Human papillomavirus oncoproteins induce a reorganization of epithelial-associated γδ T cells promoting tumor formation

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Significant statement

Of all tumor-infiltrating leukocytes, T cells bearing γδ T cell receptors have been associated with the most favorable prognosis. However, we show here, in a mouse model of carcinogenesis induced by human papillomavirus (HPV)-oncoproteins, that γδ T cells promoted the development of HPV-induced lesions. Indeed, HPV-oncoprotein expression induced an infiltration of γδ T cells producing IL-17A, a proangiogenic cytokine, and a decrease density of anti-tumor Vγ5+ γδ T subsets. Supporting the clinical relevance of our observations, IL-17A+ γδ T cells were detected in human cervical cancer, where HPV-oncoproteins are highly expressed, but not in less advanced cervical lesions. These results support the notion that viral oncoproteins can induce a switch from antitumoral to pro-tumoral γδ T subsets in solid tumors.

1. γδ T cells accelerate HPV-induced lesions development

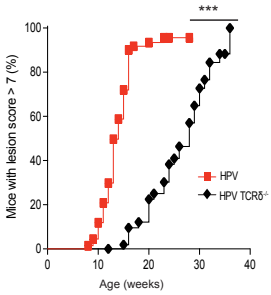

Figure 1. K14-HPV16 (n = 198, red line) and K14-HPV16 TCR δ $^{-/-}$ (n = 270, black line) mice were observed for several weeks and a score was assigned to them depending on the severity of lesions present on their skin, head, ears and tail. A score above 7 was considered as severe. *** = p < 0.0001.

2. Skint1 expression and density of resident Vγ5+ γδ T cells are reduced in mice expressing HPV oncoproteins in epidermis

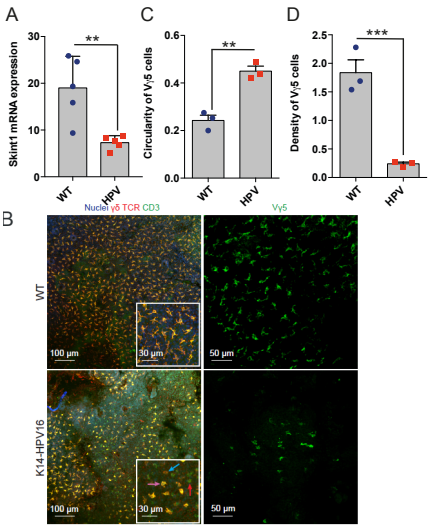

Figure 2. (A) Skint1 mRNA expression was measured by qPCR in WT and HPV mouse epidermis. (B) Confocal microscopy pictures of WT and HPV mouse epidermal sheets using anti-Vγ5 antibody (green) or panγδ (red) and anti-CD3 (green) antibodies and DAPI for nuclei staining (blue). Arrows indicate different γδTCR expression intensities: yellow, high intensity; orange low intensity; green no γδTCR expression. (C-D) Using imageJ software, confocal microscopy images of Vγ5 staining in mouse epidermis were used to quantify cell circularity (C) and density (D) (Mann-Whitney test, mean + SEM, ** = p < 0.01, *** = p < 0.005).

3. HPV-oncoprotein expression in epidermis leads to infiltration of non-Vγ5 γδ T cells expressing CCR2

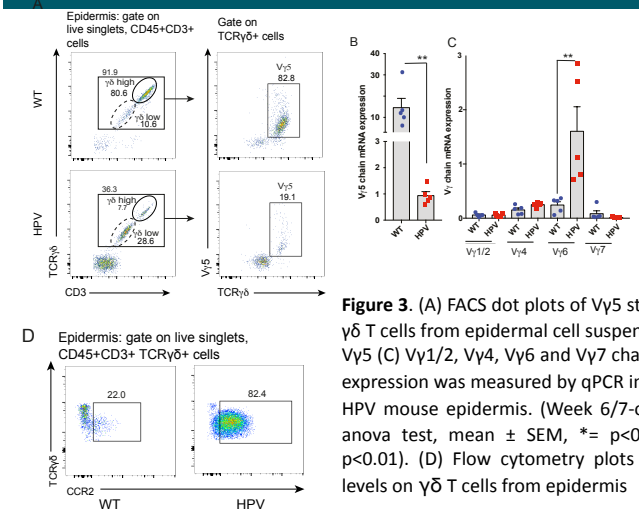

Figure 3. (A) FACS dot plots of Vγ5 staining of γδ T cells from epidermal cell suspensions (B) Vγ5 (C) Vγ1/2, Vγ4, Vγ6 and Vγ7 chain mRNA expression was measured by qPCR in WT and HPV mouse epidermis. (Week 6/7-old mice, anova test, mean ± SEM, *= p<0.05, **= p<0.01). (D) Flow cytometry plots of CCR2 levels on γδ T cells from epidermis

4. γδ^{low} T cells produce IL-17A in epidermis of HPV oncoprotein-induced lesions

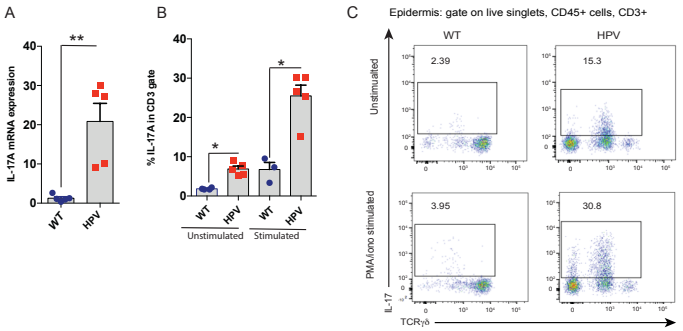

Figure 4. (A) IL-17A mRNA expression was measured by qPCR in WT and HPV mouse epidermis. (B) Percentages of IL-17A+ cells determined by FACS intracytoplasmic staining on cells isolated from epidermis and stimulated or not with PMA and ionomycin). (C) Representative dot plots of TCRγδ^{low}+ IL-17+ cells in CD3+ gate isolated from epidermis. (Week 6/7-old mice, Mann Withney tests, mean + SEM, *= p<0.05, **= p<0.01).

5. γδ T cells promote HPV oncoprotein-induced angiogenesis in the skin

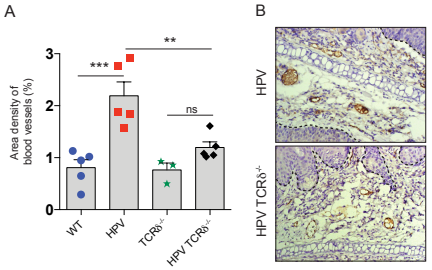

Figure 5. (A) Blood vessel density, assessed by ImageJ software, in ear sections. Each dot represents the value of one complete ear section. (B) Representative images of blood vessels detected with antibodies against VWF in HPV and HPV TCRδ$^{-/-}$ mice. anova test, mean + SEM, *= p<0.05, **= p<0.01, NS = not significant).

6. Human cervical squamous cell carcinoma (SCC) contains IL-17A producing γδ T cells

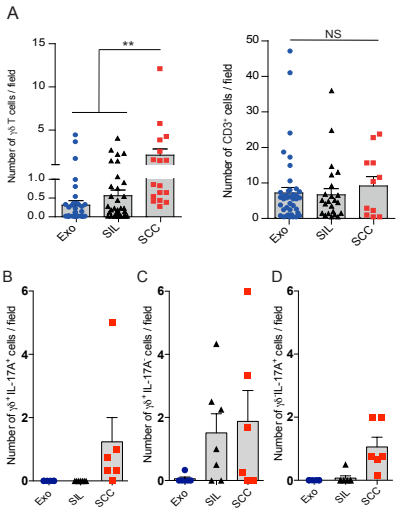

Figure 6. (A) Quantification of human γδ and total (CD3+) T cells in epidermis of paraffin sections from non-lesional (Exo), preneoplasic lesions (SIL) and tumoral (SCC) biopsies.(B-D) Quantification of human γδ+ IL-17A+ (C), γδ+ IL-17- (D) and γδ- IL-17A+ (E) T cells in epidermis of paraffin sections from non-lesional (Exo), preneoplasic lesions (SIL) and tumoral (SCC) biopsies. (Kruskal-Wallis test, followed by Dunn's tests for multiple comparisons. mean + SEM, **= p<0.01).

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Reference: Van hede et al, PNAS vol 114, E9056.