

Supplementary data

Materials and methods

Protocol details for reverse transcription (RT)-PCR. Total RNA was extracted from HPV-infected cutaneous epidermal epithelial cells using the RNA isolater Total RNA Extraction Reagent (cat. no. R401; Vazyme Biotech Co., Ltd.) as the RNA extraction buffer, and the extracted total RNA was used as the template. The Vazyme HiScript II 1st Strand cDNA Synthesis Kit (+gDNA wiper) reagent kit (cat. no. R212-01; Vazyme Biotech Co., Ltd.) was used, and the reverse transcription operation was carried out according to the instructions. We entrusted the Fudan Fuda Testing Group to conduct the HPV genotyping test.

Protocol details for PCR. i) A 0.1 ml PCR reaction plate was used, and the reaction mixture was prepared according to the PCR reaction system. For each reverse transcription product, three replicate tubes were prepared. After loading the samples, the plate was sealed with qPCR-compatible optical sealing film. The plate was then briefly centrifuged (1,000-2,000 x g for 10-20 sec) using a microplate centrifuge to collect liquid at the bottom and remove bubbles at room temperature (Table SII).

ii) PCR amplification was performed on a quantitative fluorescent PCR instrument, following the appropriate cycling

parameters that were preset based on the optimized recommendations from the reagent manufacturer, specific amplification targets, and instrument compatibility (Table SIII).

iii) Data analysis. The $\Delta\Delta CT$ method was applied as follows (1): $A=CT$ (target gene, test sample)- CT (reference gene, test sample). $B=CT$ (target gene, control sample)- CT (reference gene, control sample). $K=A-B$. Fold change in expression= 2^{-K} .

Results

PCR genotyping results. The results showed that the CT values for HPV2, HPV4 and HPV57 were all >40 or undetectable, indicating that these HPV types were not detected in the samples. By contrast, the CT values for HPV1 and HPV27 were <40 , suggesting that the samples tested positive for HPV1 and HPV27.

Reference

1. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25: 402-408, 2001.

Figure S1. Clinical follow-up photograph taken 4 years after cryotherapy. No recurrence of the doughnut wart was observed and the lesion remained completely resolved, indicating sustained long-term therapeutic efficacy of the initial cryotherapy combined with photodynamic therapy.

