J Vet Sci. 2023 Sep;24(5):e71
https://doi.org/10.4142/jvs.23036
pISSN 1229-845X·eISSN 1976-555X

Journal of Veterinary Science

Review

Molecular and Cellular Biology

Check for updates

Use of cutting-edge RNA-sequencing technology to identify biomarkers and potential therapeutic targets in canine and feline cancers and other diseases

Youngdong Choi †, **Min-Woo Nam** †, **Hong Kyu Lee**, **Kyung-Chul Choi** *

Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, Cheongju 28644, Korea

OPEN ACCESS

Received: Feb 1, 2023
Revised: Jun 16, 2023
Accepted: Jun 18, 2023
Published online: Aug 28, 2023

***Corresponding author:**
Kyung-Chul Choi
Laboratory of Biochemistry and Immunology, College of Veterinary Medicine, Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju 28644, Korea.
Email: kchoi@cbu.ac.kr
https://orcid.org/0000-0002-1925-1198

†Youngdong Choi and Min-Woo Nam equally contributed to this work.

ABSTRACT

With the growing interest in companion animals and the rapidly expanding animal healthcare and pharmaceuticals market worldwide. With the advancements in RNA-sequencing (RNA-seq) technology, it has become a valuable tool for understanding biological processes in companion animals and has multiple applications in animal healthcare. Historically, veterinary diagnoses and treatments relied solely on clinical symptoms and drugs used in human diseases. However, RNA-seq has emerged as an effective technology for studying companion animals, providing insights into their genetic information. The sequencing technology has revealed that not only messenger RNAs (mRNAs) but also non-coding RNAs (ncRNAs) such as long ncRNAs and microRNAs can serve as biomarkers. Based on the examination of RNA-seq applications in veterinary medicine, particularly in dogs and cats, this review concludes that RNA-seq has significant potential as a diagnostic and research tool. It has enabled the identification of potential biomarkers for cancer and other diseases in companion animals. Further research and development are required to maximize the utilization of RNA-seq for improved disease diagnosis and therapeutic targeting in companion animals.

Keywords: Transcriptomics; Companion animals; veterinary medicine; oncology

INTRODUCTION

Understanding the transcriptomes produced by genomes is important for interpreting the fundamental biological processes in different organisms [1]. Transcriptomic changes imply the activation of specific pathways in response to environmental stress, which helps identify complex bionetworks in different cell types. Since the RNA-sequencing (RNA-seq) technique was first used in 2005, analyzing transcriptomes has become more comprehensive and extensive [1]. RNA-seq is a powerful tool, with significant advantages compared to traditional technologies with higher sensitivity, accurate unbiased quantification of massive expression profiles of genes, and a wider dynamic range [2]. Most cancer-related drug research has focused on protein-coding genes which represent only 3% of the human genome.

ORCID iDs

Youngdong Choi
https://orcid.org/0000-0001-5121-1332
Min-Woo Nam
https://orcid.org/0000-0002-8913-0893
Hong Kyu Lee
https://orcid.org/0000-0001-6240-1553
Kyung-Chul Choi
https://orcid.org/0000-0002-1925-1198

Author Contributions

Conceptualization: Choi Y, Nam MW, Choi KC; Funding acquisition: Choi KC; Project administration: Choi KC; Supervision: Choi KC; Writing - original draft: Choi Y, Nam MW; Writing - review & editing: Lee HK, Choi KC

Conflict of Interest

The authors declare no conflicts of interest.

Funding

This work was supported by the Basic Research Lab Program (2022R1A4A1025557) through the National Research Foundation (NRF) of Korea, funded by the Ministry of Science and ICT. In addition, this study was also supported by "Regional Innovation Strategy (RIS)" through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (MOE; 2021RIS-001) in 2023.

However, RNA-seq can identify differently expressed transcriptomes, splice variants, and non-coding RNAs (ncRNAs), which have long been known as untranslated genes and have not been well understood until recently [3]. The ncRNAs are largely categorized based on their characteristics into short regulatory ncRNAs and long ncRNAs [4]. The classes of short regulatory ncRNAs include microRNAs (miRNAs), piwi-interacting RNAs (piRNAs), and small interfering RNAs (siRNAs) [5-7], while long ncRNAs include linear long non-coding RNAs (linear lncRNAs) and circular RNAs (circRNAs) (**Fig. 1**) [8]. However, with the development of computational data analysis techniques, the role of ncRNAs has been further elucidated [9]. The discovery of the functions of ncRNAs has provided a better understanding of cancer genetics and epigenetics, and the ncRNAs have the potential to serve as predictors of anticancer drug sensitivity, going beyond the limitations of protein-coding genes [10,11].

With the rising interest in companion animals in recent decades, various animal cancers and diseases have been trying to be elucidated via RNA-seq (**Fig. 2**). RNA-seq is a promising tool in animal research that can be used to discover a wide range of biological responses, including cancer research, epigenetic regulation, tissue-specific gene expression patterns at molecular level. Studying animal cancers and diseases can be very useful in understanding human diseases. Animals and humans share many common genes and physiological functions related to disease occurrence in both species. Moreover, animal research is faster and more cost-effective than human research [12,13].

This review provides a comprehensive view of RNA-seq studies in both dogs and cats, specifically focusing on identifying potential therapeutic targets of cancers and diseases. Additionally, our review incorporates the latest advancements in veterinary medicine and makes it a valuable resource for researchers in harnessing the potential of RNA-seq for disease diagnosis in companion animals.

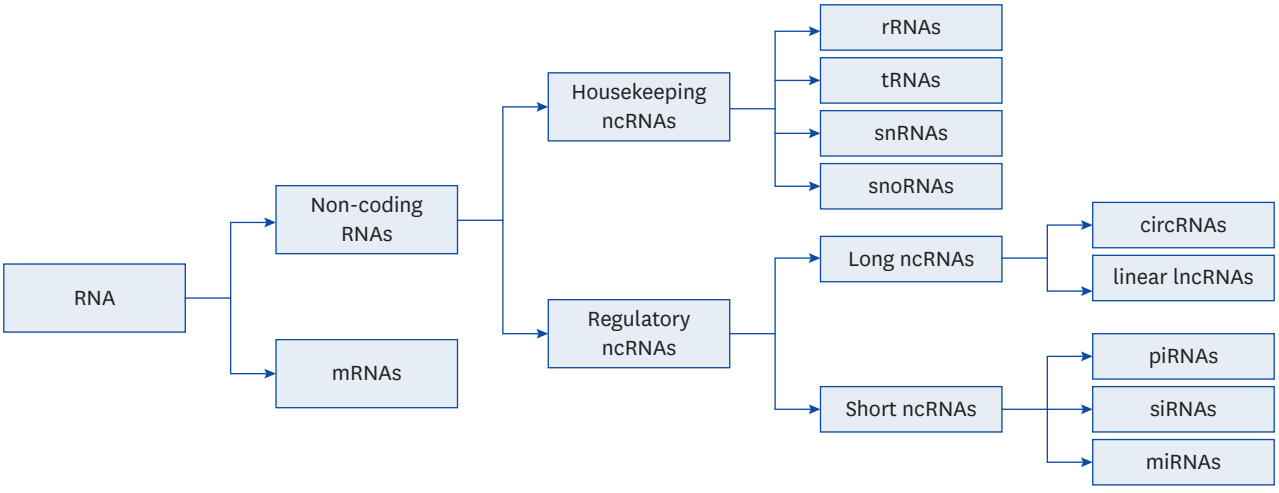

Fig. 1. Classification of RNA lineage. The proposed classification of RNA is shown. RNA is largely categorized into two classes: mRNAs and ncRNAs. In ncRNAs, regulatory ncRNAs are composed of long ncRNAs and short ncRNAs. Long ncRNAs include linear lncRNAs and circRNAs while the short ncRNAs include piRNAs, siRNAs, and miRNAs.
mRNAs, messenger RNAs; ncRNAs, non-coding RNAs; linear lncRNAs, linear long non-coding RNAs; circRNAs, circular RNAs; piRNAs, piwi-interacting RNAs; siRNAs, small interfering RNAs; miRNAs, microRNAs.

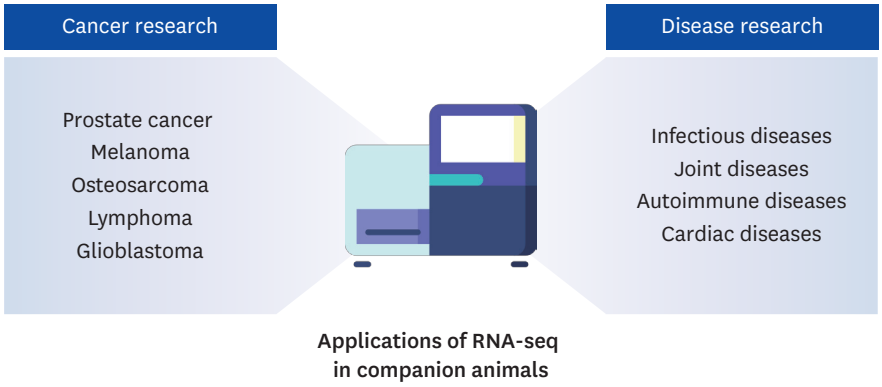

Fig. 2. Application of RNA-seq in companion animals. RNA-seq applied to cancer research includes the investigation of prostate cancer, lung cancer, melanoma, osteosarcoma, lymphoma, and glioblastoma. Infectious diseases, joint diseases, autoimmune diseases, and cardiac diseases have also been investigated.
RNA-seq, RNA-sequencing.

RESEARCH OF CANCER VIA RNA-seq

RNA-seq has been applied to investigate the pathogenesis of cancers, identify cancer-related pathways, characterize cancer progression, and identify biomarker candidate genes in dogs and cats [14,15]. This high-end technique is also used to analyze the effects of anti-cancer drugs in animals [16].

Most RNA sequences originate from protein-coding genes, wherein the information in DNA is transferred to a messenger RNA (mRNA) molecule by transcription. The mRNAs are exported to the cytosol and translated into proteins [17]. The analysis of protein-coding genes can be performed by whole transcriptome sequencing, which provides an overview of the complete gene expression landscape [18].

Previous studies have shown that the analysis of the comprehensive transcriptomic characterization of the canine prostate cancer cell line revealed distinct expression patterns between the primary epithelial cancer cells and metastatic tumors [19].

Prostate cancer

Prostate cancer cell lines exhibiting a mesenchymal marker, vimentin (VIM), and low expression of epithelial markers such as cytokeratin 8 and 18 demonstrated invasive characteristics. Each prostate cancer cell line also featured a unique individual expression of the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) signaling pathway, which is frequently targeted for cancer treatment [20].

In prostate cancer, the upregulation of five miRNAs and the downregulation of 14 miRNAs were associated with distant metastasis [21]. Specifically, two miRNAs (miR-95 and miR-18a) were overexpressed and induced cancer progression and malignant transformation [22].

Urothelial carcinoma

Other RNA-seq studies sought to understand the progression of canine invasive urothelial carcinoma (iUC). Recent findings have shown that 2,531 genes were differentially expressed in canine iUC [23]. Of those, tumor protein 53 (TP53) which is known as a tumor-suppressor gene involved in cell cycle arrest and the apoptosis pathway was downregulated in canine

iUC [24], whereas erythroblastic oncogene B 2 (ERBB2) was upregulated [25]. Interestingly, the investigation of differentially expressed genes (DEGs) in canine iUC has revealed several mutated genes [26]. A mutation of the fibroblast growth factor receptor 3 (FGFR3) oncogene was found to be present in non-invasive canine UC [26]. Another study has shown an increased expression of programmed death-ligand 1 (PD-L1) in canine UC. The gene governs different pathways linked to the inflamed tumor microenvironment. Blocking the programmed cell death protein 1/PD-L1 immune checkpoint reduces immunosuppressive signals found within the tumor microenvironment in canine bladder cancer [27].

Melanoma

Canine melanoma is a malignant cancer with a poor prognosis in dogs [28]. Downregulation of mitogen-activated protein kinase (MAPK) and the PI3K/AKT pathways leads to the progression of melanomas [29]. Recently, the MAPK and PI3K/AKT pathways have been targeted with mitogen-activated protein kinase 1/2 (MEK1/2) inhibitors which exhibited significant inhibition of tumor growth in canine melanomas [29]. Another study explored the differential expression of a number of genes in melanomas. A developing melanoma actively promotes collagen metabolism and extracellular matrix (ECM) remodeling as well as enhances cell proliferation, leading to metastasis through the action of multiple genes [30]. The results demonstrated that the nitric oxide synthase 2 (NOS2) gene, known to induce the metastatic ability of canine melanoma, was upregulated [30,31].

Several studies have shown that cancer pathogenesis and ncRNAs are closely related in canine oral melanoma. MiR-450b was found to be overexpressed in canine melanoma metastatic cells [32]. The upregulation of miR-450b induces the increase of matrix metalloproteinase-9 (MMP9) expression which is required for tumor metastasis. Also, miR-450b suppresses the expression of bone morphogenetic protein-4 (BMP4), which is known to decrease the activation of MMP9 [33].

Osteosarcoma

The intratumoral heterogeneity of canine osteosarcoma (OSA), a malignant and metastatic neoplasm was investigated to unravel the pathogenesis with transcriptomic expression patterns [34]. Fifteen pathways that had not been identified earlier, were confirmed to be related to the promotion of metastasis by using single-cell RNA sequencing (scRNA-seq) [35]. The scRNA-seq is used to examine the information on the sequence of individual cells and characterize the cells from early developmental stages to provide the differences in cellular properties and functions of a single abnormal tumor cell in comparison to a normal one [36]. The interest in understanding single-cell heterogeneity has increased. This assessment of different gene expressions between individual cells has provided the possibility of identifying rare cell populations that cannot be detected from a bulk RNA-seq [37]. In canine OSA, the expression of miR-9 was upregulated, and high miR-9 expression was associated with shorter survival compared to a low miR-9 expression [38]. The activation of miR-9 was partly mediated by the upregulation of gelsolin and the miRNA involved in its expression, which contributes to the malignant behavior of canine OSA [39].

These findings contribute to a better understanding of the expression landscape of animal cancers. Therefore, it is necessary to accumulate sequencing data and continue to perform genomic comparisons to identify novel targets. Discovering potential therapeutic candidates is the key to anti-cancer drug development and personalized medicine in the future.

Mammary tumors

Mammary tumors have been introduced as one of the major solid tumors in canine, and the studies of canine mammary tumors (CMTs) were also performed with whole genome sequencing [40]. In some studies of CMTs, PIK3CA mutations have been identified as key mutations in CMTs [40,41]. The mutations of PIK3CA have been introduced as cancer driver genes [42]. On the other hand, hundreds of circulating miRNAs have been identified from the studies on canine mammary tumors [43]. Also, the expressions of miR-29b and miR-19b were upregulated in malignant mammary tumors. It has been suggested that the evaluation of several miRNAs is needed to understand the pathology of canine mammary cancers [44].

Lymphoma

One of the common hematopoietic tumors in canines has been characterized by the expressions of miRNAs in the lymph nodes and plasma. The expressions of miR-155 and miR-21 were decreased in the lymph nodes of canines with T-cell lymphoma and miR-155 was overexpressed in B-cell lymphomagenesis [45]. Therefore, miR-21 and miR-155 have been targeted using antisense oligonucleotides to treat specific veterinary cancers [46]. Among the two miRNAs, miR-21 has been introduced as a target to induce the upregulation of phosphate and tensin homologue (PTEN) which is related to apoptosis [47].

Glioblastoma

In addition, increasing efforts are being made to discover and characterize feline miRNAs. The characterization of feline miRNAs has revealed that 88 miRNAs were specifically expressed in the brain tissue, and the role of miR-219 has been confirmed in the proliferation and migration of glioma cells [48]. MiR-124 was introduced to inhibit the proliferation of glioblastoma and activate the differentiation of brain cancer stem cells [49]. Also, the expression of miR-192 increased in all feline carcinomas and some B-cell lymphomas. Thus, the study of cancers of companion animals using RNA-seq can provide data on gene expressions. The analysis of these complex data sets will assist in a better understanding of tumor etiology and mechanisms [50].

RESEARCH OF OTHER DISEASES VIA RNA-seq

Given the vast presence of companion animals, veterinary research using RNA-seq is a significant way to comprehensively understand animal diseases and identify candidate biomarkers for diagnosis.

Infectious disease

Coronaviruses are zoonotic and can infect different vertebrates causing respiratory symptoms [51]. The canine respiratory coronavirus was first revealed in dogs with canine infectious respiratory diseases (CIRDs), and its characteristics are still being examined [52]. Some studies have revealed the epidemiology and genetic variation of canine respiratory coronavirus in Swedish dogs [53]. The study revealed the diversity of coronaviruses in dogs which can lead to determining the genetic variations and help clarify the clinical features of canine coronaviruses [53]. Another study has shown that the canine respiratory coronavirus utilizes the transmembrane protease serine 2 (TMPRSS2) to activate the coronavirus receptor for entry. This is similar to the features of the middle east respiratory syndrome coronavirus (MERS-CoV) [54].

Among the other infectious diseases in canines, the pulmonary immune responses caused by canine distemper virus infection in dogs have been investigated. The expressions of interferon-related genes were increased, leading to activation of the interferon 1 (IFN-1) pathways which are critical contributors to immune response [55]. Another infectious disease in dogs is the H5N1 virus infection, a severe lung disease, and the associated genes are known to be highly mutated [56,57]. H5N1 infection in dogs causes differential expressions of cell surface receptors (CD59) and protein-coding genes (RIB43A domain with coiled-coils 2 [RIBC2] and coiled-coil domain containing 33 [CCDC33]) [58].

Feline infectious peritonitis has been investigated to understand the feline coronavirus and 458 DEGs were seen to be related to the biology of coronavirus. Specifically, the expression of angiotensin-converting enzyme 2 (ACE2), which is known as the coronavirus receptor, was detected [59,60]. The studies on infectious diseases in cats also include the analysis of feline calicivirus (FCV), which is a common viral pathogen in cats and causes respiratory tract disease [61,62]. RNA-seq studies have revealed that the junctional adhesion molecule-1 gene, which is a cellular binding molecule of the FCV, was not expressed in FCV-infected cats [63,64].

Joint disease

An analysis of canine osteoarthritis (OA) showed that miR-542 and miR-543 were upregulated [65]. The upregulation of the two miRNAs was found in the synovial tissue of canine OA joints and was also involved in the inflammatory response [65]. As miRNAs in the synovial fluid have been known to be stable, the increased expression of the two may be indicative of canine OA [66].

Autoimmune disease

Canine histiocytic proliferative disorders are mostly found in dendritic cells [67]. Histiocytosis (HS) is an inflammatory disease that contributes to immune dysregulation [68]. Several pathways, such as the MAPK signaling pathway, are activated in canine histiocytic diseases [69]. It has also been confirmed that tyrosine-protein phosphatase non-receptor type 11 (PTPN11) mutations which are related to the dissemination of histiocytic sarcoma, play a key role in canine HS [70]. Furthermore, studies targeting MAPK signaling in canine HS have led to the development of the therapeutic drug Palbociclib [71,72].

Cardiac disease

Novel pathways and mechanisms in dogs with dilated cardiomyopathy (DCM) have also been researched, revealing that 86 genes involved in energy metabolism and cardiac function are differentially expressed in dogs with DCM. In particular, the expression of the natriuretic peptide B gene, found in dogs with late-stage heart disease, was increased [73].

DISCUSSION

High throughput sequencing technologies broaden the boundaries of molecular and cellular dynamics. As with other fields of biology, veterinary medicine has effectively embraced sequencing technologies that are used in human health. Advanced RNA-seq technologies such as scRNA-seq result from the technological integration of genomics, transcriptomics, proteomics, and computer sciences. These have made it possible to observe the individual landscape of each cell in a malignant tumor or diseased tissue [74].

As interest in companion animals increases, their diseases and cancers are also being extensively studied. A few decades ago, the diagnosis of veterinary diseases was only by judgments based on clinical symptoms and *in vitro* tests. Such processes have a significant drawback in that it takes time to diagnose the disease. When highly infectious diseases spread to livestock, it leads to considerable economic losses. To address these concerns and economic issues, the transcriptome analysis of mRNA and ncRNA has been adapted to diagnose veterinary diseases rapidly. Furthermore, the technique has also been used to identify targeted therapeutic biomarkers [75].

Despite remarkable advances in the RNA-seq technique, it still has some fundamental limitations. One of the biggest challenges is the selection of the appropriate biomarkers from a large number of differentially expressed genes, which could save expenses and time. Most of the biomarkers identified have not yet been developed into practical diagnostic kits. Large sample sizes and trials under various conditions are required to commercialize these products, and ongoing funding is essential to ensure that selected candidates have real clinical value.

Taken together, the application of RNA-seq in companion animal research is developing rapidly with the identification of many biomarkers (**Table 1**), such as mRNAs and ncRNAs, which are potent regulators of diverse oncogenic pathways in veterinary medicine.

Table 1. Coding genes and non-coding genes as biomarkers

Diagnosis	Biomarkers	References
Canine		
Cancer		
Prostate cancer	VIM, cytokeratin 8, cytokeratin 18, miR-95, miR-18a	[20, 22]
Urothelial carcinoma	TP53, ERBB2, FGFR3, PD-L1	[24, 25, 26]
Melanoma	MEK1, MEK2, NOS2, miR-450b	[33, 76, 77]
Osteosarcoma	miR-9	[39, 78]
Mammary tumor	PIK3CA, miR-29b, miR-19b	[40, 44]
Lymphoma	miR-155, miR-21, PTEN	[47, 79, 80]
Other diseases		
Coronavirus infection	TMPRSS2	[54]
Distemper virus infection	IFN-1	[55]
H5N1 infection	CD59, RIBC2, CCDC33	[58]
Osteoarthritis	miR-542, miR-543	[65]
Histiocytic proliferation disorder	PTPN11	[70]
Dilated cardiomyopathy	natriuretic peptide B	[73]
Feline		
Cancer		
Glioblastoma	miR-219, miR-124, miR-192	[49]
Other diseases		
Coronavirus	ACE2	[59, 60]
Calicivirus	JAM-1	[63, 64]

VIM, vimentin; TP53, tumor protein 53; miRNAs, micro RNAs; ERBB2, erythroblastic oncogene B 2; FGFR3, fibroblast growth factor receptor 3; PD-L1, programmed death-ligand 1; MEK, mitogen-activated protein kinase kinase; NOS2, nitric oxide synthase 2; PI3KCA, phosphatidylinositol-3-kinase catalytic subunit alpha; PTEN, phosphate and tensin homologue; TMPRSS2, transmembrane protease serine 2; IFN-1, interferon-1; RIBC2, RIB43A domain with coiled-coils 2; CCDC33, coiled-coil domain containing 33; PTPN11, phosphate non-receptor type 11; ACE2, angiotensin-converting enzyme 2; JAM-1, junctional adhesion molecule-1.

https://doi.org/10.4142/jvs.23036

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