoma (SCC)

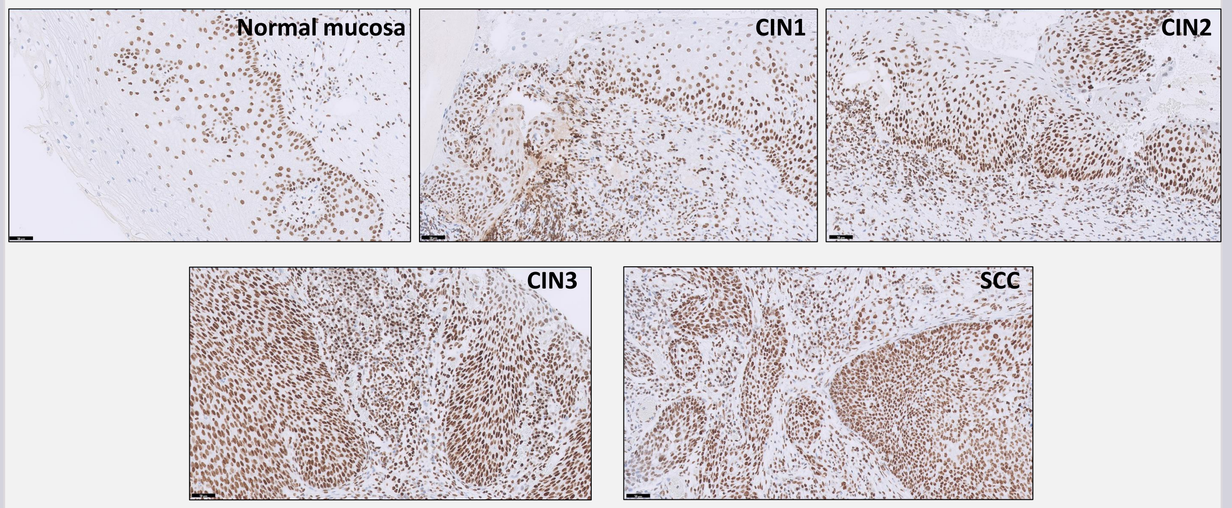
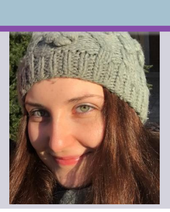


Fig. 4 PAD4 immunohistochemical photomicrographs of representative cervical tissues. In normal mucosa, PAD4 expression is limited to the basal and parabasal layer in the squamous epithelium and to the sparse stromal and inflammatory cells within the stromal compartment. With progression of cervical carcinogenesis, there is a significant increase in expression of PAD4 in both atypical squamous epithelium and in stromal compartment. Original magnification: 20X.

CONCLUSIONS

- The expression of E6 and E7 HPV oncoproteins is strongly impaired in the presence of the pan-PAD inhibitor BB-Cl-amidine;
- p53 and p21, the main targets of HPV oncoproteins, are upregulated by PAD inhibitors;
- PAD4 expression is significantly associated with cervical cancer progression.

CONTACT



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