Review
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Comparative aspects of microRNA expression in canine and human cancers

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MicroRNAs (miRNAs) have important roles in all biological pathways in multicellular organisms. Over 1,400 human miRNAs have been identified, and many are conserved among vertebrates and invertebrates. Regulation of miRNA is the most common mode of post-transcriptional gene regulation. The miRNAs that are involved in the initiation and progression of cancers are termed oncomiRs and several of them have been identified in canine and human cancers. Similarly, several miRNAs have been reported to be down-regulated in cancers of the two species. In this review, current information on the expression and roles of miRNAs in oncogenesis and progression of human and canine cancers, as well the roles miRNAs have in cancer stem cell biology, are highlighted. The potential for the use of miRNAs as therapeutic targets in personalized cancer therapy in domestic dogs and their possible application in human cancer counterparts are also discussed.

Keywords: dogs, gene expression, microRNAs, neoplasms, stem cells

Introduction

The dog not only shares its environment with its best friend, man, but they also share similar diseases. Several infectious and non-infectious diseases with remarkably similar clinical and pathological characteristics have been reported in both dogs and humans, which indicates the value of the dog as a large animal model of human diseases. Cancer affects both humans and dogs with similar clinical and pathological features. Diagnoses of these cancers in humans and dogs follow the same approach, which includes history taking, cytology, imaging, histopathology, and, in some cases, immunohistochemistry. Surgical excision followed by adjuvant chemotherapy and radiotherapy is the recommended therapeutic procedure for most cancer treatments. Radiation therapy is not commonly used as the primary therapy in the management of canine cancers, except in nonresectable tumors or in incomplete resection of large tumors with poor clean achievable margins [52]. The advent of the genomic era has redirected cancer therapy to a more 'personalized' approach, in which each patient is considered an individual case at the molecular level in order to determine the cancer-driving mutations and alterations in the cancer's genome and transcriptome that differ between individuals. This new approach allows the design of treatment regimens and approaches that best suit a particular patient based on targetable changes in cancer cells [47]. Altered changes in gene expression occur randomly in cancer cells, and such changes can provide cancer cells with an advantageous phenotype for further invasion of host tissues and development of metastases. Generally, gene expression changes within a cell could be due to transcriptional or post-transcriptional gene regulation. These changes could serve as a basis for the initiation and progression of cancer and must be identified and reversed or circumvented for successful anticancer therapy.

Oncogenesis or tumor initiation is the result of derailment of one or several signaling pathways involved in the maintenance of normal cell proliferation [82]. These changes generally affect the production and function of several proteins and factors synthesized by cells for use in normal physiologic activities, resulting in subsequent alteration of the phenotype of the affected cell or group of cells. Alterations in protein expression due to microRNA (miRNA) regulation of messenger RNA (mRNA) are described as the most common mode of post-transcriptional gene regulation, given that approximately 60%

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of all mRNAs are predicted to be under the control of miRNAs [5].

The miRNAs are short (20–24 nucleotides long) non-coding RNAs that have important roles in all biological pathways in multicellular organisms, including mammals [35]. Approximately 1,881 precursor and 2,588 mature human miRNAs have been identified, and many of them are conserved among vertebrates and invertebrates [1,31]. RNA polymerase II generates primary miRNA transcripts from transcriptional units embedded within the introns of protein-coding genes or as separate transcriptional units [15,23]. While still in the nucleus, the primary transcript, which is hundreds of nucleotides long, is trimmed by a multiprotein microprocessor complex, comprised of RNase III enzymes Drosha and Pasha to release the pre-miRNA hairpin [7]. The 70- to 120-nucleotide long pre-miRNA is then transported to the cytoplasm by the transport protein exportin 5 (XPO5) and the Ran-GTP complex [15,35]. The pre-miRNA is trimmed further in the cytoplasm to an 18- to 22-nucleotide long miRNA duplex. Together with protein kinase RNA activator (PACT), Argonaute 2 (Ago2), trinucleotide repeat-containing gene 6A (TNRC6A), transactivation response RNA binding protein (TRBP), and other proteins, this miRNA duplex forms the RNA-induced silencing complex (RISC) [7,15,35]. Helicase separates the RNA duplex to produce single-stranded mature miRNA, and the passenger strand is, in most cases, degraded [7,23,64]. The miRNA then tethers the RISC to the complementary sequence motifs (seed-site) in the target mRNA, mostly found within the 3′ untranslated regions [21]. The seed-site sequence is responsible for the specificity of miRNA-mRNA interactions [19]. When there is perfect complementarity, the mRNA becomes degraded by the RISC, while in the case of partial (non-perfect) complementarity, translation of mRNA by the ribosome is impaired [19]. Either way, effective impairment of post-transcriptional gene silencing is achieved. IsomiRs are miRNAs that differ by one or two nucleotides at their 5′ or 3′ ends and have been reported to target different mRNAs in different cell types [72], depending on the seed-site sequence of the target mRNA.

miRNAs are essential in processes such as cell-cycle control, cell proliferation, apoptosis, differentiation, migration, metabolism, and stem cell maintenance in both normal and cancerous cells [35,62]. Their involvement in oncogenesis and cancer progression have made them attractive targets in oncological research since their discovery almost three decades ago [3,57,67,71,74,85], with the activities of a few already being modulated for therapeutic applications [62,86]. In some cases, a single miRNA could target several hundreds of mRNAs. Therefore, an aberrantly expressed miRNA may affect a multitude of transcripts, which could influence cancer-related signaling pathways [22]. Overall expression of miRNAs has been reported to decrease in several human cancers compared to normal tissues, with poorly differentiated tumors having lower expressions of miRNAs compared to well-differentiated tumors and normal tissues [66]. Expression of miRNAs can be deregulated due to alterations in transcription, epigenetic modifications, or as a result of DNA copy number aberrations, leading to changes in miRNA-controlled gene regulatory networks [18,75]. Overall, miRNAs involved in cancers act as either tumor suppressors or oncogenes, with each category exerting its effect depending on its level of expression and activity [18,59].

In animal models of cancers, studies have illustrated the roles of miRNAs in oncogenesis and progression. These characteristics include the hallmarks of cancer such as sustaining proliferation independent of normal growth signals, evading suppressors of cellular growth, resistance to apoptotic signals, immortality, sustained angiogenesis, and ability to invade tissue and establish metastasis [32]. In these studies, the intricate nature of post-transcriptional gene silencing and the interplay between several miRNAs to control the major pathways involved in cancers were demonstrated [45,77]. In this review, current information on the expression and roles of miRNAs in oncogenesis and the progression of canine cancer are highlighted. The potential for miRNAs as therapeutic targets in personalized cancer therapy in domestic dogs and possible applications in human cancer counterparts are also discussed.

MicroRNA Expression and Regulation in Canine and Human Tumors

Studies performed on normal canine tissues have mostly focused on studying miRNA signatures in physiological processes that occur during normal development [20]. miRNAs that are involved in the initiation and progression of cancers are termed oncomiRs [57], and several have been identified in canine tumors [8,30,48]. Some miRNAs are tumor suppressors, and their expression inhibits the development of tumors by targeting mRNAs that initiate or maintain the cellular tumor phenotype. Several studies have assessed cohorts or single miRNAs that are down-regulated in various tumors of dogs [44,75,77]. Of importance when identifying deregulated miRNAs in cancers is to identify dogs and patients who can benefit from RNA interference (RNAi) or other means that will resume/inhibit the expression and activity of an miRNA. Ectopically expressed miRNA will target the specific oncogene that drives cancer initiation and/or progression, as previously successfully demonstrated in animal species including dog [14], whereas silencing of an overexpressed miRNA in cancer will allow the expression of mRNAs that can suppress tumor progression (tumor suppressor genes) [57].

Lymphoma

Canine lymphoma is a commonly diagnosed and studied tumor in dogs and has been suggested as a model for the study of human T-cell and B-cell lymphomas [77]. A miRNA microarray

kit targeting 95 human miRNAs (all of which are known to be involved in differentiation, apoptosis, and cancer) was used to identify several miRNAs that are dysregulated in canine T- and B-cell lymphomas compared with peripheral blood mononuclear cells (PBMC). The study identified up-regulation of miRNA-19a, -19b, and -17-5p in their tumor samples and cell lines. Both miRNA-19a and miRNA-19b were overexpressed in B-cell and T-cell tumors compared with PBMC. miRNA-19a + miRNA-19b belongs to the oncomiR-1 cluster (miRNA17-92), an oncogene that is known to effectively inhibit apoptosis. Hence, increased expression of miRNA-19a and miRNA-19b contribute to oncogenesis of canine T- and B-cell lymphomas. miRNA-17-5p is similarly reported to be overexpressed in canine B-cell lymphomas [48], although the study suggested a dual role for the oncomiR as a tumor suppressor due to its interaction with several known promoters of cellular proliferation and other transcriptional regulators. This suggestion of a dual role for miRNA-17-5p has been previously made in human breast cancer [34]. Another study reported the ability of miRNA-17-5p to differentiate between high- and low-grade samples of canine splenic lymphomas, with intermediate/high-grade samples expressing significantly higher levels of miRNA-17-5p [2]. This example demonstrates that a miRNA can be a tumor suppressor or an oncomiR depending on the mRNA whose translation the miRNA is regulating.

In addition, a tumor suppressive effect of miRNA-203 has been reported in canine lymphoma [77]. Decreased expressions of miR-181a, miRNA-218, and miR-203 were noted in the canine lymphoma cell lines and tissues investigated. miRNA-203 was similarly reported as down-regulated in human acute lymphoblastic leukemia and chronic myelogenous leukemia [12]. When expressed, miRNA-203 is shown to target the ABL1 mRNA and prevent the fusion of ABL1 and BCR to form the Philadelphia chromosome and inhibit cell proliferation. The ability to inhibit tumor cell growth or invasion following ectopic expression of down-regulated miRNAs indicates the value of this class of non-coding RNAs in the treatment of canine and human lymphoma and leukemias. miRNA-155 was down-regulated in canine splenic lymphomas [2] and in intermediate/high-grade samples compared with low-grade samples. In contrast, overexpression of miRNA-17-5p was correlated with the mitotic index of the tissues, suggesting the use of miRNA-17-5p (increased expression) and miRNA-155 (low to absent expression) as potential markers for canine lymphomas.

Mammary gland/breast cancers

In canine mammary gland tumors, up-regulation of miRNA-21 and miRNA-29b was reported in 6 mammary gland tumor tissues compared with that in 10 normal mammary gland tissues [8]. These oncomiRs (miRNA-21 and miRNA-29b) were also reported to be involved in the regulation of invasion, migration, and metastasis in human breast cancers [16]. However, in the canine study [8], miRNA-21 and miRNA-29b were involved in the inhibition of tumor cell apoptosis. The miRNA-21 oncomiR acts as a regulator of phosphatase and tensin homolog (PTEN) and tropomyosin-1 (TPM1) tumor suppressor genes [54,60], whereas miRNA-29b targets T-cell leukemia/lymphoma 1 (TCL1) and induces myeloid leukemia cell differentiation protein (MCL1) oncogenes [56,58] to regulate apoptosis, tumorigenesis, and cancer progression. This scenario of one miRNA targeting multiple pathways is an indication of the complexity of post-translational gene regulation by miRNAs and demonstrates how miRNA modulation for application in targeted therapy must be studied in detail to avoid unforeseen deleterious effects following the inhibition or ectopic expression of a particular miRNA with several mRNA targets.

A quantitative polymerase chain reaction array of tissues and cell lines from canine mammary gland tumors [44] revealed that among the 277 miRNAs investigated, miRNA-141 was overexpressed in the assessed cell lines and was experimentally validated to target tumor suppressor INK4 mRNA (INK4/CDNK2 family of tumor suppressors). Thus, miRNA-141 is considered a potent oncomiR. The work demonstrated a direct correlation between the expression of miRNA-141 and expression of its target mRNA (p16/INK4A). Two cell lines (CMT12 and CMT27) overexpressing the miRNA do not express its target mRNA, whereas the cell line that is negative for the miRNA expresses the tumor suppressor gene. In the same study [44], miRNA-21, -155, and -9 were overexpressed in both human and canine breast cancer tissues examined, whereas miRNA-31, -34a, and -143/145 were down-regulated in the tissues. It was also reported that miRNA-429 and -200c were overexpressed by more than 1000- and 150-fold, respectively, in the canine mammary tumor cell lines studied. Both miRNA-429 and miRNA-200c were predicted to target the ERBB receptor feedback inhibitor 1 (ERRI1), which is another tumor suppressor gene; hence, the overexpressed miRNAs are oncomiRs.

Similarly, in human breast cancer, miRNA-9 has been implicated in aiding metastasis by targeting the E-cadherin encoding mRNA, given that its expression levels are correlated with tumor grade and metastatic status [46]. E-cadherin silencing resulted in the activation of β-catenin signaling, which enhances angiogenesis via vascular endothelial growth factor activation. When miRNA-9 expression was induced in non-metastatic breast cancer cells, it resulted in the formation of lung micrometastasis in mice. Similarly, when the expression was silenced by using miRNA sponges in highly malignant breast cancer cells, no metastases were established in the mouse model. This finding indicates the role that the targeted miRNA-9 could play in cancer therapy by preventing the formation of metastases in both dogs and humans affected by solid tumors.

Although some miRNAs are important in tumorigenesis and

might not be required for tumor progression, others are required and expressed throughout all stages of tumor development and progression. miRNA-210 demonstrated a persistent and progressive increase in expression from normal canine mammary tissue to adenomas (7.01-fold) to non-metastasizing carcinomas (10.41-fold) to metastasizing carcinomas (10.72-fold) and, lastly, to metastatic tissue (19.63-fold) [80]. As explained in that report, miRNA-210 was previously reported to be up-regulated as a result of hypoxia in tissues due to the action of hypoxia-inducible factor in facilitating metabolic adaptation as a result of anaerobic conditions and has been termed a 'hypoxamir'. Given that the steady increase in miRNA-210 expression from normal to metastatic (larger mass) tissues could result from hypoxia developing in the tumor mass and its reported role in the formation of capillary-like structures, the authors hypothesize that miRNA-210 enhances tumor metastasis by neovascularization of the tumor mass. The activity of miRNA-210 in neovascularization has made it a potential diagnostic marker in human malignancies [40,70]. The interest in miRNA-210 is due to its presence in the peripheral circulation of patients as opposed to that of healthy controls. Diagnostically significant miRNA-210 serum levels have been recently reported in renal cell carcinoma [24], gastric cancer [87], and breast cancer [6]. However, such studies that look for miRNAs in the peripheral circulation have yet to be conducted in canine malignancies.

The same study that reported that miRNA-210 overexpression in canine mammary gland tumors drives metastasis [80], also reported a down-regulation of miRNA-125a in metastasizing canine breast carcinoma compared with that in adenomas and normal glands. This finding indicates the complex nature of miRNA-mediated post-transcriptional gene regulation, whereby more than one miRNA is transcriptionally altered, with some being down-regulated and others up-regulated to control the signaling of single or multiple pathways and achieve an advantageous phenotype in cancer cells. Another cohort of miRNAs that are dysregulated in Mardin-Darby canine kidney (MDCK) cells that have undergone the epithelial-mesenchymal transition (EMT) are miRNA-200 (miRNA-200a and -200b) and miR-429 [10] which are reported to down-regulate these miRNAs in MDCK cells. miRNA-200 (miRNA-200a and -200b) and miR-429 are encoded on the same polycistronic transcript and regulate the expression of zinc finger E-box-binding homeobox 1 (ZEB1) and survival of motor neuron protein-interacting protein 1 (SIP1), which also regulate the expression of miRNAs via a double-negative feedback loop aimed at regulating EMT in MDCK and human breast cancer cells [73]. The feedback mechanism in the expression of miRNAs must be in constant equilibrium to avoid the consequences of derailed expression of the miRNA or its target, such as tumor development.

Mast cell tumor

In canine mast cell tumors (MCT), miRNA-9 enhances tumor progression [25]. That study identified miRNA-9 overexpression as a characteristic of malignant cell lines and high-grade canine tumors compared with normal canine bone marrow-derived mast cells (BMMCs) and low-grade tumors, respectively. In an experiment using mouse BMMCs, the study demonstrated that forced overexpression of miRNA-9 in these cells significantly enhanced tissue invasion and metastasis by up-regulating CMA1 expression, which subsequently activated matrix metalloproteinases, an enzyme that degrades the extracellular matrix and triggers matrix remodeling. The involvement of miRNAs in tumor progression and, especially, metastasis make them attractive candidates for targeted therapy. This is particularly true in cases where resistance to conventional chemotherapeutic drugs have developed. MCTs are rare in humans (mastocytomas) [41], and reports related to miRNA expression in human MCTs were not found during literature searches.

Transitional cell/urothelial carcinomas

The expression and activities of miRNAs in canine bladder transitional cell carcinoma (TCC) have been studied [78]. In that study, miRNA-34a and -106b exhibited increased expression in TCC samples compared with normal bladder samples. In addition to miRNA-34a, -106b, -16, and -103b exhibited increased expression in TCC samples compared with samples from dogs with inflammatory lower urinary tract disease [78]. The report proposed the use of miRNA expression assays as a means of differentiating TCC from inflammatory disease in dog. In another recent study on human bladder cancer cells [83], miRNA-433 expression was involved in the regulation of proliferation, colony formation, invasion, and migration in bladder cancer cells, and its expression was down-regulated in most bladder cancer cases. Its involvement in EMT was via its regulatory effect on the c-Met/Akt/GSK-3β/Snail signaling pathway. Therapeutic expression of miRNA-433 would involve inhibiting bladder cancer's ability to invade tissue and establish metastasis, thereby increasing the chance of increased survival time of the patients. In another study in urothelial carcinoma [84], miRNA-142 and -200a detection in the urine of 207 patients compared with 144 controls was used to classify patients based on recurrence and invasion prediction. miRNA-200a expression predicted recurrence in patients with non-invasive bladder cancer, with patients with reduced miRNA-200a levels exhibiting an increased risk of recurrence compared with those with increased expression levels. On the other hand, miR-145 was able to distinguish patients from controls with a sensitivity of 84.1% and a specificity of 61.1% in patients with muscle-invasive TCC. miRNA-200a could serve as a non-invasive diagnostic/screening tool for TCC diagnosis and monitoring by assays for miRNAs in urine samples, as suggested elsewhere

for bladder cancer in general [40].

Osteosarcoma

A miRNA cluster at 14q32 is one of the largest miRNA-enriched loci, containing more than 40 different miRNAs [75]. This locus was investigated in canine and human osteosarcoma (OS) tissues. Both miRNA-134 and miRNA-544 were selected as representatives of the 14q32 locus miRNAs in the canine OS tissues, and miRNA-382 was selected for human OS tissues [65]. The work demonstrated correlations between decreased expressions of miRNA-134, -544, and -382 and aggressive behavior (including metastasis and shorter survival time) of tumors in affected dogs and patients. Sarver *et al*. [65] suggested that down-regulation of the 14q32 locus could be used as a marker of the biological behavior of OS in both dogs and humans, emphasizing the significance of the dog as a model of aggressive human OS, as previously suggested [68]. In another study on canine and human OS [76], the expression of the 14q32 locus inhibited the expression of c-Myc. The c-Myc gene codes for a transcription factor with a well-established role in multiple cancers, including canine cancers [9], and is involved in apoptosis, cellular transformation, and cell-cycle progression [27]. Decreased expression of the miRNA cluster in the 14q32 locus aids in tumor cell survival through inhibition of apoptosis via c-Myc, whereas re-expression of the cluster results in enhanced apoptosis in OS cells [75]. The identification of miRNAs that regulate its expression has presented more options in therapeutic targeting of the gene, which is still not entirely successful [69]. In another recent study [55], miRNA-196a expression was investigated in human and canine OS cell lines. The study analyzed tissues from 15 human OS patients and 20 canine OS cases and reported a decrease in miRNA-196a expression in human OS tissues compared with that in canine OS tissues. In addition, two human and one canine OS cell lines were investigated for miRNA-196a expression, and the results indicated a decreased miRNA-196a expression in all cell lines compared with expression in species-derived mesenchymal cells [55]. Upon ectopic expression of miRNA-196a in the cell lines, decreased cell proliferation and increased apoptosis were observed in one of the human cell lines (143B), but not in the other cell lines. The study also reported a transient decrease in cell motility in 143B human OS cell line and the canine OS cell line (DAN) and a more sustained (24 h) decrease in the other human OS cell line (MG63) with ectopic overexpression of the miRNA. The report identified annexin 1 as the miRNA-196a target as demonstrated by the effects observed in the cell lines and tissues investigated. miRNA-9 has been reported to be overexpressed in canine OS primary cells [26]. The expression of miRNA-9 resulted in enhanced invasion and migration in cells that enhance metastasis. These changes are the result of miRNA-9 interference with gelsolin (GSN), an actin filament involved in cytoskeletal remodeling of cells. The therapeutic targeting of miRNA-9 has the potential to decrease the metastatic capacity of canine OS cells.

Melanoma

Another miRNA that targets c-Myc mRNA that is down-regulated in canine tumors is miRNA-145 [51]. That study assessed the expression of miRNA-145 in canine melanoma tissues, as well as in canine and human melanoma cell lines, and reported a significantly decreased expression of this miRNA compared with that in normal canine oral mucosa. When miRNA-145 was ectopically expressed in melanoma cells, it resulted in the inhibition of cellular growth in both human and canine melanoma cells. This significant reduction in growth was partly attributed to the inhibition of c-Myc by miRNA-145. Moreover, a reduced migratory ability in canine melanoma (KMeC) and human melanoma (A2058) cells was attributed to the inhibitory effect that miRNA-145 exerts on the actin-bundling protein FASCIN1, clearly indicating the tumor suppressive function that miRNA-145 has on canine and human melanomas [51]. In a similar study by the same group [50], miRNA-203 and -205 were down-regulated in canine malignant melanoma compared with that in normal oral mucosa, and they associated the shorter survival time in the affected dogs with the down-regulation of miRNA-203 expression. In addition, decreased cell growth was observed in melanoma cells of both species and was attributed to the inhibitory effect of miRNA-205 on human epidermal growth factor receptor 3 (*errb3*) mRNA, thus making miRNA-205 a tumor suppressor and miRNA-203 a prognostic factor in canine malignant melanoma [50].

Leukemia

One of the most commonly diagnosed and studied hematologic malignancies in the dog is leukemia [29]. Chronic lymphocytic leukemia (CLL) is a form of leukemia that affects B- and T-lymphocytes and has been studied in both humans and canines, in which miRNA deregulations have been reported in CLL [11,13,29]. One of the studies described deregulation of miRNAs in canine CLL and suggested its usage as a differentiating factor between CLL immunophenotypes [29]. A potential use of the miRNA-125b, -150, and -155 gene expression rates to distinguish T-cell CLL, B-cell CLL, and normal blood samples has been suggested [29]. Preliminary evidence suggests that miRNA-125b was significantly overexpressed in B-cell CLL compared with that in T-cell CLL, whereas miRNA-155 was preferentially expressed in T-lymphocytes and overexpressed in some B-cell CLL. On the other hand, miRNA-150 exhibited increased expression in normal T-cells compared with that in normal B-cells and overexpression in canine T-cell CLL compared with that in B-cell CLL [29].

Hemangiosarcoma

miRNA-124 expression was investigated in canine

hemangiosarcoma clinical samples and cell lines [33]. The authors reported a down-regulation of miRNA-214 in all of the clinical samples and cell lines studied, indicating that miRNA-214 has a role in the pathogenesis of canine hemangiosarcoma. The study further revealed growth inhibition in the cell lines due to ectopic expression of the miRNA in a dose-dependent manner, confirming the role that miRNA has in the disease. In addition, miRNA-214 enhanced apoptosis in the cell lines by increasing the expression of p53-regulated genes. miRNA-214 regulates p53 by targeting the COP1 E3 ubiquitin-protein ligase, which negatively regulates p53 activity, thus making COP1/miR-214 modulation a potentially useful approach in the treatment of canine hemangiosarcoma and other malignant endothelial proliferative diseases [33].

MicroRNAs in Cancer Stem Cells

Cancer stem cells (CSC) or cancer-initiating cells are a subpopulation of cancer cells that have the ability to initiate or repopulate tumors and other neoplasia with new differentiated cancer cells [53]. Although CSCs only represent approximately 1% of a cancer cell population, they are responsible for metastasis and recurrence observed in most cancers after seemingly successful treatment [4,42]. These characteristics of CSCs are as a result of their ability to live for a long time, self-renew, resist apoptosis, undergo dormancy, and differentiate into tumor cells

Table 1. Summary of selected miRNA expression patterns and their role in regulating genes/pathways in canine and human cancers

miRNA	Expression	Gene/pathway regulated in human	Gene/pathway regulated in dog	Reference
miRNA cluster 14q32	Down-regulated	Regulates c-Myc expression to decrease metastasis and increase survival time in patients with OS	Regulates c-Myc expression to decrease metastasis and increase survival time in dogs with OS	[65]
miRNA-17-5p	Up-regulated	Enhance breast cancer cell proliferation via inhibition of AIB1 mRNA	Overexpressed in canine B-cell lymphomas. Increased expression identifies high-grade canine lymphomas	[2,34,48]
miRNA-21	Up-regulated	Enhance tumor Proliferation in breast cancer patients by targeting PDCD4	Overexpression targets PTEN and TPM1 in canine mammary tumors	[8,28]
miRNA-34	Down-regulated	Blocks tumor growth via repression of c-Met, Bcl-2 in lung cancer; regulates anti-apoptotic Bcl-2 and SIRT 1 in breast cancer stem cells; blocks Notch1 pathway in breast cancer	Lost in mammary tumor cells	[36,38,44,81]
miRNA-124a	Down-regulated	Inhibition of cyclin D kinase 6 in colon cancer cells	Enhances apoptosis by regulating p53 via COP1 E3 ubiquitin-protein ligase in canine hemangiosarcoma	[11,43]
miRNA-145	Down-regulated	Inhibits cell growth via c-Myc and reduced cell migration via FASCIN1in melanoma cells	Inhibits cell growth via c-Myc and reduced cell migration via FASCIN1in melanoma cells	[51]
miRNA-200	Down-regulated	Predicted recurrence in urothelial carcinoma patients	Regulate EMT via inhibition of ZEB1 and SIP1 expression in MDCK cells	[73,10,84]
miRNA-203	Down-regulated	Suppresses cell proliferation via ABL1 in leukemia cells	Suppresses cell proliferation in canine lymphoma cells	[12,77]
miRNA-205	Down-regulated	Down regulated in breast cancer	Down-regulated in malignant melanoma	[50,61]
miRNA-210	Up-regulated	Aids tumor development by increasing hypoxia-inducible factor expression	Overexpressed in canine mammary tumors	[61,79]
miRNA-221/ miRNA-222	Up-regulated	Tumor cell proliferation via inhibition of p27 in triple negative breast cancer	Cell proliferation in canine prostate cancer tissues	[37,61]

miRNA, microRNA; OS, osteosarcoma; mRNA, messenger RNA; PDCD4, programmed cell death 4; PTEN, phosphatase and tensin homolog; TPM1, tropomyosin-1; Bcl-2, B-cell lymphoma 2; EMT, epithelial-mesenchymal transition; ZEB1, zinc finger E-box-binding homeobox 1; MDCK, Mardin-Darby canine kidney.

[4,17]. In addition to these advantageous characteristics, one critical factor that makes CSCs important in anticancer therapy is the activity of their drug efflux pumps, which can expel most if not all chemotherapeutic agents that enter the cell, thereby making CSCs resistant to conventional chemotherapy. The overall effect of CSCs in a tumor cell population is that even after an apparent successful treatment that killed the differentiated tumor cells (the bulk of the mass), such tumors almost certainly recur due to the drug-resistant, self-renewing, and tumor-differentiating CSCs within the general population of tumor mass. The drug/radiotherapy resistant property of stem cells poses the greatest hurdle in successful anticancer therapies. There is undoubtedly a need for anticancer agents that can specifically target and kill CSCs in addition to killing differentiated cancer cells.

Differential miRNA expression was investigated in canine mammary gland tumor stem cell-like cells [63]. That report identified miRNA-451 as the most significantly up-regulated and miRNA-135b as the most significantly down-regulated in stem cell-like cancer cells compared with that in differentiated tumor cells. Overall, the down-regulated miRNAs were involved in regulating genes in the mitogen-activated protein kinase signaling pathway, specifically the transforming growth factor beta pathway [63]. Thus, the down- regulation of miRNA-135b is essential for EMT and tissue invasion as described previously [49].

A similar study reported that miRNA-1 is involved in breast CSC proliferation and migration [39]. That study reported an increased number of CSCs following inhibition of miR-1 expression in MCF-7 cells, and reduced numbers were noted following active expression of miR-1. miRNA-1 expression inhibited Wnt/β-catenin signaling by down-regulating frizzled 7 and tankyrase-2 expressions. miRNA-1 expression was also negatively correlated with the expressions of Oct4, Nanog, and c-Myc (stemness factors) as well as with cancer aggressiveness in 45 cases. Furthermore, enhanced miRNA-1 expression altered the nuclear to cytoplasmic ratio of β-catenin and its dependent luciferase activity and impaired proliferation, migration, and wound-healing abilities in breast CSCs *in vitro* and tumorigenicity *in vivo* compared with control cells. This single study demonstrated the significance of miRNAs in CSC function and how their therapeutic targeting could revolutionize cancer therapy.

miRNA-34a is down-regulated in both canine mammary gland tumors and human breast tumors [44]. miRNA-34a down-regulated the expressions of the anti-apoptotic B-cell lymphoma 2 (Bcl-2) and a member of silent information regulator (SIRT 1) (involved in cell metabolism and proliferation), making it a tumor suppressor miRNA [38]. However, in CSCs, miRNA-34a inhibits stem cell propagation and regulates tissue invasion and migration by targeting and down-regulating Notch1 [36]. In addition, miRNA-34 expression was negatively correlated with tumor stage, metastasis, and Notch1 expression in the breast cancer tissues examined. Moreover, induced expression of miRNA-34a in breast cancer cells reduced their ability to form mammospheres *in vitro*, reduced ALDH 1 (stemness factor) expression, and more importantly, reduced chemosensitivity to the therapeutic agent paclitaxel [36]. It is therefore evident that miRNAs are important in cancer therapy due to their potential dual roles of altering the phenotype (stemness and invasion) and improving the chemosensitivity of CSCs. The involvement of miRNAs in human and canine cancer is being investigated, and considerable information has already been documented (Table 1) [2,8,10−12,28,34,36−38,43, 44,48,50,51,61,65,73,77,79,81,84]. However, the involvement of miRNA in altering CSC characteristics is still not significantly elucidated and warrants further research.

Conclusions

miRNAs are the largest single class of post-transcriptional regulators of gene expression and have roles in virtually all cellular processes. Their involvement in cancer initiation, proliferation, invasion, and metastasis in human and canine cancers is well established, and their potential diagnostic, prognostic, and therapeutic significance has been exploited. However, more research is required to determine how miRNAs aid in maintenance of CSC phenotypes and chemoresistance in order to therapeutically benefit from silencing and/or reducing ectopic expression of specific miRNAs in both human and canine cancers. The similarities and differences between the two species in terms of miRNA function and dynamics in cancer should be exploited in the quest for more effective cancer prevention and treatment.

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Conflict of Interest

The authors declare no conflicts of interest.

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