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Equine papillomavirus-associated genital tumors: a possible animal model for human cancers

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Equine genital squamous cell carcinomas (egSCCs) are common tumors in older horses, with poor prognosis mostly due to local invasion and recurrence[1]. These tumors are recognized to be mainly caused by Equus caballus papillomavirus type 2 (EcPV-2) in both females and males[2].
The **aim of this study** is to better characterize the tumor immune microenvironment (TIME) in equine genital SCCs.
Twenty-three equine genital epithelial tumors were retrospectively selected; immune infiltrate was assessed by histology and immunohistochemistry; RT-qPCR tested the expression of EcPV-2 DNA and RNA, selected chemokines, and RANKL.

1. Viral detection, gene expression and immunohistochemical characterization of immune infiltrate in genital epithelial tumors

Case ID	Histological Diagnosis	Sex	DNA			cDNA		
			B2M	L1	E6	L1	E6	E2
1	SCC	M	+	+++	++	33.5±0.2	30.4 ± 0.2	>48
2	SCC	M	+	+	+	ND	>48	>48
3	SCC	M	+	+++	+++	34.3±0.3	32.8 ± 0.3	>48
4	CIS	M	+	++++	++++	35.1±0.6	36.2 ± 0.6	44.2 ± 2.4
5	SCC	F	+	++++	++++	ND	31.5 ± 0.7	34.1 ± 0.4
6	P	M	+	+	+	ND	>48	>48
7	SCC	M	+	+	+	35.9±0.7	>48	>48
8	SCC	M	+	++++	+++	32.3±0.3	33.5 ± 0.4	>48
9	SCC	M	+	++++	++++	34.9±0.9	32.7 ± 0.9	36.1 ± 0.6
10	SCC	M	+	++++	+++	ND	33.5 ± 0.3	39.3 ± 1.1
11	SCC	M	+	+	+	33.0±0.3	>48	>48
12	SCC	M	+	++++	++++	ND	33.7 ± 0.4	>48
13	SCC	M	+	+	+	36.6±0.1	>48	>48
14	SCC	M	+	++	+	31.3±2.7	>48	>48
15	SCC	M	+	++++	++++	35.6±0.1	33.9 ± 1.9	38.5 ± 0.6
16	SCC	F	+	++++	++++	ND	31.9 ± 0.4	>48
17	SCC	M	+	++++	+++	ND	35.3 ± 1.5	39.2 ± 0.9
18	SCC	M	+	++	++	31.3±2.7	>48	>48
19	SCC	F	+	++++	++++	35.3±0.1	32.1 ± 1.1	35.6 ± 0.7
20	SCC	M	+	-	-	ND	ND	ND
21	CIS	M	+	+++	++++	33.5±0.2	30.4 ± 0.6	>48
22	SCC	M	+	+++	++++	ND	32.3 ± 0.1	41.9 ± 2.6
23	SCC	M	+	-	-	34.3±0.3	ND	ND

Table: Histological diagnosis: SCC: squamous cell carcinoma; CIS: carcinoma in situ; P: papilloma. RT-PCR data for beta-2-microglobulin (B2M) are expressed as + (amplified) or – (not amplified); Viral amount, L1 and E6 Cq for positivity: - (>38 Cq), + (34–38 Cq), ++ (29–33 Cq), +++ (23–28 Cq), and ++++ (18–22Cq). RT-qPCR data for viral gene expression are indicated as mean Cq ± 1-standard deviation of three replicates. ND indicates no amplification.

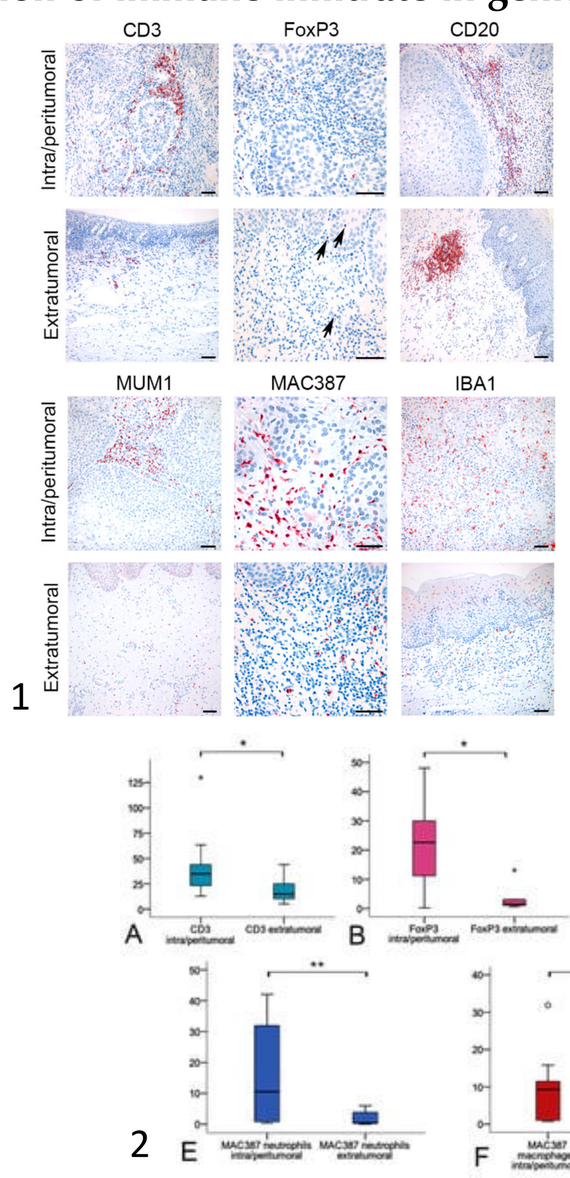

Figure 1: Immunohistochemical expression of different immunohistochemical markers for immune cell population in intra/peritumoral areas of epSCCs and in the extratumoral tissues. Scale bars: 200 microns.

Figure 2: Box plots of the number of CD3 (**A**), FoxP3 (**B**), CD20 (**C**), MUM1 (**D**), MAC387 neutrophils (**E**), MAC387 macrophages (**F**), and IBA1 (**G**) positive cells in the intra/peritumoral areas and in the extratumoral areas. * $p < 0.05$, ** $p < 0.01$ (Mann–Whitney tests).

Results showed an **increased infiltration** of CD3+lymphocytes, macrophages (MAC387; IBA1), plasma cells (MUM1), and FoxP3+lymphocytes in the intra/peritumoral stroma when compared to extratumoral tissues ($p<0.05$), indicating a vivacious TIME in egSCCs. IBA1 and CD20 were intratumorally increased in cases where IL-10 was expressed ($p < 0.005$), possibly indicating the presence of **immunosuppressive mechanisms**.

2. Selected chemochines and RANKL gene expression

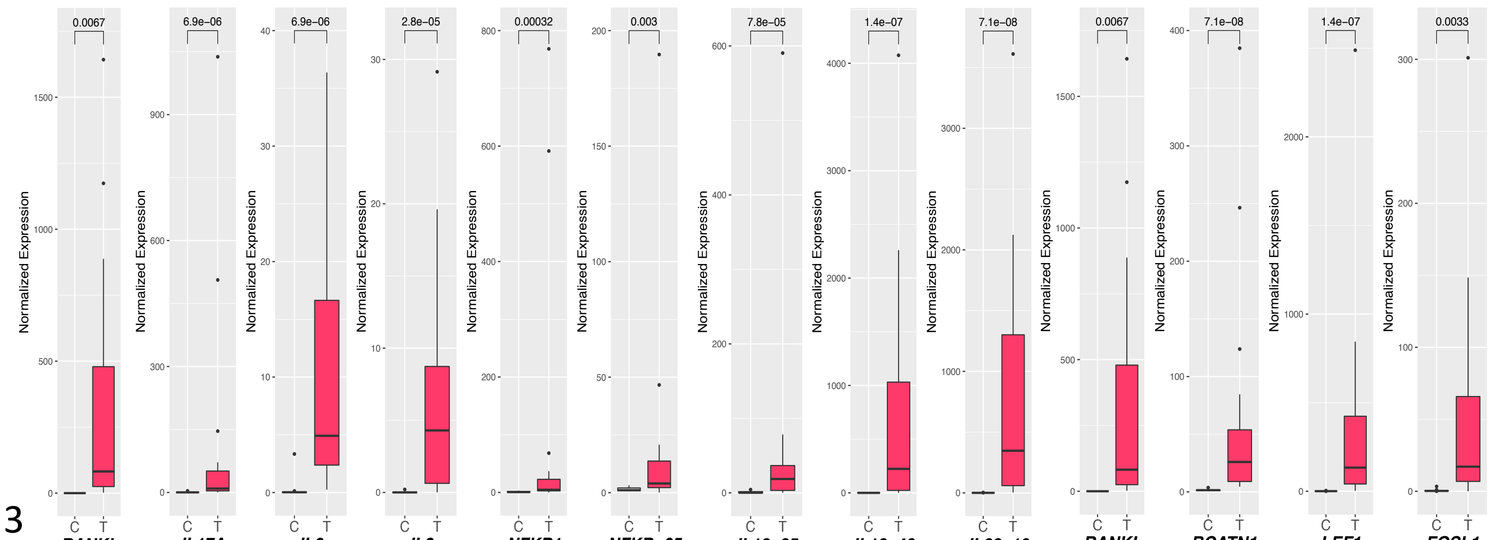

Figure 3. Relative normalized gene expression, The statistically significant ($P < 0.05$) values for each gene are reported on the horizontal bars in the upper part of the. C: control group; T: tumor group.

The SCCs in this study showed the **overexpression** of *RANKL*, indicating that the related signaling pathway could be considered crucial for both prognostic and therapeutic reasons, since SCC cells in both in humans and animals express RANK mRNA/protein at various levels. Moreover, the upregulation of *BCATN1*, *FOSL1*, and *LEF1* suggest the activation of Wnt signaling pathway that could be critical for carcinogenesis and tumor progression, often a feature of oncogenic transformation in human cancer.

Our results describe a complex inflammatory TIME characterized by the **activation of RANKL/RANK and IL17** pathways leading to the upregulation of proinflammatory cytokines, such as *IL6* and *IL8*. Many of these molecules are involved in **Th17 differentiation** and **Treg/Th17 imbalance**.

Conclusions

Our results describe an inflammatory environment similar to human counterpart[3,4] and characterized by a marked infiltration of immune cells, particularly T and B lymphocytes, the activation of RANKL/RANK and IL17 with the relative downstream pathways, and a positive modulation of inflammatory cytokines genes such as *IL6* and *IL8*. Equine genital squamous cell carcinomas may represent a **good spontaneous model** for the human counterpart. Further prospective studies are needed to confirm these preliminary results.

References:
[1] Sykora S et al.Papillomavirus infection and squamous cell carcinoma in horses. Vet. J. 2017, 223, 48–54. [2] Arthurs, C et al. Equine Penile Squamous Cell Carcinoma: Expression of Biomarker Proteins and EcPV2. Sci. Rep, 10,2020. [3] Ottenhof S.et al.The prognostic value of immune factors in the tumor microenvironment of penile squamous cell carcinoma. Front. Immunol. 2018, 9, 1253. [4] Suárez-Bonnet, A et al. Molecular Carcinogenesis in Equine Penile Cancer: A Potential Animal Model for Human Penile Cancer. Urol. Oncol. Semin. Orig. Investig. 2018, 36, 532.e9–532.e18.