Liquid biopsy analysis in canine subjects reveals actionable somatic cancer variants shared with humans

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INTRODUCTION

The genomic landscapes of human and canine cancers have shown high homology, and discoveries in one species often have value for the other. Characterizing actionable variants in humans and dogs can expand the knowledge base of comparative oncology and support the development of novel treatments for the benefit of both species. Blood-based liquid biopsy offers unprecedented opportunities to profile the canine cancer genome noninvasively across large patient cohorts.

METHODS

The top 100 variants (defined by the number of times the variant was independently observed in human cancer subjects) from the Catalogue of Somatic Mutations in Cancer (COSMIC) database (v91)[1] were compiled and evaluated for canine orthologs. A >70 kilobase targeted next-generation sequencing (NGS) panel was then developed that incorporated the top 100 COSMIC variants for which a canine ortholog was identified. NGS of the targeted libraries was performed on matched cell-free DNA (cfDNA) and genomic DNA (gDNA) extracted from whole blood samples of 245 dogs with cancer enrolled in the CANcer Detection in Dogs (CANDiD) study, an all-comers cohort representing over 40 different cancer types.[2] The panel was also used to sequence matched tumor tissue samples from a subset of the cancer-diagnosed dogs. Somatic variant calling was performed using a tumor/normal approach in which the gDNA was used as a baseline normal sample to subtract out the germline variants.

RESULTS

Of the top 100 COSMIC variants in humans, 94 had identifiable orthologs in the canine genome, which were incorporated into the larger targeted NGS panel. When analyzed at the variant level, a total of 25 of these 94 variants were identified in the cfDNA samples of the cancer-diagnosed subjects; additional variants not in the top 100 COSMIC were also identified in many of the samples [data not shown], with some subjects testing positive for more than one of these variants. The 25 variants were detected across 11 canine oncogenes and tumor suppressor genes, including *BRAF, PIK3CA, KRAS,* and *TP53*; and orthologs of most of these variants were determined to have a level of evidence (LOE) for actionability in human cancers.[3]

When analyzed at the patient level, 17% (42/245) of cancer-diagnosed patients had at least one of the 25 COSMIC orthologs detected, and over 10 canine cancer types were represented in the patients with positive results. In these 42 subjects, matched tumor tissue was tested for 9 patients. The same targeted NGS panel used for liquid biopsy testing was used for concordance testing of the tissue samples; 6 of the 9 subjects (67%) were found to have at least one concordant variant in both cfDNA and tissue. Concordance was evaluated by first detecting the variants in the cfDNA and then evaluating for their presence in the tissue samples.

Table 1: Top 100 COSMIC mutations (variants) ranked based on the number of times the variant has been detected in human cancer subjects. 94 of these variants were bioinformatically identified as having a canine ortholog; the 6 variants highlighted did not have an identifiable canine ortholog.

Rank	Gene	Human Mutation	Rank	Gene	Human Mutation	Rank	Gene	Human Mutation	Rank	Gene	Human Mutation
1	JAK2	p.V617F	26	TP53	p.R273H	51	TP53	p.Y220C	76	KRAS	p.Q61H
2	BRAF	p.V600E	27	TP53	p.R273C	52	RET	p.M918T	77	NRAS	p.G12S
3	KRAS	p.G12D	28	KIT	p.D816V	53	CTNNB1	p.S45P	78	SRSF2	p.P95H
4	KRAS	p.G12V	29	IDH1	p.R132C	54	APC	p.T1556Nfs*3	79	MPL	p.W515L
5	KRAS	p.G13D	30	NRAS	p.G12D	55	NRAS	p.G13D	80	CTNNB1	p.S33C
6	KRAS	p.G12C	31	TP53	p.R248W	56	HRAS	p.G13R	81	HRAS	p.G12V
7	IDH1	p.R132H	32	DNMT3A	p.R882H	57	KCNJ5	p.L168R	82	TP53	p.V157F
8	PIK3CA	p.H1047R	33	GNAS	p.R844C	58	KRAS	p.G13C	83	ACVR2A	p.K437Rfs*5
9	NPM1	p.W288Cfs*12	34	IDH2	p.R140Q	59	EGFR	p.T790M	84	NPM1	p.W288Cfs*12
10	EGFR	p.L858R	35	FOXL2	p.C134W	60	TP53	p.R196*	85	PIK3CA	p.E545A
11	PIK3CA	p.E545K	36	CTNNB1	p.S45F	61	H3F3A	p.K28M	86	TGFBR2	p.K153Sfs*35
12	KRAS	p.G12A	37	TP53	p.R282W	62	APC	p.R1450*	87	KRAS	p.A146T
13	KRAS	p.G12S	38	MED12	p.G44D	63	DNMT3A	p.R882C	88	RHOA	p.G17V
14	MYD88	p.L273P	39	BRAF	p.V600K	64	MED12	p.G44S	89	CDKN2A	p.R80*
15	NRAS	p.Q61R	40	GNAS	p.R844H	65	FLT3	p.D835Y	90	TP53	p.E285K
16	CALR	p.L367Tfs*46	41	AKT1	p.E17K	66	TP53	p.R342*	91	TP53	p.H179R
17	TP53	p.R175H	42	TP53	p.R213*	67	IDH2	p.R172K	92	FBXW7	p.R465C
18	PIK3CA	p.E542K	43	EGFR	p.E746_A750del	68	NRAS	p.Q61L	93	PIK3CA	p.Q546K
19	FGFR3	p.S249C	44	TP53	p.G245S	69	MED12	p.G44V	94	KCNJ5	p.G151R
20	KRAS	p.G12R	45	NOTCH1	p.P2514Rfs*4	70	HRAS	p.Q61R	95	IDH1	p.R132G
21	NRAS	p.Q61K	46	SF3B1	p.K700E	71	FGFR3	p.R248C	96	NPM1	p.W288Cfs*12
22	CTNNB1	p.T41A	47	ASXL1	p.G646Wfs*12	72	CTNNB1	p.S37F	97	APC	p.R876*
23	EGFR	p.E746_A750del	48	FGFR3	p.Y375C	73	GNA11	p.Q209L	98	TP53	p.Y163C
24	TP53	p.R248Q	49	TP53	p.R249S	74	GNAQ	p.Q209L	99	PIK3CA	p.R88Q
25	CALR	p.K385Nfs*47	50	PIK3CA	p.H1047L	75	TP53	p.R306*	100	TP53	p.C176F

No canine ortholog identified

Table 2: List of canine ortholog variants identified in cfDNA samples from canine cancer patients in the CANDiD Study.

Gene Name	Human p.	Canine p.	Number of Cancer Positive Subjects	Canine Cancer Types	OncoKB highest LOE in human cancers
BRAF	p.V600E	p.V588E	2	Lymphoma, Transitional Cell Carcinoma	L1/L2 Therapeutic
CTNNB1	p.S33C	p.S33C	2	Lymphoma	NA
EGFR	p.L858R	p.L792R	2	Lymphoma, Hepatocellular Carcinoma	L1 Therapeutic
FBXW7	p.R465C	p.R512C	6	Lymphoma	L2 Diagnostic Lymphoma
GNAS	p.R844C	p.R156C	1	Lymphoma	NA
GNAS	p.R844H	p.R156H	1	Lymphoma	NA
KRAS	p.G12D	p.G106D	1	Mammary Gland Carcinoma	L3/L4 Therapeutic
KRAS	p.G12V	p.G106V	1	Pulmonary Carcinoma	L3/L4 Therapeutic
KRAS	p.G13D	p.G107D	2	Lymphoma, Malignant Melanoma	L3/L4 Therapeutic
KRAS	p.Q61H	p.Q155H	1	Lymphoma	L3/L4 Therapeutic
MED12	p.G44D	p.G44D	1	Lymphoma	NA
NPM1	p.W288Cfs*12	p.W288C	1	Osteosarcoma	L1/L2 Diagnostic
NRAS	p.G13D	p.G13D	1	Transmissible Venereal Tumor	L3/L4 Therapeutic
NRAS	p.Q61L	p.Q61L	1	Transarticular Sarcoma	L3/L4 Therapeutic
PIK3CA	p.E542K	p.E542K	2	Lymphoma, Hepatocellular Carcinoma	L1 Therapeutic
TP53	p.C176F	p.C227F	2	Lymphoma	L1 Prognostic
TP53	p.E285K	p.E337K	1	Lymphoma	L1 Prognostic
TP53	p.R175H	p.R226H	8	Lymphoma, Osteosarcoma, Adenocarcinoma	L1 Prognostic
TP53	p.R196*	p.R248*	1	Hemangiosarcoma	L1 Prognostic
TP53	p.R213*	p.R265*	5	Lymphoma, Osteosarcoma, Hemangiosarcoma, Squamous Cell Carcinoma	L1 Prognostic
TP53	p.R248Q	p.R300Q	2	Lymphoma, Pulmonary Adenocarcinoma	L1 Prognostic
TP53	p.R248W	p.R300W	1	Lymphoma	L1 Prognostic
TP53	p.R273C	p.R325C	5	Lymphoma, Osteosarcoma, Soft Tissue Sarcoma	L1 Prognostic
TP53	p.R273H	p.R325H	9	Lymphoma, Osteosarcoma, Adenocarcinoma	L1 Prognostic
TP53	p.V157F	p.V121F	1	Lymphoma	L1 Prognostic

Figure 1: Flowchart depicting the development of a targeted NGS panel for canine cancer, and results of testing

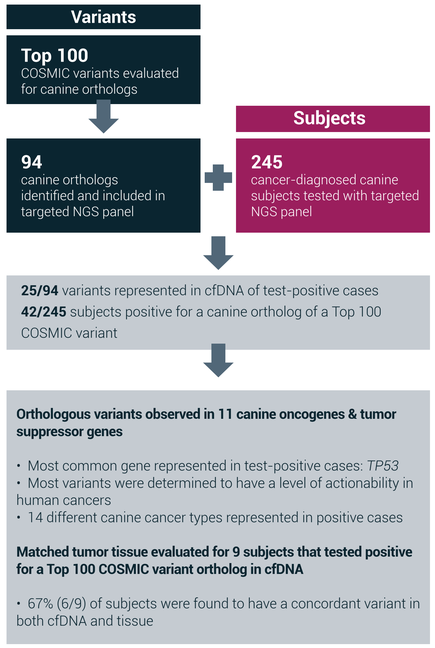

Table 3: Six cancer-diagnosed subjects in which a concordant variant was found in both cfDNA and tissue

Gene Name	Human p.	Canine p.	OncoKB Highest LOE in Human Cancer	Diagnosis
TP53	p.R273H	p.R238H	L1 Prognostic	Lymphoma
TP53	p.R213*	p.R201D	L1 Prognostic	Osteosarcoma
NRAS	p.Q61L	p.Q61L	L3/L4 Therapeutic	Transarticular Sarcoma
TP53	p.R248Q	p.R300Q	L1 Prognostic	Pulmonary Adenocarcinoma
TP53	p.R273H	p.R325H	L1 Prognostic	Adenocarcinoma
KRAS	p.G12D	p.G106D	L3/L4 Therapeutic	Mammary Gland Carcinoma

Figure 2: Representation of genes in canine patients positive for orthologs of the Top 100 COSMIC variants

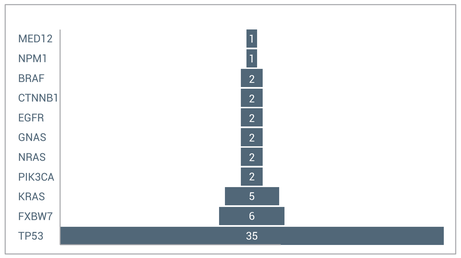

Figure 3: OncoKB Levels of Evidence[3]

DISCUSSION

This study demonstrates that dogs and humans with cancer share common driver mutations, and adds to a growing body of evidence suggesting that dogs are a suitable model organism for the development of diagnostics and therapeutics that can be used to manage cancer in humans. Further, this study demonstrates that liquid biopsy can be used to detect orthologous variants in canine cancer subjects with a routine blood draw, and that the same variants are often present in both cfDNA and tissue samples from the same subject.

CONCLUSION

Certain cancer pathways have been evolutionarily conserved to a considerable degree across humans and dogs, and many of the same somatic mutations driving cancer development in one species are present in the other. Liquid biopsy can serve as a powerful tool for the noninvasive detection of cancer-specific genomic alterations in both species.

KEY POINTS

1. Canine and human cancers share orthologous mutations that are actionable and drive cancer development in both species
2. Dogs may be a suitable model for understanding the biological underpinnings of cancer in humans and for the development of diagnostics and therapeutics to benefit both species
3. Cancers that are rare in humans (such as osteosarcoma and angiosarcoma) are common in dogs, offering the ability to study the genomic landscapes of these cancers in much larger cohorts
4. Liquid biopsy is a powerful tool for detecting cancer in both dogs and humans

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Presented at the AGBT Precision Health Meeting, September 9-11, 2021, San Diego, CA