Skin coat color-dependent risks and risk factors of squamous cell carcinoma and deafness of domestic cats inferred via RNA-seq data

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Abstract
The ribonucleic acid-sequencing (RNA-seq) data of skin cells from domestic cats with brown, orange, and white coats were analyzed using a public database to investigate the relationship between coat color-related gene expression and squamous cell carcinoma risk, as well as the mechanism of deafness in white cats. We found that the ratio of the expression level of genes suppressing squamous cell carcinoma to that of genes promoting squamous cell carcinoma was significantly lower than the theoretical estimation in skin cells with orange coats in white-spotted cat. We also found frequent production of KIT lacking the first exon (d1KIT) in skin cells with white coats, and d1KIT production exhibited a significant negative correlation with the expression of SOX10, which is essential for melanocyte formation and adjustment of hearing function. Additionally, the production of d1KIT was expected to be due to the insulating activity of the feline endogenous retrovirus 1 (FERV1) LTR in the 1st intron of *KIT* and its CTCF binding sequence repeat. These results are expected to contribute to basic veterinary research to understand the relationship between cat skin coat and disease risk, as well as the underlying mechanism.

Keywords: domestic cats, *Felis catus*, coat color, squamous cell carcinoma, deafness, white locus, transcriptome, insulator

Introduction

Domestic cats are among the most important companion animals, and feline health occupies an important position in human society. It has also recently been suggested that dementia and breast cancer in cats share various similarities with Alzheimer's disease (Chambers et al., 2015; Sordo et al., 2021) and triple-negative breast cancer in humans (Wiese et al., 2013; Caliari et al., 2014; Cannon, 2015; Thomas, 2015; Adega et al., 2016; Howard et al., 2020; Sommerville et al., 2022), respectively. Therefore, advances in feline medicine are expected to make important contributions to advances in human medicine.

In recent years, research on various feline diseases has progressed, leading to the development of vaccines for various lethal infectious diseases caused by virus such as Feline leukemia virus (FeLV) (Hofmann-Lehmann et al., 2020) and Feline immunodeficiency virus (FIV) (Westman et al., 2019; Andrade et al., 2022), therapeutic drugs for Feline infectious peritonitis (FIP) (Pedersen et al., 2019; Cook et al., 2022), and drugs for kidney disease (Sugisawa et al., 2016; Arai et al., 2016) that have been put into practical use, consequently extending the lifespan of domestic cats. However, these methods have only solved some diseases in cats. Basic medical research focusing on the genetics and physiology of cats is essential for establishing preventive and therapeutic methods for various feline diseases.

Domestic cats have different colors and patterns on their skin and coat, which vary from individual to individual depending on the combination of genes related to color (white, orange, brown, black, etc.) and patterns (agouti, tabby, dilute, etc.) (Lyons et al., 2005; Schmidt-Küntzel et al., 2005; Imes et al., 2006; Ishida et al., 2006; Schmidt-Küntzel et al., 2009; Eizirik et al., 2010; David et al., 2014; Lyons, 2021), and the pattern of X-chromosome inactivation in female. In addition, certain skin colors in cats have been suggested to be associated with specific diseases. For example, light-skinned individuals have been suggested to have a higher risk of squamous cell carcinoma than dark-skinned individuals (Dorn et al., 1971; Thomson, 2007; Murphy, 2013). This tendency has been attributed to the fact that light-colored individuals with less melanin have a weak resistance to ultraviolet (UV) rays. However, squamous cell carcinoma also develops in the lining of the oral cavity, tongue, and tonsils and is less likely to be directly exposed to UV light. Therefore, there may be other marked factors besides UV light (Baer et al., 1993; Bertone et al., 2003; Sabattini et al., 2010; Munday et al., 2011; Munday et al., 2012; Dermatology et al., 2016, Sequeira et al., 2022).

It has also been known that individuals with a white coat all over their bodies develop deafness at a high rate (Bergsma et al., 1971; Strain, 1996; Geigy et al., 2007;

Ryugo et al., 2012; Strain, 2015; Kral et al., 2015; Strain, 2017; Mari et al., 2019; Bianchi et al., 2020). This disease is thought to result from the inhibition of melanin synthesis and the differentiation of melanocytes form the neural crest cells when *White* allele is present at white locus (Geigy et al., 2007; David et al., 2014; Strain, 2017). In a cat with white coat, a sequence derived from feline endogenous retrovirus 1 (*FERV1*) is inserted at the white locus in the first intron of the tyrosine protein kinase (*KIT*) gene (David et al., 2014). The *white spotting* allele of an individual with white-coated skin on a part of the body surface (white-spotted cat) contains the full-length sequence of *FERV1* (7125 bp). In contrast, the *White* allele of an individual whose whole body is completely covered with a white coat (dominant white cat) contains only the long terminal repeat (LTR) of *FERV1* (622 bp) and not the full length. This has suggested that the presence of LTR caused a decrease in melanin synthesis. However, the role of LTR and the mechanism through which melanin synthesis is suppressed remain unclear. White coat skin formation with decreased melanin production is also associated with deafness in other animals, such as dogs, miniature pigs, and yaks (Strain, 2015; Strain, 2017; Qi et al., 2022). However, the underlying mechanisms have not been elucidated.

Thus, the relationship between the mechanisms that determine skin coat color and disease risk remains poorly understood. Therefore, in this study, we analyzed the RNA-seq data obtained from differently colored domestic cat skin cells published in a public database. Through this analysis, the relationship between skin color and squamous cell carcinoma risk and deafness as well as the mechanism by which genes that determine skin coat color is involved in these diseases were inferred.

Materials and Methods

Quantification of transcriptome data in 3 types of skin cells of *Felis catus*

From the Ensembl public database, specifically the file transfer protocol (ftp) site, mapped RNA-seq data on the *Felis catus* genome, Fca126_mat1.0, were obtained in bam and bam.bai formats. Data were retrieved from the orange skin cells of cats with orange and white coats (referred to as cell O), white skin cells of cats with orange and white coats (referred to as cell W), and skin cells of cats ticked with white torties (referred to as cell T). Each cell line had one replicate and the original Sequence Read Archive (SRA) identifiers were SRS1335428, SRS1319391, and SRS1319719. Here, O and W cells were obtained from the same cat, whereas T cells were obtained from a different cat (https://www.ebi.ac.uk/biosamples/samples/SAMN04498546; https://www.ebi.ac.uk/biosamples/samples/SAMN04498547; https://www.ebi.ac.uk/biosamples/samples/SAMN04498539).

We should note the following facts for the samples:
I) Both cats belonged to the white-spotted cat classification, which means that the cell genomes contained the full-length sequence derived from *FERV1* in the first intron of the *KIT* gene.
II) Most of the body surface of cats with white tortie-ticked tabby is covered with a dark-colored coat, such as brown or black. Therefore, for the analysis, it was assumed that cell T represented a typical dark-colored skin cell. As mentioned below, this assumption was supported by the expression levels of *tyrosinase* (*TYR*) and *tyrosinase related protein 1* (*TYRP1*) genes, which are located in the "color locus" and "brown locus" loci.
III) Cats with O and W cells are carriers of lipoprotein lipase deficiency.

The annotation file of the *F. catus* genome (Fca126_mat1.0) in the gene transfer format (GTF) was obtained from the National Centre for Biotechnology Information (NCBI) database (Bethesda, MD, USA). In the file, the chromosome numbers were converted from NC_058368.1, NC_058369.1, etc. to A1, A2, etc.

Using featurecounts with the options -M -O --fraction, the number of reads for each gene described in the GTF file was obtained from the bam and bam.bai formatted files for cells T, O, and W. From these counts, the transcript level of each gene was calculated by dividing the read count by the total exon length. The total transcript level was defined as the sum of all the transcript levels of the genes. Based on this result, the transcripts per million (TPM) of each gene were calculated as 1000000 * [transcript level] / [total transcript level] (see Table S1).

It is worth noting that the TPM values of the *TYR* and *TYRP1* genes in T cells were more than ten times higher than those in W cells (Table S1). This observation supports the previous assumption that T cells were sampled from dark-colored skin rather than white skin in cats with a ticked tabby and white tortie.

Quantification of exon transcription levels of *KIT* gene in 3 types of skin cells of *F. catus*

A GTF file specifying each exon region of *KIT* was obtained from the GTF file Fca126_mat1.0. The read count in each exon region of *KIT* was obtained from bam and bam.bai formatted files with featurecounts (using options: -t feature -M -O --fraction) (Liao et al., 2014) for T, O, and W cells. The transcript level of each region was calculated as the ratio of read count to region length. From the results, the TPM in each

exon region was obtained as 1000000 * [transcript level] / [total transcript level] (Table S2), where the total transcript level uses the value calculated in the previous section.

Extraction of skin coat color-dependent differentially expressed genes (SCDEGs)

The median TPM values (Table S1) obtained for all genes in all the cells (T, O, and W) were approximately 0.987. Based on this fact, we considered genes that met the following conditions as skin coat color-dependent differentially expressed genes (SCDEGs):

- The maximum expression levels obtained for T, O, and W were more than twice the minimum value.
- The maximum expression levels obtained for T, O, and W were 2 (approximately twice the median value) or higher.

Additionally, the SRY-Box Transcription Factor 10 (*SOX10*) was included as an SCDEG. Although the maximum expression level obtained from T, O, and W was 1.9 times the minimum value, which does not satisfy the above requirement, *SOX10* is known to be one of the predominant factors in determining skin coat color (Mort et al., 2015; Strain, 2015; Qi et al., 2022). Furthermore, the maximum expression of *SOX10* was nearly twice the minimum value. Therefore, *SOX10* was specifically considered an SCDEG (Table S3).

Enrichment analysis

Pathway enrichment analysis of the SCDEGs was conducted using g:Profiler (https://biit.cs.ut.ee/gprofiler/gost) (Raudvere et al., 2019). For this analysis, "felis catus" was chosen as the target organism, and "only annotated genes" were selected as the target gene set. The focus of the analysis was to identify significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways with a false discovery rate (FDR) threshold of less than 0.1.

Classification into squamous cell carcinoma-promoting and suppressing genes

First, SCDEGs associated with the KEGG pathway were identified from the results obtained through the aforementioned pathway enrichment analysis. Next, using databases and prior literature, the extracted SCDEGs were categorized into the following groups:

1. Carcinoma-promoting genes: These genes exhibited an excessive increase in expression leading to promotion of squamous cell carcinoma.

2. Carcinoma suppressor genes: These were expressed at decreased levels or were mutated; these genes contributed to the development of squamous cell carcinoma.
3. Other genes: SCDEGs that did not fall into the above categories.

Categorization was based on the information obtained from databases and previous studies (Table S5).

Theoretical ratio of the number of "tumor-promoting genes" and "tumor-suppressor genes"

A file named cancermine_collated.tsv, which contained cancer gene roles and citations, was obtained from the data download page (https://zenodo.org/record/7689627) of the public database CancerMine (http://bionlp.bcgsc.ca/cancermine/) (Lever et al., 2019). This file provided a list of oncogenes and tumor suppressor genes for each of the 526 cancer types covered in the database. The total number of oncogenes and tumor suppressor genes in all carcinomas was 18,994 and 11,295, respectively. Therefore, we assumed that the "theoretical ratio" between the number of "carcinoma-promoting genes" and the number of "carcinoma-suppressor genes" could be estimated based on these numbers.

Although the database data used for estimating this theoretical ratio are specific to human cancer, it is reasonable to assume that the ratio of the number of genes would be similar between the two species because humans and cats are both mammals.

Evaluation of skin coat color dependent squamous cell carcinoma risk

To assess the cancer risk, the expression levels of carcinoma-promoting and carcinoma-suppressor genes within the SCDEGs, specifically those contributing to pathways related to squamous cell carcinoma, were compared among T, O, and W cells using the following criteria:

1. The number of carcinoma-promoting genes whose expression level in cell X (X = T, O, or W) was at least twice the minimum expression level among cells T, O, or W was denoted as P_X.
2. The number of carcinoma-suppressor genes whose expression level in cell X (X = T, O, or W) was at least twice the minimum expression level in cells T, O, or W was denoted as S_X.
3. If P_X was greater than S_X, a comparison was made with the theoretical ratio obtained earlier using an exact test of goodness-of-fit. If the resulting *p*-value is

less than 0.05, it indicates that cell X is in a state where squamous cell carcinoma is more likely to develop and progress than in the other cells.

Evaluations of motif sequence of CTCF binding and promoter activity in white locus LTR

T, O, and W cells were derived from individual cats partially surrounded by white skin and fur. In the genomes of these white-spotted cats, an LTR sequence derived from *FERV1* was located in the first intron of *KIT*. To determine the specificity of this LTR sequence, we searched for binding motif sequences of the CCCTC-binding factor (CTCF) and motif sequence for core promoter elements within the LTR.

For this purpose, we utilized the CTCFBS Prediction Tool available in CTCFBSDB 2.0 (https://insulatordb.uthsc.edu/) (Ziebarth et al., 2013) and Eukaryotic Core Promoter Predictor (YAPP: http://www.bioinformatics.org/yapp/cgi-bin/yapp.cgi). The former tool is based on a database containing the CTCF-binding motif sequences from humans and mice, where the given sequence is considered to involve CTCF-binding activity if the score of this tool is larger than 3. The latter tool is based on the statistical analysis of data sets of eukaryotic core promoter elements (Cartharius et al., 2005; Gershenzon et al., 2005; Jin et al., 2006), where the sequence with the score larger than 0.8 ($0 < \text{score} < 1$) is considered to exhibit promoter activity; the sequences with the score equal to or larger than 0.9 were focused in this study.

Although the CTCFBSDB database is primarily constructed based on research conducted in humans and mice, given that cats are also mammals, it is reasonable to assume that there might be similarities in CTCF-binding motifs and therefore we considered them in the analysis.

Statistical analysis

P-values for various statistical analyses were calculated using R (version 4.2.3).

Result

SCDEGs and results of KEGG pathway enrichment analysis

To investigate the relationship between SCDEGs (Table S3) and the risk of squamous cell carcinoma, KEGG pathway enrichment analysis was conducted using g:Profiler (Table S4). The analysis identified the following pathways related to

squamous cell carcinoma in which significantly larger number of SCDEGs were involved.

1. Pathways in cancer (FDR ~ 3.58×10^{-3})
2. The p53 signaling pathway (FDR ~ 0.013×10^{-2})
3. Human papillomavirus (HPV) infection (FDR ~ 8.33×10^{-2})

It has been reported that HPV infection is implicated in the development of human squamous cell carcinoma and cervical cancer (Hennessey et al., 2009; Husain et al., 2017; Augustin et al., 2020; Bao et al., 2021; Chrysovergis et al., 2021; The et al., 2021; Karina, 2022; Kwon et al., 2022). Feline papillomavirus (FPV) type-2 has been proposed as the primary cause of squamous cell carcinoma in domestic cats (Munday et al., 2008; Munday et al., 2011; O'Neill et al., 2011; Munday et al., 2012; Thomson et al., 2016; Munday et al., 2017; Carrai et al., 2020; Yamashita-Kawanishi et al., 2021; Teh et al., 2021; Altamura et al., 2022). HPV and FPV are distinct viruses; however, their *E5*, *E6*, and *E7* oncogenes of HPV and FPV exhibit similar features (Jiang et al., 2014; Altamura et al., 2016; Karina, 2022). Additionally, the pathogenesis of FPV-2 infection has been found to share pathways with HPV-induced pathogenesis and carcinogenesis (Teh et al., 2021; Karina, 2022). Consequently, genes involved in processes driven by HPV infection should also be considered genes associated with squamous cell carcinoma.

Assessment of squamous cell carcinoma risk by comparing the expression of carcinoma-promoting and carcinoma-suppressing genes

SCDEGs that contributed to cancer pathways, the p53 signaling pathway, and human papillomavirus infection were classified into squamous cell carcinoma-promoting and squamous cell carcinoma-suppressing genes based on previous studies (Table 1, Table S5). For each of the T, O, and W cells, the P_X and S_X that exhibited stronger expression than the others were counted. The relationship between P_X and S_X (X = T, O, W) was compared to the theoretical ratio of the number of carcinoma-promoting and carcinoma-suppressor genes using the exact goodness of fit test (Table 2).

The analysis revealed that the P_O in O cell was significantly higher than the number of carcinoma-suppressor genes (S_O) (*P* value ~ 0.02246). The relationship between P_W and S_W displayed similar features, although the *P*-value obtained was slightly larger (*P* value ~ 0.05194). The values for P_T, P_O, and P_W were similar. In contrast, S_O and S_W were lower than S_T (*P* value of Fisher's exact test ~ 0.1296),

indicating that the expression levels of carcinoma-suppressing genes were much lower in O and W cells than in T cells.

CTCF binding motif repeat in LTR at white locus in 1st intron of *KIT* gene

White-coated cats possessed a DNA sequence derived from *FERV1* located in the first intron of the *KIT* gene. Furthermore, both *White* and *white spotting* alleles includes a 5' LTR region, which is expected to inhibit melanocyte differentiation (David et al., 2014). In this study, we examined the characteristics of the LTR sequence by evaluating the presence of CTCF-binding motifs in this region using CTCFBS Prediction Tool. The analysis identified putative seven CTCF-binding motifs within the LTR that showed similarity to known CTCF-binding sites (Fig. 1a). Furthermore, these motif sequences were arranged in a repeat pattern with a spacing of 46 base pairs between them.

1st exon-lacking *KIT* (*d1KIT*) expression level increased in white skin cell

The repeat region of the putative CTCF-binding motif within the LTR of the first *KIT* intron suggested a potential role of the LTR by binding of CTCF and functioning as an insulator, thereby acting as a boundary for topologically associated domains (TADs). *CTCF* has been found to be enriched at the edges of chromatin loops that form TADs and cooperates with cohesins to regulate TAD boundary formation (Parelho et al., 2008; Wendt et al., 2008; Merkenschlager et al., 2016). Genes located within neighboring TADs are generally known to be independently regulated in their expression.

Retroviral LTRs typically function as transcription promoters (Klaver et al., 1994; Pereira et al., 2000; Dunn et al., 2003; Cohen et al., 2009). The *FERV1* LTR may also possess promoter activity because the prediction tool YAPP found an initiator motif (INR), which is a typical consensus motif sequence of core promoter region, at the downstream of the repeat region of the putative CTCF-binding motif in LTR (Fig. 1a). Additionally, a downstream promoter element (DPE), which is also typical consensus motif sequence of core promoter region, was also found at the downstream of the INR (Fig. 1a). The DPE and the INR can function as TFIID-binding site cooperatively, leading to accurate and efficient transcription (Burke and Kadonaga, 1996, 1997). Therefore, if CTCF-binding motives exhibits insulating activity, it is possible that the second exon and downstream regions of *KIT* gene can be transcribed independently of the native promoter, utilizing the region regulated by above pair of INR and DPE as a core promoter (Fig. 1b). Now, we tentatively named the *KIT*

transcribed in such a manner and lacking the first exon *d1KIT* and quantified its expression level in each cell type in the next section.

Based on the estimated TPM values provided in Tables S1 and S2, the ratio of [TPM of the 1st exon] / [TPM of KIT] in T and O cells was in the range of 0.8 and 0.84, respectively. Additionally, the ratio of [Average TPM of 2nd-22nd exons] / [TPM of KIT] in T, O, and W cells was close to 1. However, in W cell, the ratio of [TPM of 1st exon] / [TPM of KIT] was approximately 0.48, which was close to half of the values observed in the other cells (Fig. 2). This suggests that approximately half of the *KIT* mRNAs generated by W cells lacked the first exon.

The approximate transcript levels (TPM) of normal *KIT* and *d1KIT* in T, O, and W cells were estimated as follows. First, since the amount of normal *KIT* mRNA was expected to consist of those of the first exon and the second and subsequent exons, [TPM of normal KIT] was assumed to be the minimum value between [TPM of 1st exon] and [Average TPM of 2nd-22nd exons]. Second, the TPM of d1KIT was estimated as follows: [TPM of d1KIT] = [TPM of KIT] × [TPM of normal KIT]. The TPM of normal *KIT* in T, O, and W cells was approximately 9.35, 20.50, and 9.87, respectively, whereas that of *d1KIT* in T, O, and W cells was approximately 1.67, 5.00, and 10.49, respectively (Fig. 2).

d1KIT expression correlated negatively to gene expressions for melanocyte formation

To determine how normal KIT and d1KIT are involved in melanocyte formation, we examined the correlation between their expression levels and those of typical genes involved in melanoblast and melanocyte formation (Table S6). The expression level of *d1KIT* showed a significant negative correlation with the expression level of *SOX10*, with a correlation coefficient of approximately -0.99957 (*P*-value ~ 0.01876) (Fig. 3). Furthermore, strong negative correlations were observed with the expression levels of melanocyte-related *DKK4* and *TYRP1*, with correlation coefficients of approximately -0.98777 (*P*-value ~ 0.09968) and -0.98000 (*P*-value ~ 0.12763), respectively. In contrast, the correlation coefficient between the expression levels of normal *KIT* and melanocyte protein *PMEL* ~ 0.94548 (*P*-value ~ 0.20302) that exhibited the largest absolute value of correlation coefficient (Fig. 3). This fact suggested the expression level of normal KIT did not strongly correlate with the expression of genes involved in melanoblast and melanocyte formation.

Discussion

Squamous cell carcinoma tends to occur more frequently in light-coated cats, which may be due to a lack of melanin production, resulting in poor UV resistance. If this is the case, it would be expected that white-coated skin, without melanin production, would be most susceptible to carcinoma. However, in our analysis, we found that, in terms of gene expression, the expression level of a factor that suppresses squamous cell carcinoma was significantly lower in cells with orange-coated skin with *white spotting* allele. In other words, when considering gene expression, cells with orange skin may have a higher risk of cancer than cells with other skin colors.

We also observed that the ratio of the expression level of genes suppressing squamous cell carcinoma to that of genes promoting squamous cell carcinoma was lower than the theoretical estimation in cells with white coated skin with *white spotting* allele. It is worth noting that the orange- and white-coated skin cells analyzed in this study were sampled from the same individuals and were derived from carriers of lipoprotein lipase deficiency. These findings suggest two possible mechanisms underlying the risk of squamous cell carcinoma.

1. Cells for light-colored skin, such as white- and orange-coated skin, tend to exhibit low expression of carcinoma suppressor genes.
2. Cells derived from carriers with lipoprotein lipase deficiency tended to exhibit low expression of carcinoma-suppressor genes.

Validation of these possibilities through analysis of more accumulated data and additional experiments should be conducted in future.

In white-coated skin cells, a large amount of *d1KIT* mRNA, in which the 1st exon of the *KIT* gene is deleted, was produced. Furthermore, we found that the LTR of *FERV1*, which was present in *White* and *white spotting* alleles at white locus in the first intron of the *KIT* gene in the genome of a domestic cat with white skin, contains a repeated CTCF-binding site. This finding suggests that the transcription from the 1st exon and that from the 2nd exon are separately regulated by the presence of the promoter and insulator activities of this LTR, which provided the mechanism for d1KIT production.

Additionally, the amount of *d1KIT* transcript was found to exhibit a significant negative correlation with the expression levels of *SOX10*, as well as strong negative correlations with genes that induce differentiation into melanocytes. These results suggest that the insulating activity at the white locus, specifically the LTR of *FERV1*, resulted in the production of d1KIT and subsequent inhibition of the *SOX10* expression, which was crucial for melanocyte formation.

Notably, Sox10 mutations has been known to cause deafness in miniature pigs, leading to degeneration and a significant decrease in the density of utricular hair cells, cochlear abnormalities, and saccular hypofunction (Qi et al., 2022). Therefore, when the aforementioned LTR activities occur throughout the body, it is conceivable that the entire skin becomes covered with white fur and deafness is induced. We hypothesized that this is the mechanism underlying dominant white cat deafness.

However, in white-spotted cats, such as the individual from which the cells sampled in this study were derived, since the full-length of *FERV1* is present at white locus, LTR activity and its influences on the *KIT* gene expression might be limited depending on cell lineages or cell types. We also anticipate that the remaining regions of *FERV1*, other than the LTR, are involved in its regulation. However, the precise mechanism remains unclear, and its elucidation is an issue for future research.

Furthermore, for both dominant white and white spotted cats, it was difficult to show a causal relationship between d1KIT production and *SOX10* expression only in the present study. Such relationship should be confirmed by future experiments.

In this study, only one replicate per skin cell color was available for RNA-seq data, which limited the application of conventional methods to assess differentially expressed genes (DEGs). Consequently, we provide a projected scenario based on an analysis that can be conducted using such limited data. Unfortunately, the availability of experimental data for *F. catus* (domestic cats) is currently significantly limited compared to that for humans and canines, which are also common companion animals. For instance, the number of *F. catus* RNA-seq samples registered in the public database COXPRESDB (https://coxpresdb.jp/RNA-seq) (Obayashi et al., 2023), which allows the search of co-expressed genes, is only 267, whereas the numbers for humans and canines are much larger (235,187 and 1,361, respectively). Additionally, the specific roles of each gene in the KEGG pathways obtained through enrichment analysis have rarely been directly confirmed in domestic cats, with most predictions being extrapolated from results in humans and mice.

To advance the field of basic veterinary medicine and support clinical veterinary practices not only for domestic cats, but also for various domestic and companion animals, it is crucial to accumulate a substantial amount of diverse basic data.

Author Contributions

AA, KW and NS conceived and designed the study. AA and DK performed the analyses. AA and NS drafted the manuscript.

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Table 1: List of squamous cell carcinoma promoting and suppressor SCDEGs.

Carcinoma promoting					Carcinoma suppressor	
AKT3	CRB3	GNB4	MGST1	VEGFC	AIFM2	LAMA2
ATP6V1C2	CXCL8	GNG11	MMP9	WNT10A	BBC3	MFNG
CAMK2A	E2F1	HEY1	PDGFA	WNT10B	CD82	MGST2
CCNA1	ERBB2	IBSP	PDGFB	WNT16	CDKN1A	PARD6G
CCNA2	FGF1	IGF1	PGF	WNT2	CDKN2A	PERP
CCNB2	FGF2	IL7R	PIM1	WNT2B	CHEK1	PIK3R1
CDK1	FGFR1	ITGA5	PLCB1	WNT3A	EDNRB	RASGRP2
CDK2	FGFR2	ITGA7	PRKCI	WNT4	ESR1	RASSF5
CEBPA	FGFR3	ITGB4	PTGS2	WNT6	FLT3LG	RXRG

CKS2	FLAI-K	ITGB6	ROCK2	WNT7B	FOXO1	SESN1
COL1A1	FLT3	JUN	RRM2		GADD45G	TGFBR2
COL4A1	FLT4	JUP	SERPINE1		GSTM3	TP53
COL4A5	FOS	KIT	SPI1		ITGA8	ZBTB16
COL4A6	FZD10	LAMA4	VEGFB		ITGA9	

Table 2: Number of squamous cell carcinoma promoting and suppressor genes, which are more strongly expressed, for cells T (P_T and S_T), O (P_O and S_O), and W (P_W and S_W), and *P*-values of exact test of goodness-of-fit between promoting-suppressor genes of each case and the theoretical ratio. *P*-value of Fisher's exact test for a 2×3 table with P_T, S_T, P_O, S_O, P_W, and S_W was obtained ~ 0.1296.

	T	O	W	Theoretical ratio
Carcinoma promoting genes	$P_T = 27$	$P_O = 32$	$P_W = 31$	18994
Carcinoma suppressor genes	$S_T = 17$	$S_o = 8$	$S_W = 9$	11295
P-value	0.7584	0.02246	0.05194	

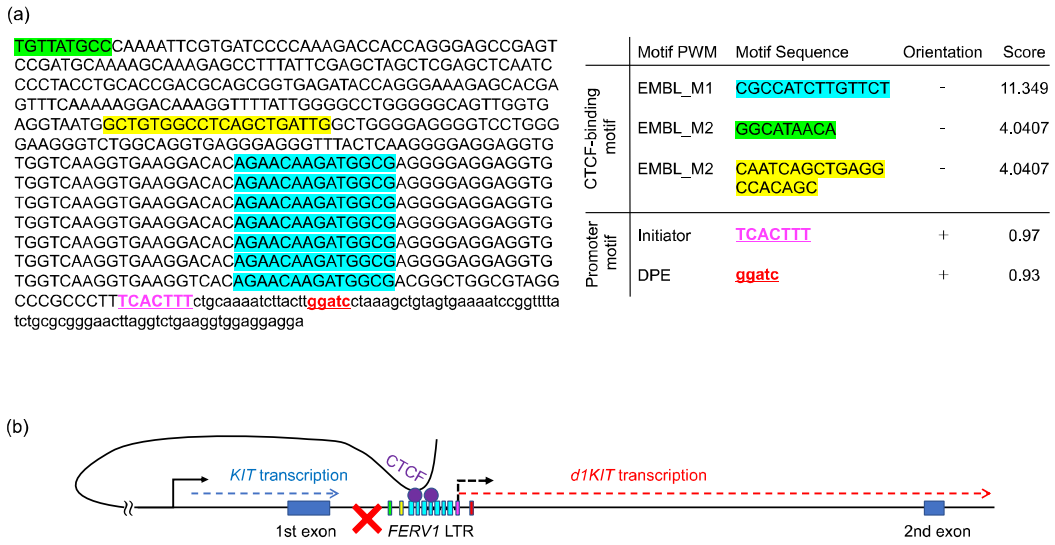

Figure 1: Positions of motif sequences with higher scores of CTCF-binding and core promoter activity in *FERV1* LTR in the first intron of the *KIT* gene. Detailing position and sequence of each CTCF-binding motif (cyan, green, and yellow shadowed) and

each core promoter motif (magenta and red texts) (a), and illustration of inferred promoter and insulator activities of *FERV1* LTR (b). Capital letters indicated the sequence in LTR and lower case letters indicated outside of LTR.

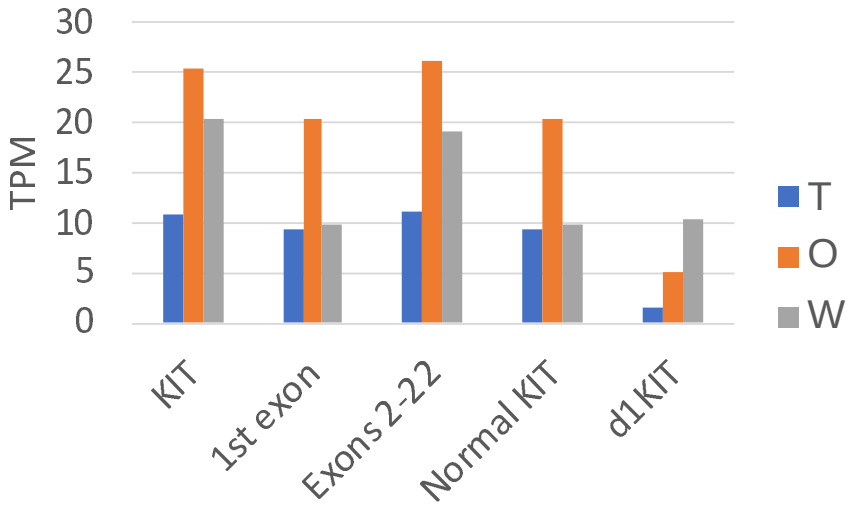

Figure 2: TPM of the following: entire KIT, 1st exon of KIT, averages over 2nd ~ 22nd exon, normal KIT, and d1KIT of T, O, and W cells. Values indicate TPM and length of each green bar is proportional to TPM.

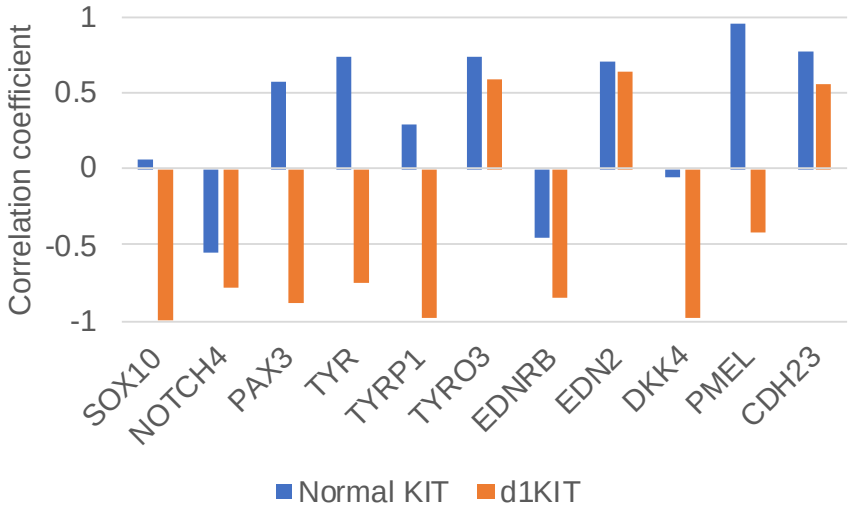

Figure 3: Correlation coefficients between TPMs of typical SCDEGs that are involved in melanoblast and melanocyte formation and TPM of normal KIT (Blue) or d1KIT

(Orange). P-values of correlation coefficient between normal *KIT* and *PMEL* ~ 0.20302, that between *d1KIT* and *SOX10* ~ 0.01876, that between *d1KIT* and *Dkk4* ~ 0.09968, and that between *d1KIT* and *TYRP1* ~ 0.12763.

Supplementary Materials

Supplementary Table S1: TPM of each gene in each cell type: T, O, and W.
Table_S1.xlsx

Supplementary Table S2: TPM of each exon in the KIT gene in T, O, and W.
Table_S2.xlsx

Supplementary Table S3: List of SCDEGs and their TPMs in each cell, T, O, and W.
Table_S3.xlsx

Supplementary Table S4: Results of pathway enrichment analysis of SCDEGs
Table_S4.xlsx

Supplementary Table S5: List of pathways and roles of SCDEGs that are related to squamous cell carcinoma (top) and references (bottom).
Table_S5.xlsx

Supplementary Table S6: List of genes that are related to melanoblast and melanocyte formation (top) and references (bottom).
Table_S6.xlsx