Investigating Variants in Tumor Suppressor Genes in Histiocytic Sarcoma

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Introduction/Background

- Histiocytic sarcoma (HS) is a malignant cancer involving histiocytes, which includes macrophages and dendritic cells that function as a part of the immune system.
- HS is a rare disease found in both humans and dogs, but in certain dog breeds such as Bernese mountain dogs (BMD), the frequency can be as high as 25%.[1]
- HS is hypothesized to have an inherited predisposition likely involving the inactivation of tumor suppressor gene(s) (TSG).
- Previous studies of HS in BMDs have found mutations in PTPN11 and KRAS associated with the activation of MAPK signaling, which is a major cell proliferation pathway.[2]

Fig 1. Bernese Mountain Dog

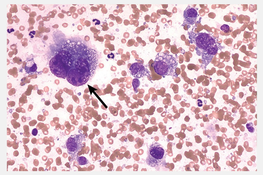

Fig 2. Canine histiocytic sarcoma[3]

Objectives

- To analyze if variants in various tumor suppressor genes contribute to the high incidence of histiocytic sarcoma in Bernese Mountain dogs.

Methods

- **HS cell line variants**- Variant data was generated from next-gen sequencing of 3 canine HS cell lines (BD, OD, PJ, developed in the Yuzbasiyan-Gurkan lab).
- **ROSALIND**- RNAseq data from the BD, OD, and PJ cell lines with HS were compared to an unaffected cell line.
- **Clustal Omega-** Multiple sequence alignment tool used to identify regions of similarity that may indicate functional/structural relationships.
- **Ensembl**- Genome browser used to identify protein sequences for genes of interest from human, canine, mouse, and rat genomes.
- **BLAST**- Tool used to identify regions of similarity between proteins of interest to sequence databases and calculate the statistical significance.
- **cBioPortal**- Database referenced for specific gene mutations found in humans for various types of cancers. Matches in protein change between the BMD data and known human genes were further investigated.
- **SIFT/PolyPhen**- Sorting Intolerant From Tolerant (SIFT) and PolyPhen were used to evaluate the predicted effects of amino acid substitutions on protein function.

Results

Variant calling on HS cell line DNA sequences → Variants in tumor suppressor genes → Ensembl protein sequence (human and canine) → Multiple Sequence Alignment → cBioPortal reference gene in human → ROSALIND differential analysis SIFT/PolyPhen protein function prediction

	Chr	Fold Change	Expression	Variant	BD	OD	PJ	Known Variant	Dog SIFT Score	Reported Variant Allele Frequency	Human AA Position
RASAL1	26	-10.44	yes	R101P	1/1	0/1		yes	0.25 (0.33)	20-40%	R109
				T263M	1/1		1/1	yes	0.14 (0.17)	30-50%	M in human (M271)
				S566L		0/1		yes	0.02 (0)	<2%	S565
PALB2	6	4.87	yes	D486N		1/1	1/1	yes	1	40-50%	N in human (N330)
				G497E		1/1	1/1	yes	1	40-50%	E in human (E341)
ANXA7	4		yes	V144I	1/1			yes	0.58	40-50%	V120
				M294V	0/1			yes	0.09	40-50%	M248
				R413Q		0/1		yes	0.03	10-13%	R367
BLM	3		yes	D231N	1/1	1/1		yes	0.1 (0.09)	10-20%	D234
CD82	18		yes	I257V	0/1	0/1		yes	0.83 (0.86)	50-70%	V in human (V256)
FANCG	11		yes	E158G	0/1	1/1		yes	0.58	15-25%	same
				Q649R	0/1	1/1		yes	0.15	NA	Q465
IGF2R	1		yes	T132N	1/1	1/1		yes	1	<2%	A in human
PARP1	7		yes	A451E			0/1	yes	0.93	<1%	V in human (V493)
PER2	25		yes	P27L			1/1	yes	0.35 (0.23)	20-40%	P27
				R740Q	0/1	1/1		yes	0.02 (0.07)	70-80%	R749
				T782N	0/1			yes	0.26 (0.46)	~8%	T791
				P891L	0/1			yes	0.03 (0.04)	3-4%	P895
				K1251E	0/1	1/1	1/1	yes	1 (1)	40-50%	D in human (D1241)
RPS6KA2	1		yes	Y702C			1/1	yes	0.01 (0.16)	<1%	Y677
SETD2	20		yes	D253N	0/1			no			D257
				A1719S		0/1		no			A1721
SP100	25		yes	R860C	1/1	1/1	1/1	yes	0.04	NA	R838
TP53BP2	7		yes	A611V		0/1	0/1	yes	0.48 (0.58)	30-60%	P in human (P544)
				Q646L			0/1	yes	0.06	NA	Q580
				A850V	0/1			yes	0.37 (0.51)	~10%	T in human (T784)
				V876L		0/1	0/1	yes	0.64 (0.83)	20-50%	V810
UVRAG	21		yes	R535Q	0/1	0/1	1/1	yes	0.87 (0.29)	60-70%	same
ZBTB48	5		yes	K184E	1/1	1/1	0/1	yes	0.33 (for E>K)	70-80%	E in human (E184)

Fig 3. Matching TSG variants of interest in HS cell lines.

	Variant	Dog SIFT Score	Dog PolyPhen Score	Reported Variant Allele Frequency	Human AA Position
RASAL1	S566L	0.02 (0)	0.992	<2%	S565
ANXA7	R413Q	0.03	0.99	10-13%	R367
PER2	R740Q	0.02 (0.07)	0.048	70-80%	R749
	P891L	0.03 (0.04)	0.877	3-4%	P895
RPS6KA2	Y702C	0.01 (0.16)	0.804	<1%	Y677
SP100	R860C	0.04	1	NA	R838

Fig 4. Deleterious variants with SIFT and PolyPhen scores (SIFT <0.05 and PolyPhen >0.85 are predicted deleterious).

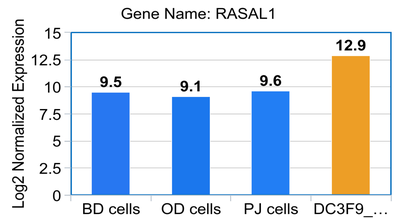

Fig 5. RASAL1 log normalized expression using ROSALIND

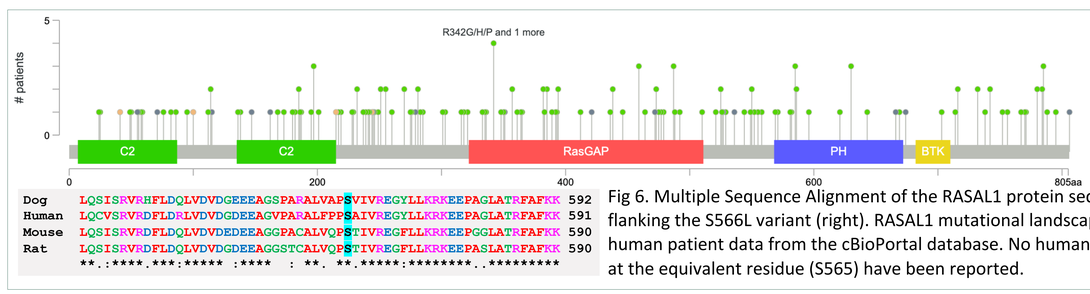

Fig 6. Multiple Sequence Alignment of the RASAL1 protein sequence flanking the S566L variant (right). RASAL1 mutational landscape in human patient data from the cBioPortal database. No human variants at the equivalent residue (S565) have been reported.

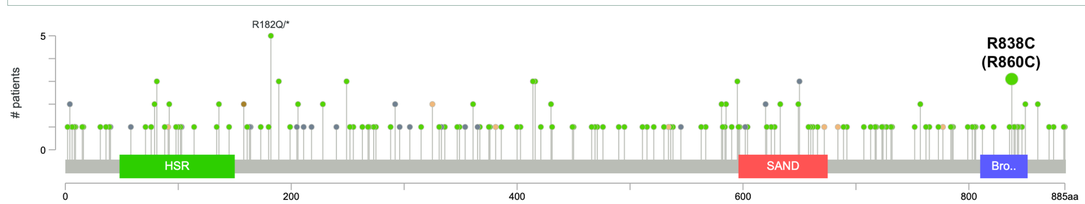

Fig 7. SP100 mutational landscape in human cancer patient data from the cBioPortal database. Equivalent variant (R838C) reported in human data. Variant located in the Bromodomain of the SP100 protein.

Discussion

- SIFT and PolyPhen analysis of variants from 3 HS cell lines revealed predicted deleterious substitutions in 5 genes of interest: ANXA7, PER2, RASAL1, RPS6KA2, and SP100.
- Out of these 5 genes, we had indication of differential expression only for RASAL1.
- Findings on ROSALIND indicated a -10.44-fold change in expression compared to normal. The associated pathway was DNA replication (p-adj=2.6e-11).
- The specific protein mutation found from BMD data was S566L, which corresponds to the S565 amino acid position in the human RASAL1 protein sequence.
- RASAL1 functions as tumor suppressor gene through inhibition of RAS function as a GTPase-activating protein, which may have potential implications in the RAS/RAFF/MAPK pathway.[4]
- Literature review indicates that altered expression of RASAL1 has also been associated with gastric and colorectal carcinoma in humans.[4]

Conclusion

- We identified a number of variants in TSGs, in our HS cell lines. Of note, a predicted deleterious variant resulting in a S566L substitution was identified in one of our cell lines. Importantly, the expression of this gene was downregulated in all three cell lines.
- Additionally, a predicted deleterious mutation was identified in the SP100 gene, homozygous in all three cell lines, which has been reported in human cancer patients.
- Frequency of these variant in HS tumors will need to be further investigated, along with variants in other genes.
- Orthogonal studies to probe downstream effects of RASAL1 on the RAS/RAF/MAPK pathway will need to be conducted.

References

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