Immuno-Oncological Characterisation of Canine Cutaneous Histiocytoma

by Gene Expression Profiling Utilising NanoString nCounter® RNA Hybridisation Technique

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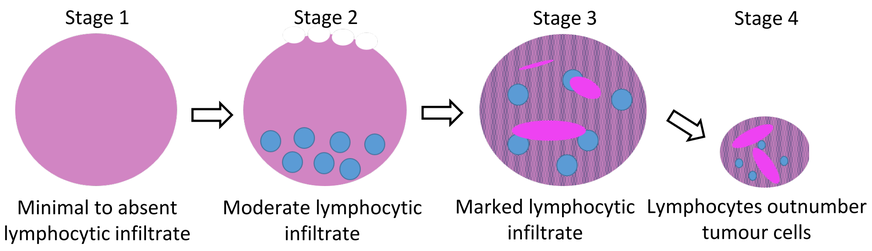
Introduction

The stereotypic regression of canine cutaneous histiocytoma (CCH) is a unique phenomenon of larger comparative oncological interest. However, its mechanisms have only partially been investigated. In this study, we asked which specific immuno-oncological dynamics on the transcriptome level may underlie regression of CCH. In addition to general pathways such as apoptosis, proliferation and hypoxia, spatiotemporal dynamics of expression of co-stimulatory molecules CD80 and CD86 were examined.

Staging of the canine cutaneous histiocytoma:

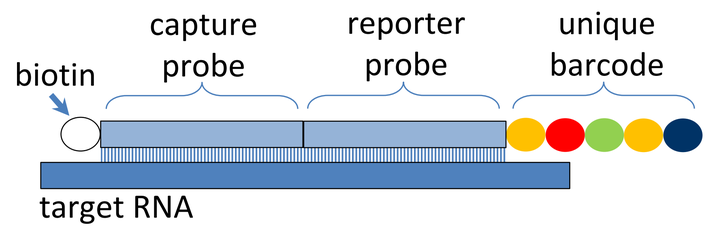


Cockerell & Slauson, 1979

Materials & Methods

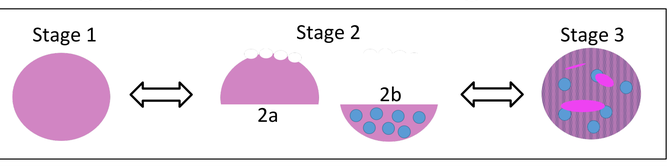
HE-stained FFPE sections from 35 CCH were staged and RNA isolated for analysis with the nCounter® Canine IO Panel, an RNA hybridisation assay which allows 800 genes to be analysed. 45 immuno-oncologically relevant pathways were compared between stages 1, 2 and 3. Expression of CD80 and CD86 was measured by in situ hybridisation (ISH) followed by quantitation of positive tumour cells and finally compared to 3 samples from canine histiocytic sarcomas (HS).

nCounter® RNA hybridisation technique



Results

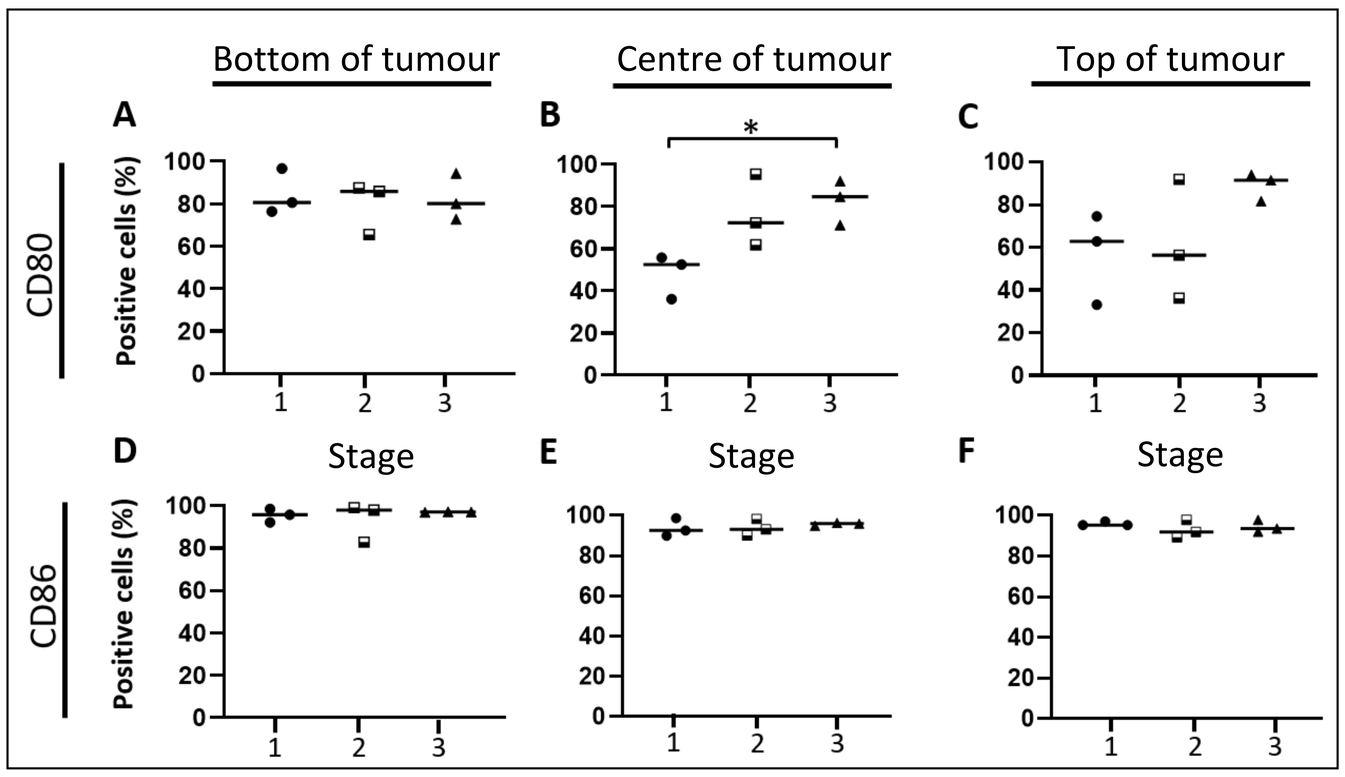
Investigated nCounter® gene sets



2a versus 1		Gene Set	3 versus 2b	
DEG*	No DEG		DEG	No DEG
	X	Adhesion		X
	X	Angiogenesis		X
	X	Antigen Presentation		X
	X	Antigen Processing		X
	X	Apoptosis		X
	X	Autophagy		X
	X	B Cell Functions		X
	X	Cell Cycle		X
	X	Cell Functions		X
	X	Cell Proliferation		X
	X	Chemokines		X
	X	Complement		X
	X	Complement System		X
	X	Costimulatory Signaling		X
	X	Cytokine and Chemokine Signaling		X
	X	Cytokines		X
	X	Cytotoxicity		X
	X	DNA Damage Repair		X
	X	Epigenetic Regulation		X
	X	Hedgehog Signaling		X
	X	Hypoxia		X
	X	Immune Cell Adhesion and Migration		X
	X	Interferon Signaling		X
	X	Interleukins		X
	X	JAK-STAT Signaling		X
	X	Leukocyte Functions		X
	X	Lymphoid Compartment		X
	X	Macrophage Functions		X
	X	MAPK		X
	X	Matrix Remodeling and Metastasis		X
	X	Metabolic Stress		X
	X	Microglial Functions		X
	X	Myeloid Compartment		X
	X	NFkB Signaling		X
	X	NK Cell Functions		X
	X	Notch Signaling		X
	X	Pathogen Defense		X
	X	PI3K/Akt		X
	X	Regulation		X
	X	Senescence		X
	X	T Cell Functions		X
	X	TGF-beta Signaling		X
	X	TLR		X
	X	TNF Superfamily		X
	X	Wnt Signaling		X

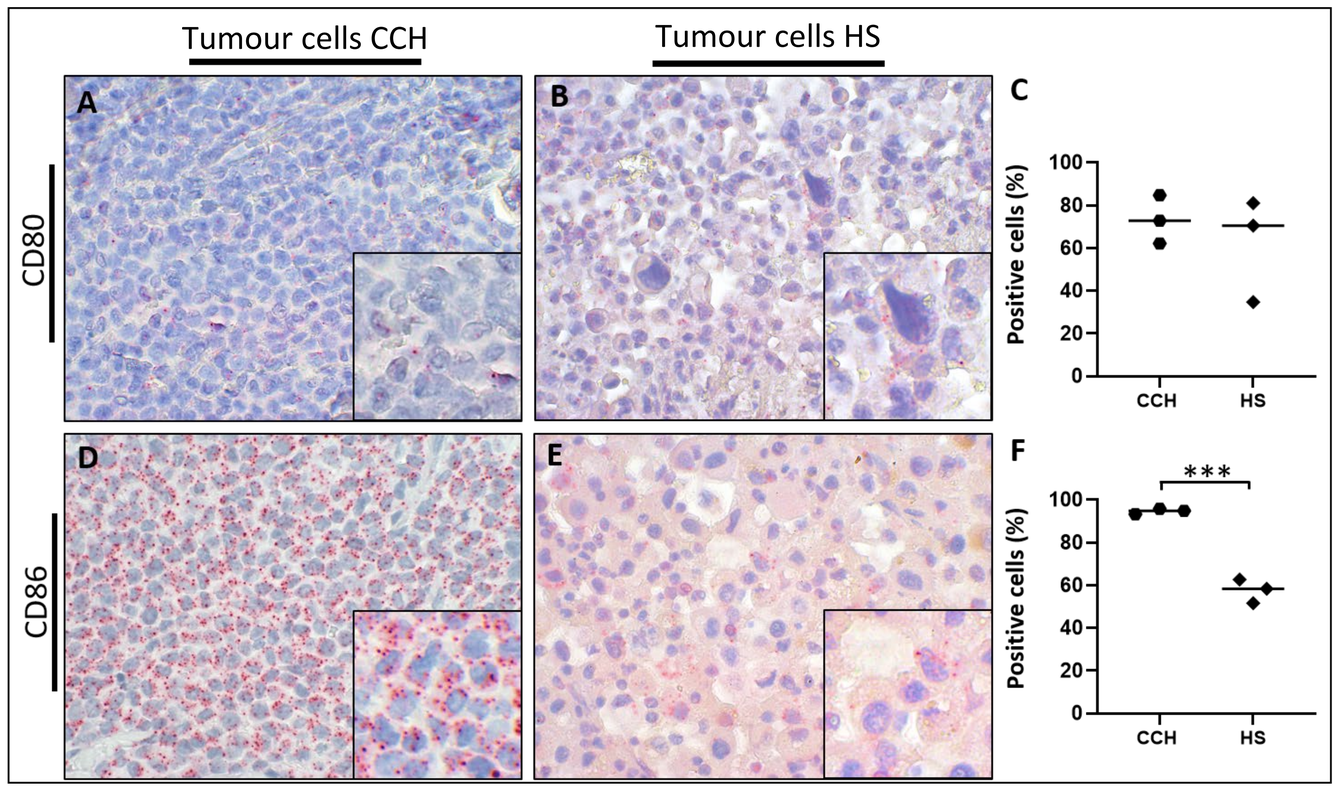
*** DEG = differentially expressed genes**

Spatial and time-dependent mRNA expression levels of co-stimulatory B7 family ligands CD80 and CD86 in CCH analysed via ISH



(**A**, **B**, **C**) Percentages of CD80 positive tumour cells among all tumour cells in bottom, centre, and top areas of stage 1 (dots), 2 (squares), and 3 (triangles) of CCH. (**D**, **E**, **F**) Percentage of CD86 positive tumour cells among all tumour cells in bottom, center, and top areas of stage 1. Each symbol represents the mean of all counted high power fields of a biological replicate of the respective group. Asterisks indicates statistically significant difference between groups 1 und 3 in the central area (unpaired t-test, p = 0.0161).

Comparison of mRNA expression patterns of co-stimulatory B7 family ligands CD80 and CD86 in CCH compared to HS



(**A**, **B**) CD80 expression pattern (red dots) in tumour cells of CCH and HS, respectively. (**C**) Means of percentages of CD80 positive tumour cells among all tumour cells in CCH and HS. (**D**, **E**) CD86 expression pattern (red dots) in tumour cells of CCH and HS. (**F**) Means of percentages of CD86 positive tumour cells among all tumour cells in CCH and HS (unpaired t-test, p = 0.0004). In situ hybridisation with fast red (chromogen, red) and hemalaun (blue) counterstain. Magnification: 600x, Inserts: 1800x.

Surprisingly, no significant differences were found for any of the pathways tested between the three stages. Over time, CD80 displayed an increase in expression from bottom to top, while CD86 remained unchanged (average: 95.1% expressing tumour cells). Overall, expression of CD80 in CCH (73.3%) was similar to HS (62.1%), while CD86 was significantly less expressed in HS (57.6%) compared to CCH.

Conclusion

Our data reveal that major immuno-oncological pathways are seemingly not regulated on the mRNA level during the course of CCH. We speculate that key processes leading to tumour regression may occur at an earlier time point than examined here. Our data further support a role of co-stimulatory molecules in CCH regression involving cytotoxic lymphocytes.

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