Background

Urothelial carcinoma (UC) comprises approximately 2% of all naturally occurring cancers in dogs, making UC the most common urogenital cancer in this species. Many patients present with nonspecific signs, similar to other lower urinary tract diseases, such as stranguria, pollakiuria, and/or hematuria. Due to the trigonal location of most tumors in dogs and the metastatic potential of this disease, the goal of treatment is to improve quality of life rather than to cure the disease. It has been reported that the disease can be controlled in up to 75% of dogs; however, the ability to control the disease relies on early diagnosis. Studies using microRNAs (miRNAs) as diagnostic markers for canine UC have been recently reported. MiRNAs are small noncoding RNA that post-transcriptionally regulate RNA expression and play a significant role in carcinogenesis by influencing the expression of oncogenes and tumor suppressor genes. The stable nature of miRNAs and their ubiquitous presence in tissues and body fluids make them promising candidates for diagnostic and prognostic markers of disease. The differing expression pattern of miRNAs, where a single miRNA may be upregulated in one disease process and downregulated in another, complicates their use as markers of disease, demonstrating a need for thorough investigation and validation of miRNAs in both diseased and healthy patients prior to their use as diagnostic or prognostic markers.

Dogs are an established model of human muscle-invasive UC; however, evaluation of the similarity of the miRNA profiles and behavior between human and canine UC has not been established. However, there are also notable differences that suggest potential variations in the pathophysiologic mechanism of tumor development such as the location of the tumor in humans (apex of the bladder) versus dogs (trigone area), sex predilection (humans, males; dogs, females), and the predilection of dogs to form muscle-invasive tumors whereas humans tend to develop the less invasive form. MiRNAs have been frequently researched in human medicine as diagnostic tools, prognostic tools, and therapy targets for various diseases, and animal models are often used to elucidate the roles...
<table>
<thead>
<tr>
<th>miRNA</th>
<th>Canine (sample, regulation)</th>
<th>Human (sample, regulation)</th>
<th>Experimentally validated targets in humans (sample[s], investigated, target)</th>
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<tr>
<td>Let-7c</td>
<td>Urine, downregulated⁵</td>
<td>Urine, upregulated⁽⁵,⁶⁾/tissue, downregulated⁽⁴,⁵⁾</td>
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<td>miR-7-3</td>
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<td>Tissue, downregulated⁹</td>
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<td>miR-34a</td>
<td>Tissue, upregulated¹⁵</td>
<td>Tissue, downregulated⁶/cell culture, downregulated¹⁶</td>
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<td>miR-99a-2</td>
<td>Tissue, downregulated⁶</td>
<td>Tissue, downregulated¹⁸/cell culture, downregulated¹⁸</td>
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<tr>
<td>miR-103b</td>
<td>Blood and urine, downregulated⁵</td>
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<tr>
<td>miR-105a</td>
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<td>miR-106b</td>
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<td>miR-143/</td>
<td>Tissue, downregulated⁶</td>
<td>Urine, downregulated⁵⁵⁵⁶/tissue, downregulated²⁴²⁶²⁷²⁸⁴¹⁴²⁶³⁶³⁷</td>
<td>Tissue (in situ hybridization), PAI-1²⁴; cell culture, PI3K/AKT²⁵,³⁵; cell culture, MAPK²⁶ cell culture, IGF-1R²⁷ tissue and cell culture (immunoblot assay) KRT⁷²⁶; tissues and cell culture, PAK1²⁷</td>
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<td>miR-145</td>
<td>cluster</td>
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<td>miR-181a-1</td>
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<td>miR-214</td>
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<td>Tissue, upregulated²⁸</td>
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<td>miR-374b</td>
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<td>miR-429</td>
<td>Tissue, upregulated⁶</td>
<td>Tissue, downregulated⁴⁰⁴¹⁹⁹/tissue, upregulated¹⁹/cell culture, upregulated¹⁹</td>
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<td>miR-450a</td>
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<td>Tissue and cell lines, HMGB3/Wnt/β-catenin²² Not reported</td>
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<td>miR-582</td>
<td>Tissue, downregulated⁶</td>
<td>Tissue, downregulated⁵⁵/cell culture, downregulated⁵⁵</td>
<td>Tissue and cell culture (dual luciferase reporter and RNA pull-down assays), TTK⁶⁵ Cell lines, KCNN³³¹</td>
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<td>miR-802</td>
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<td>miR-874</td>
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<tr>
<td>miR-876</td>
<td>Tissue, downregulated⁶</td>
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Samples compared include tissue (bladder mucosa), urine, blood, and cell cultures. All samples were comparing patients or cell cultures with urothelial carcinoma (UC) to aired samples that had no evidence of disease. All comments on upregulation/downregulation are indicating UC in comparison to nondiseased samples. Verification of targets was, unless otherwise noted, done using a combination of luciferase reporter assay and western blot evaluation analysis.
of miRNAs in oncogenesis and progression, leading to the identification of parallels between miRNA expression in human pathologic states and their canine counterparts. Additionally, miRNAs are conserved across numerous species, including dogs and humans, where the function of miRNAs, their targets, and regulation are generally similar across all species studied. In addition, particularly in UC, several studies suggest an evolutionarily conserved molecular pathogenesis in tumor development and behavior. These similarities in miRNA expression support that canine UC may be a natural model to study human UC, but functional studies are lacking in the canine model.

In this review, we identify the miRNAs that are dysregulated in dogs and compare these miRNAs to the expression profiles and experimentally validated targets of miRNAs reported in the human literature as there are no studies available that experimentally validated miRNA targets in bladder cancer in dogs. Our goal is to identify similarities and highlight differences that can be evaluated further to better understand the pathophysiology and behavior of UC in dogs.

Methods

A search was performed on both PubMed and Google Scholar for the terms “canine” or “dog”; “urothelial carcinoma,” “bladder cancer,” “transitional cell carcinoma,” “TCC,” “MIBC,” “IMBUC,” or “BLCA”; and “miRNA” or “microRNA.” TCC is an abbreviation for transitional cell carcinoma, MIBC and IMBUC are abbreviations for muscle-invasive bladder cancer, and BLCA is an abbreviation for bladder cancer. The search was limited to publications in the English language. Publications were screened for potential applicability, and 3 reports were identified.

Inclusion criteria for this review were 1) the manuscript had to be an original peer-reviewed research manuscript (no reviews or meta-analyses were included in this study), and 2) all findings must be experimentally validated; studies relying on prediction software without further validation were not included in the scope of this review.

The reported dysregulated miRNAs were then compared with expression profiles reported in human literature. The literature was also searched for experimentally validated targets of miRNA in human UC of the dysregulated miRNA reported in dogs, evaluating for manuscripts that demonstrated the dysregulation of the miRNA investigated resulted in the predicted effect through evaluation for changes in miRNA transcription or protein expression in samples where the miRNA is dysregulated. No manuscripts were identified that experimentally validated targets of miRNA in canine UC.

Results

Three manuscripts meeting the criteria for inclusion in this review were identified. Thirty-one miRNAs were found to be dysregulated in canine UC: 1 (mir-103b) in blood, 3 in urine (let-7c, mir-103b, and mir-106b), and 30 in bladder tissue samples (Table 1). Four miRNAs were upregulated in tissues (miR-34a, miR-106b, miR-374b, and miR-450), and the remaining miRNA identified were downregulated (Table 1). Ten miRNAs dysregulated in canine UC were not reported to be dysregulated in human UC (miR-105b, miR-105a, miR-216a, miR-361, miR-544, miR-568, miR-764, miR-802, miR-974, and miR-876), 1 miRNA (miR-105a) was not reported to be dysregulated in human UC, and no target was reported. Six miRNAs have differing expression patterns reported in the human versus dog literature: 2 in urine (let-7c and mir-106b) and 4 in bladder tissue (miR-34a, miR-301 A, miR-429, and miR-652). Fifteen experimentally validated targets, from the human literature, are reported for 31 miRNAs identified to be differentially expressed in canine UC literature (Table 1). In the human literature, there are 15 miRNAs with targets validated in urothelial tissue (hepatocyte nuclear factor 4 gamma for miR-544MI, factor last growth factor receptor 3 for miR-99a-217 and miR-10010; cyclin-dependent kinase inhibitor 2B for miR-42019; protooncogene c-FOS for miR-49020; high mobility group box 3/wingless type/B-catenin22 for miR-532; phosphotyrosine picked threonine protein kinase for miR-58222; potassium channel, calcium-activated intermediate/small for miR-65223; plasminogen activator inhibitor-124 phosphatidylinositol-4,5-bisphosphate 3-kinase/RAC-α serine/threonine-protein kinase, and mitogen-activated protein kinase [MAPK]25 for miR-143 and miR-14518; keratin 726 and p21/Cdc24/Rac1-activated kinase27 for miR-145; and phosphate and tensin homolog [PTEN] for miR-301A.28

Discussion

This review highlights the similarities between canine and human UC and, arguably more importantly, identifies notable differences between the expression profiles of miRNA in canine and human UC. The differing miRNA expression profile, such as miR-34a, miR-106b, and miR-652, which is upregulated in canine UC tissue but downregulated in human UC, and no target was reported. Six miRNAs have differing expression patterns reported in the human versus dog literature: 2 in urine (let-7c and mir-106b) and 4 in bladder tissue (miR-34a, miR-301 A, miR-429, and miR-652). Fifteen experimentally validated targets, from the human literature, are reported for 31 miRNAs identified to be differentially expressed in canine UC literature (Table 1). In the human literature, there are 15 miRNAs with targets validated in urothelial tissue (hepatocyte nuclear factor 4 gamma for miR-544MI, factor last growth factor receptor 3 for miR-99a-217 and miR-10010; cyclin-dependent kinase inhibitor 2B for miR-42019; protooncogene c-FOS for miR-49020; high mobility group box 3/wingless type/B-catenin22 for miR-532; phosphotyrosine picked threonine protein kinase for miR-58222; potassium channel, calcium-activated intermediate/small for miR-65223; plasminogen activator inhibitor-124 phosphatidylinositol-4,5-bisphosphate 3-kinase/RAC-α serine/threonine-protein kinase, and mitogen-activated protein kinase [MAPK]25 for miR-143 and miR-14518; keratin 726 and p21/Cdc24/Rac1-activated kinase27 for miR-145; and phosphate and tensin homolog [PTEN] for miR-301A.28
Small RNA sequencing is a global method of miRNA detection and uses global normalization methods to determine differential expression, whereas RT-qPCR is limited by the chosen number of miRNAs tested, and normalization methods rely on finding a suitable normalizer for relative expression. Thus, more miRNA were identified as being differentially expressed in the study performed by Varvil et al. versus the targeted evaluation performed by Kent et al.

In canine and human UC, disruptions of cell cycle pathways are important in the development of disease. Alterations in tumor protein 53 (TP53) expression are linked to high-grade tumors, whereas alterations in the Ras-MAPK signal transduction pathway have been more frequently associated with low-grade tumors, more commonly seen in human UC. PTEN expression has also been shown to be altered in human UC, correlating with high tumor grade. These findings are similar in both humans and dogs. Additionally, the alterations within these pathways reflect which subclassification of the tumor arises; TP53 alterations result in a more aggressive basilar tumor, and alterations in the Ras-MAPK pathway result in the less aggressive luminal tumor, shown in both the human and canine literature relating to UC. Within this review, none of the miRNAs identified as having differential expression in canine UC have been experimentally shown to target TP53 in the human literature. However, 4 miRNAs have been experimentally shown to target PTEN in human literature in other cancers, including miR-181A, miR-214, miR-301A, and miR-374b. Additionally, both miR-143 and miR-145 have been experimentally shown to target PTEN in the human literature, to alter the Ras-MAPK pathways. Of these, miRNAs miR-143, miR-145, and miR-181A have the same expression pattern in UC tissue in both humans and dogs. Mir-301A, however, is upregulated in canine UC and downregulated in human UC in tissues. This may represent a differing miRNA target in canine UC versus human UC; more studies are needed in canine UC tissue to determine the cause of this discrepancy.

The limitations of this study include the low number of manuscripts available for review regarding miRNA expression in canine UC. Additionally, the manuscripts relating to human UC often did not specify whether the results were related to high-grade muscle-invasive UC (such as seen in dogs) or low-grade forms. The lack of manuscripts relating to miRNA targets in the canine literature, let alone in canine bladder tissue, raises concern for the potential of a single miRNA to be acting on a different pathway in dogs and humans, which may relate to the difference in pathophysiologic disease development and behavior that is noted between canines and humans. Another inherent limitation is the diverse expression pattern of a single miRNA in differing disease processes. Expression of a single miRNA can be altered in numerous disease processes and can also be upregulated or downregulated depending on the disease or even disease progression. As such, thorough investigation of miRNA expression patterns in disease processes compared to nondiseased tissues and in the same tissues affected by other diseases that may be considered for the patients presenting signs is recommended prior to the use of miRNAs as diagnostic or prognostic markers.

The findings in this review highlight a gap in the literature on what is known of canine UC and how it compares to human UC. Additionally, numerous miRNAs are dysregulated in human and canine UC where the miRNA target has not been experimentally validated in bladder tissue, which may affect our understanding of this disease. Ultimately, more research is needed into both canine and human UC for a better understanding of tumorigenesis and to determine additional avenues of disease monitoring and treatment.

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References


AJVR


