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Candidate genes in canine hepatocellular carcinoma for molecular targeted therapy

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Abstract

Objectives Unresectable canine hepatocellular carcinoma (HCC) has limited nonsurgical treatment options. Sorafenib is a targeted therapy for unresectable canine HCC. However, there are limited reports on the expression of target genes. Therefore, the efficacy of the targeted therapies for canine HCC remains unclear.

Data description Liver specimens were obtained from 11 dogs with HCC and four dogs without HCC. We performed RNA seq using the mRNA extracted from the specimens. Differentially expressed genes (DEGs) between canine HCC and normal liver were explored based on previously reported molecular-targeted agents for human tumours. ***PARP3***, ***DNMT1***, ***FGF19***, ***FGF23***, and ***RET*** DEGs were upregulated, whereas ***KIT***, ***FGFR2***, and ***FGF21*** DEGs were downregulated.

Keywords RNA sequence, *FGF19*, *PARP3*, *DNMT1*, *FGF23*, *RET*

Objective

In canine hepatocellular carcinoma (HCC), the prognosis is generally good when complete surgical resection is possible [1]. Unresectable nodular and diffuse HCC have a poor prognosis and limited non-surgical treatment options [2]. In humans, systemic therapies including targeted therapies are indicated when curative treatment is difficult [3]. Targeted therapy includes the use of conventional molecular targeted agents, hormonal agents, immune checkpoint inhibitors, and targeted cytotoxic therapy [4]. Despite the high anticancer activity of the targeted therapy, the agents can only be applied to patients with targeted gene mutations or abnormalities [5, 6]. For these targeted genes, the therapeutic agents exert antitumor effects by inhibiting cell proliferation, metastasis, and angiogenesis, reversing multidrug resistance, and inducing apoptosis [4]. In canine unresectable HCC, sorafenib is used as targeted therapy [2]. However, there are limited reports on the expression status of the target gene [7]. Moreover, up regulation of *PDGFB* is reported in canine HCC as a potential gene for targeted therapy [7]. However, the application of targeted therapy in canine HCC is not clear. Therefore, in this study, we assessed the expression of target genes in canine HCC based on their expression in human tumours.

Data description

Specimens were obtained from eleven dogs histopathologically diagnosed with HCC and four dogs with normal livers by surgical resection at the Osaka Metropolitan University Veterinary Medical Center. Dogs with HCC

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consisted of two neutered male dogs and two intact male dogs, and two neutered female dogs and five intact female dogs. The mean age of the dogs was 10.5±2.3 y (mean±SD). The dog breeds chosen for the study were as follows: three Shiba, two Border Collies, one Brussels griffon, one West Highland White Terrier, one Toy Poodle, one Welsh corgi, one Shih Tzu, and one Dachshund. The dogs with normal livers consisted of four intact female dogs. The mean age was 2.8±1.3 y (mean±SD). All dogs were beagles. Information of dogs in this study was shown in Data file1 [8].

The liver specimens used for tissue banking were placed in a liquid nitrogen bath and snap-frozen. The specimens were stored in freezers maintained at -80 °C. Total RNA was isolated from frozen liver tissues using NucleoSpin® RNA Plus (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. The quality and quantity of purified RNA were determined by measuring the absorbance at 260 nm and 280 nm (A260/A280 ratio) using an Eppendorf Biophotometer (Eppendorf).

In the isolated RNA samples, the RNA integrity number was measured using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and RNA samples with an RNA integrity number greater than 7.0 were used for analysis. RNA-Seq (75 bp paired-end) was conducted using NextSeq 500 (Illumina) with the High Output Kit (Illumina), and a minimum of 43 million read pairs were generated for each sample covering more than 96% of the canine genome. Quality controls and adaptor trimmings of fastq files were performed using the default parameters of Trim Galore software (v0.6.10) based on FastQC and Cutadapt. Trimmed fastq was mapped to CanFam3.1, using HISAT2 (v2.2.1), and transcript abundance was estimated using a published pipeline [9] with a gene transfer file for Ensembl (CanFam3.1.102, https://www.ensembl.org). Differentially expressed genes (DEGs) were estimated from the obtained gene count data using the EdgeR-based analysis tool, TCC-GUI [10]. Data Set 1 contains the RNA sequencing results for this publication, which have been deposited in DDBJ Sequence Read Archive and are accessible through bioproject accession number PRJDB18013 [11].

We investigated DEGs between the canine HCC liver and normal liver, based on previously reported targeted genes of human tumours including *EGFR*, *ERBB2*, *VEGF*, *VEGFR*, *PDGFR*, *FGFR*, *KIT*, *Flt-3*, *RET*, *RAF*, *BCR-ABL*, *ALK*, *mTOR*, *CTLA-4*, *PD-1*, *BRAF*, *BTK*, *CD20*, *CD30*, *CD33*, *CD52*, *CCR4*, *DNMT*, *HDAC*, *JAK*, *MEK*, *RANKL*, *SLAMF7*, *IGF-1R*, *FGF*, *MET*, *AXL*, *ROS1*, *TRK*, *Src*, *FLT3*, *CSF-1R*, *Tie2*, *ET*, *SFK*, *ARG*, *DDR1*, *NQO2*, *EPHB4*, *BTK*, *KRAS*, *PI3K*, *CDK4/6*, *PARP*, *EZH2*, *IDH1*, *IDH2*, *Bcl-2*, *NFE2L2*, *RB1*, *MT1G* and *SIGMAR1* [4, 12–15]. The targeted agents reported for these genes are shown in Data file2 [8]. The cut-off criteria for the DEGs were defined as the absolute value of log fold changes>1 and p-value<0.05.

DEGs met the cut-off criteria were 1991, including 1207 upregulated genes and 784 downregulated genes, compared to the normal liver. Among the 1991 DEGs, eight DEGs for molecular targeted agents were identified in HCC, five of which were upregulated and three were downregulated. The upregulated DEGs included *PARP3*, *DNMT1*, *FGF19*, *FGF23*, and *RET*, whereas the downregulated DEGs included *KIT*, *FGFR2*, and *FGF21* (See Data file3 and 4) [8]. In canine HCC, cirrhosis is detected in 7% [16]. Because liver cirrhosis is not involved in the occurrence of canine HCC, canine HCC may involve different mechanisms of carcinogenesis and different gene expression level compared to human HCC in cirrhosis. Our results may be useful for comparative studies of gene expression status for HCC in dogs and humans (see Table 1).

Limitations

This study has some limitations. First, this study have small sample size, biased sample size between normal liver and hepatocellular carcinoma. A small sample size lead to reduce the statistical power. Particularly limited sample sizes in normal liver may not reflect representativeness. Second, there is an age bias between normal liver and hepatocellular carcinoma. As DNA damage accumulates with aging, genetic and epigenetic alterations occur [17]. Therefore, DEGs in hepatocellular carcinoma may be affected by aging. Third, we did not

Table 1 Overview of data files/data sets

Label	Name of data file/data set	File types (file extension)	Data repository and identifier (DOI or accession number)
Data set 1	Candidate genes in canine hepatocellular carcinoma for molecular targeted agent	FASTQ files	DDBJ; https://identifiers.org/bioproject:PRJDB18013 [11]
Data file 1	Information of dogs in this study	Data file1(excel file)	Harvard Dataverse; https://doi.org/10.7910/DVN/1AQOKY [8]
Data file 2	Previously reported target genes and targeted agents for human tumours	Data file2(word file)	Harvard Dataverse; https://doi.org/10.7910/DVN/1AQOKY [8]
Data file 3	Up- or downregulated DEGs in HCC compared to normal liver	Data file3(word file)	Harvard Dataverse; https://doi.org/10.7910/DVN/1AQOKY [8]
Data file 4	Cluster heatmap of eight DEGs	Fig1 (PDF file)	Harvard Dataverse; https://doi.org/10.7910/DVN/1AQOKY [8]

evaluate DEGs in canine HCC and normal liver specimens from the same dog.

Abbreviations

DEGs Differentially expressed genes
HCC Hepatocellular carcinoma

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13104-024-07016-y.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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Author contributions
TT was the principal investigator and first author of the manuscript. TT and HA conceived the study. HA-supervised surveillance components. MM, NS, KM, and YI performed specimen sampling and RNA isolation. TM validated and analysed the RNA-seq data. TM, TT, MM, and NS interpreted the RNA-Seq data. TT prepared initial drafts, figures, and tables. All authors contributed to writing and editing the manuscript.

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Data availability
The data have been deposited with links to BioProject accession number PRJDB 18013 in the DDBJ BioProject database.

Declarations

Ethics approval and consent to participate
Informed consent for sample collection of HCC in this study was obtained from all clients at first examination of their dogs. The normal liver was a piece of liver tissue that had been collected and cryopreserved in another project that was properly conducted according to the Guidelines of Animal Care and Use in Osaka Metropolitan University.

Consent for publication
Informed consent for publication of HCC in this study was obtained from all clients at first examination of their dogs.

Competing interests
The authors declare no competing interests.

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