Animal Science Papers and Reports vol. 42 (2024) no. 2, 217-230
DOI: 10.2478/aspr-2023-0032
Institute of Genetics and Animal Biotechnology
of the Polish Academy of Sciences, Jastrzębiec, Poland

Altered miRNA pattern in canine mammary tumors - pilot study

Luciana-Mădălina Gherman[1,2], Oana Zanoaga[1], Liviuta Budisan[1], Ekaterina Isachesku[1], Raduly Lajos[1], Cristina Ciocan[1], Cornelia Braicu[1]*, Atanas G. Atanasov[3,4], Ioana Berindan-Neagoe[1]

[1] Research Center of Functional Genomics, Biomedicine, and Translational Medicine Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

[2] Animal Facility University of Medicine and Pharmacy Iuliu-Hatieganu, Cluj-Napoca, Romania

[3] Ludwig Boltzmann Institute Digital Health and Patient Safety, Medical University of Vienna, Spitalgasse 23, 1090 Vienna, Austria

[4] Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Jastrzebiec, 05-552 Magdalenka, Poland

(Accepted April 30, 2024)

Canine mammary tumors (CMTs) represent a prevalent malignancy in female dogs. MicroRNAs (miRNAs) have emerged as critical regulators of gene expression and are implicated in various cancer types, including CMTs. This study aimed to investigate the altered miRNA expression patterns in CMTs and their potential role in tumorigenesis. We analyzed miRNA profiles in a cohort of CMT samples and matched normal tissues using a custom canine panel microarray slide (Agilent technology). The bioinformatics analysis overlapped the altered miRNA signature in CMT with human breast cancer miRNA (TCGA patient cohort). The biological significance of this altered miRNA signature was evaluated using Ingenuity Pathway Analysis. Our results revealed a distinctive miRNA expression signature associated with CMTs compared to normal mammary tissues, and when overlapped with human breast cancer miRNA data (TCGA cohort), we identified a common

*Coresponding author:cornelia.braicu@umfcluj.ro

signature composed of one overexpressed transcript and eight downregulated transcripts. In conclusion, our study provides comprehensive insights into the altered miRNA expression patterns in CMTs, shedding light on their potential contribution to the pathogenesis of these tumors. Further investigation into the specific roles of these dysregulated miRNAs is warranted to elucidate their precise involvement in CMT progression and to explore their therapeutic implications.

KEYWORDS: canine mammary tumors, miRNA, biomarkers

Canine mammary tumors (CMT) are the most common type of cancer in intact (non-spayed) female dogs. These tumors originate from the mammary gland tissue and can occur in both benign and malignant forms. CMT can be classified into various types, including benign tumors (such as adenomas and fibroadenomas) and malignant tumors (such as carcinoma and sarcoma) - Gray *et al.* [2020]. Diagnosis of canine breast tumors involves a combination of physical examination, fine-needle aspiration or biopsy for cytology or histopathology, and imaging techniques to evaluate tumor size and involvement of adjacent tissues [Giambrone *et al*. 2022, Burrai *et al.* 2023].

Treatment options for CMT depend on various factors, including the tumor type, size, grade, stage, and overall health of the dog [Levi *et al*. 2021]. Treatment may involve surgical removal of the tumor (lumpectomy or mastectomy), sometimes combined with lymph node removal. In malignant tumors or advanced disease cases, additional treatments such as chemotherapy or radiation therapy may be recommended [Valdivia *et al.* 2021].

Naturally occurring CMT represent a valuable translational model for human breast cancer research [Gray *et al*. 2020, Inglebert *et al*. 2022, Kwon *et al*. 2023]. The prognosis for CMT depends on several factors, including tumor type, grade, stage, presence of metastasis, and the completeness of the surgical removal [Cassali 2013]. Generally, malignant tumors have a higher risk of recurrence and metastasis than benign tumors[Sorenmo *et al*. 2009, Klopfleisch *et al*. 2010]. Early detection and novel biomarkers prompt treatment to improve the prognosis, overcoming human cancer through animal studies [Oh and Cho 2023].

CMTs offer a unique opportunity for comparative oncology research, as they share similarities with human breast cancer regarding histopathology, molecular features, and clinical behavior [Sorenmo *et al*. 2009, Klopfleisch *et al*. 2010, Graim *et al*. 2020]. Microarray studies in canine models can contribute to our understanding of the underlying molecular mechanisms of CMT and facilitate the translation of findings from dogs to humans [Bulkowska et al. 2017]. The obtained data can be further analyzed to identify enriched biological pathways and functional transcript sets associated with canine breast cancer. This information can provide insights into the molecular processes and cellular pathways involved in disease development and progression [Sahabi *et al*. 2018].

MicroRNAs (miRNAs) have been extensively studied in human breast cancer and are known to play important roles in regulating gene expression. MiRNA research in cancer research is an emerging area [Petri and Klinge 2020]; the study aimed to evaluate the altered miRNAs in CMT and their implication in key signaling pathways. These dysregulated miRNAs can impact various cellular processes, including cell proliferation, differentiation, apoptosis, and metastasis (Petri and Klinge 2020). Although the research in this specific field is relatively limited compared to human breast cancer, only several miRNAs have been investigated in canine models *[Boggs et al.* 2008, Sahabi *et al*. 2018].

Microarray technology allows for simultaneous measurement of the expression levels of thousands of transcripts in a single experiment. Our study aims to use the microarray analysis in CMT to investigate altered transcriptomics patterns and identify molecular signatures associated with this disease. Then, we can identify genes and pathways dysregulated in CMT by comparing transcriptomic profiles between normal and cancerous tissues or different tumor subtypes.

However, it's important to note that miRNA profiling studies in canine breast cancer are still in their early stages; we developed for the first time a custom microarray slide using Agilent technology and Genotypic. This is an important step. Microarray platforms do not cover the desired miRNA targets for canine models.

Material and methods

RNA Extraction

Total RNA was extracted from four breast tumors (two Bichon dog breeds and two Metis dog breeds) tumoral and normal adjacent canine breast cancer tissue using a TriReagent, according to the protocols recommended by the manufacturer. The quality of RNA extracted from tissue was evaluated by spectrophotometry according to the absorbance at 260 and 280 nm, respectively. The study included samples with A260/A280 ratios between 1.80 and 2.10.

Microarray evaluation

Form miRNA microarray evaluation from breast cancer canine samples using 100 ng of total RNA for labeling using miRNA Complete Labeling and Hyb Kit (Agilent Technologies, Santa Clara, CA, USA) following the manufacturer's instructions. The microarray slide (8x 60K Agilent Human miRNA Microarray, Amaid: 0872721) was hybridized with 100 ng Cy3-labeled RNA using miRNA Complete Labeling and Hyb Kit (Agilent Technologies, Santa Clara, CA, USA) in hybridization Oven (Agilent Technologies, Santa Clara, CA, USA) at 55°C, 20 rpm for 20 h according to the manufacturer's instructions, as are presented for the hybridization section. Then, the slide was washed in staining dishes (Thermo Shandon, Waltham, MA, USA) with

Gene Expression Wash Buffer Kit (Agilent Technologies, Santa Clara, CA, USA) and scanned by Agilent Microarray Scanner (Agilent Technologies, Santa Clara, CA, USA).

Bioinformatic analysis of the canine miRNA microarray data

The data were extracted using Feature Extraction software 10.7 (Agilent Technologies, Santa Clara, CA, USA) with default settings. Raw data were normalized by Quantile algorithm, Gene Spring Software 11.0 (Agilent Technologies, Santa Clara, CA, USA). The microarray experiments were performed by following the protocol of Agilent technologies. Fold changes of miRNA expression values were calculated between tumor tissue and normal adjacent tissue. Differentially expressed miRNAs *(p*-value <0.05 and with a fold change of at least 1.5 or more) were considered statistically significant.

Data validation

Additional overlapping of the microarray data with PCR-array data (Qiagen) of the expression profiles of 317 microRNAs in 146 canine mammary tumours (GSE103093, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE103093) - [Boggs *et al*. 2008].

Ingenuity pathway analysis (IPA)

Additional analysis was done to see the biological significance of altered miRNA transcripts, considering the structural similarities of the canine miRNAs listed in miRbase (Sanger Institute), which were kept only those that show absolute sequence complementarity to the human counterparts. The sequence identity of the canine mature miRNA sequences with the corresponding human homologs was confirmed by miRbase, single sequence search (http://www.mirbase.org/search.shtml) as was described in a recent paper [Wagner *et al.* 2013].

Results and discussion

miRNA Microarray profiling of CMT

Genome-wide microarray analysis was employed to uncover the miRNA expression profiles of CMT (tumor tissue versus normal adjacent tissue) using microarray technology based on a custom microarray slide developed by Genotyping compatible with Agilent technology. The miRNAs with significant changes in expression level in the pair-wise comparison, fold change (FC) ≥1.5 and *p*-values <0.05 were presented as a heatmap in Figure 1; 34 differentially expressed miRNAs were identified between the tumor tissue versus the normal group of which 23 were downregulated, and 11 were upregulated, data presented in Table 1.

Table 1. miRNA list with an altered expression level using an FC cut-off of 1.5 and *p*-values<0.05

Systematic name	Active_sequence	Mirbase accession no	FC	p -value
cfa-miR-582	AGTAACTGGTTGAACAACTGTA	MIMAT0009916	-56.5567	0.02056
cfa-miR-95	TGCTCAATAAATACCCGT	MIMAT0009878	-40.0286	0.003191
cfa-miR-126	CGCGTACCAAAAGTAATAATG	MIMAT0006730	-4.20373	0.008801
cfa-let-7f	AACTATACAATCTACTACCTC	MIMAT0006610	-2.11104	0.000337
cfa-miR-26b	AACCTATCCTGAATTACTTG	MIMAT0006678	-2.03821	0.003167
cfa-miR-30e	GCTGTAAACATCCGACTG	MIMAT0006627	-2.0142	0.000283
cfa-miR-30b	AGCTGAGTGTAGGATGTT	MIMAT0006617	-1.9827	0.010541
cfa-let-7a	AACTATACAACCTACTACCT	MIMAT0006594	-1.94466	0.000975
cfa-let-7g	AACTGTACAAACTACTACCTC	MIMAT0006637	-1.91721	0.000966
cfa-miR-181a	CTCACCGACAGCGT	MIMAT0006707	-1.83611	0.037553
cfa-miR-20a	CTACCTGCACTATAAGCAC	MIMAT0006651	-1.82924	0.010127
cfa-miR-98	AACAATACAACTTACTACCTC	MIMAT0006756	-1.77255	0.044172
cfa-miR-1271	AGTGCTTACTAGGTGCC	MIMAT0006685	-1.73921	0.005384
cfa-miR-151	ACTAGACTGTGAGCTCC	MIMAT0006615	-1.72982	0.005345
cfa-let-7c	AACCATACAACCTACTACC	MIMAT0006669	-1.69366	0.004659
cfa-miR-92a	ACAGGCCGGGACAAGT	MIMAT0006653	-1.6774	0.041103
cfa-miR-195	TGCCAATATTTCTGTGCT	MIMAT0006692	-1.65527	0.03345
cfa-miR-99a	ACAAGATCGGATCTACGG	MIMAT0006668	-1.65206	0.016683
cfa-let-7b	AACCACACAACCTACTACC	MIMAT0009836	-1.63947	0.044522
cfa-miR-30c	AGCTGAGAGTGTAGGATG	MIMAT0006605	-1.59224	0.022074
cfa-miR-16	CGCCAATATTTACGTGCTG	MIMAT0006648	-1.57236	0.001031
cfa-miR-374b	CACTTAGCAGGTTGTATTA	MIMAT0006754	-1.52962	0.031465
cfa-miR-186	AGCCCAAAAGGAGAATTCTTT	MIMAT0006694	-1.51889	0.001482
cfa-miR-8808	GTCGCGGCTCTGT	MIMAT0034298	87.19109	3.45E-07
cfa-miR-8859a	TCCGGACCCCGG	MIMAT0034354	54.44715	7.16E-07
cfa-miR-489	GCCGCCGTATATGTG	MIMAT0009860	33.66676	0.016667
cfa-miR-1844	CTCAGCCCGTCCG	MIMAT0006740	22.74058	0.027116
cfa-miR-8898	GGGAGCTGCTACCA	MIMAT0034408	18.30344	0.029398
cfa-miR-210	TCAGCCGCTGTCACAC	MIMAT0009846	3.321926	0.034952
cfa-miR-8834b	AGCACCCCCGCTG	MIMAT0034431	3.123015	0.024326
cfa-miR-8815	CCGTCCCCCGC	MIMAT0034305	2.628619	0.032118
cfa-miR-8834a	CCCCGGAGCCTC	MIMAT0034327	2.318919	0.011147
cfa-miR-1185	ATAAGAGTCTCCCCCTG	MIMAT0034383	1.800138	0.048796
cfa-miR-8818	CAGGAAGGTCTCCCA	MIMAT0034308	1.725456	0.001557

Data validation

Additional analysis of the expression levels for altered miRNA in CMT was downloaded from the paper published by Bulkowska *et al*. [2017], data publicly available, revealing one overexpressed transcript (cfa-miR-210) and 59 downregulated miRNAs (adjusted *p*-values <0.05). When we overlapped this data (GSE103093) with our microarray data, we observed a common signature composed of one overexpressed transcript and 19 downregulated miRNAs (Fig. 2).

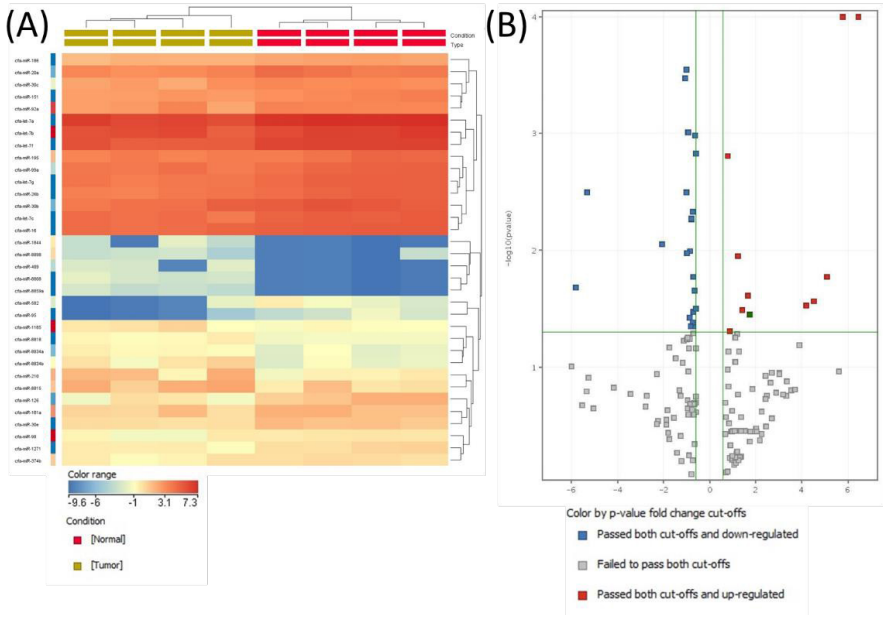

Fig. 1. Altered miRNA pattern in canine breast cancer. (A) Heatmap graphical representation of modified miRNA pattern in canine breast cancer evaluated using microarray Agilent technology differentially expressed miRNA with $p < 0.05$ and FC ≥ 1.5; (B) Volcano plot in canine mammary tumors, tumor tissue versus adjacent normally mammary tissue. Red dots indicate upregulated miRNAs, blue dots downregulated miRNAs, and grey dots indicate miRNAs with no alteration in the expression levels.

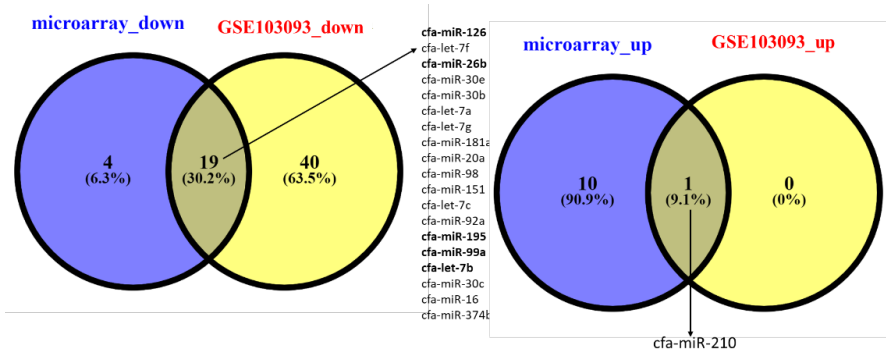

Fig. 2. Venny diagram emphasizes common and specific miRNA signatures in human and canine breast cancer, overlapping our microarray data with those obtained from GSE103093.

Ingenuity Pathway Analysis (IPA)

IPA tool was used to identify pathways and diseases associated with miRNAs with altered expression levels. The main molecular and Cellular Functions limited in CMT, based on extrapolated miRNA signatures to humans, are displayed in Table 2.

Table 2. Molecular and cellular functions

Name	p-value range	# Molecules
Cell Cycle	4.60E-02 - 1.68E-09	7
Cellular Movement	3.72E-02 - 4.93E-07	11
Cell Death and Survival	4.38E-02 - 2.32E-06	10
Cellular Development	4.38E-02 - 3.37E-05	14
Cellular Growth and Proliferation	4.38E-02 - 3.37E-05	14

An additional miRNA-mRNA network was generated using IPA related to Cancer, Organismal Injury and Abnormalities, and Reproductive System Disease is presented in Figure 3. According to the IPA database, some of these transcripts were related to advanced malignant tumors, invasive cancer and metastasis (Fig. 4).

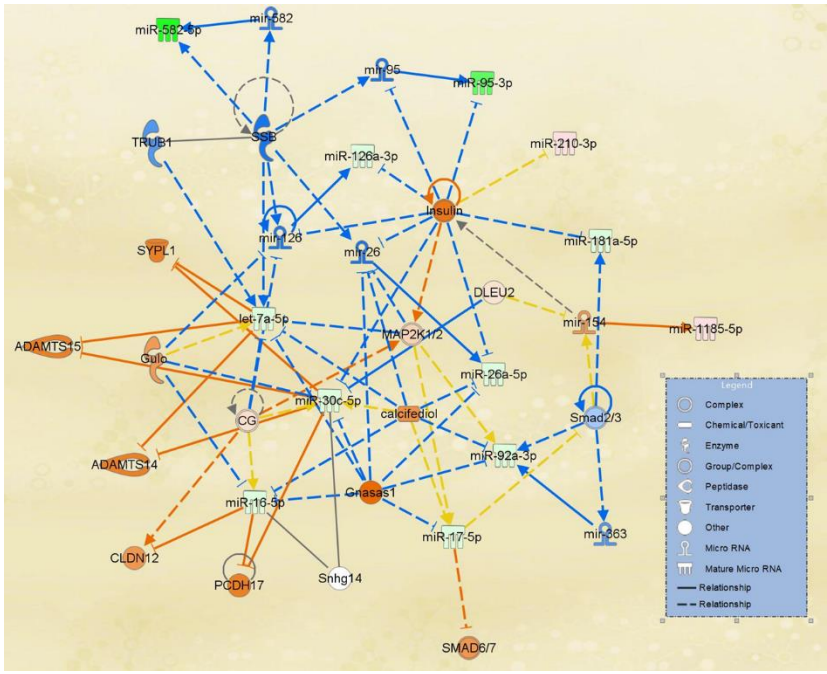

Fig. 3. Ingenuity pathway analysis of the significantly altered miRNA found in breast cancer shows that the transcripts are related to Cancer, Organismal Injury and Abnormalities, and Reproductive System Disease. Strongly upregulated and downregulated miRNA is represented with dark red and green color and vice versa.

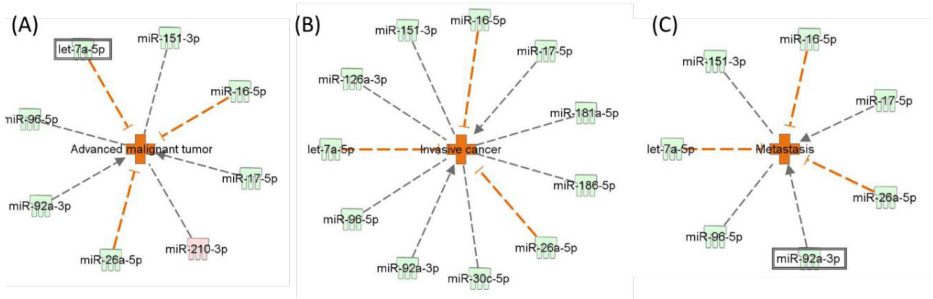

Figure 4. Ingenuity Pathway Analysis of differentially expressed miRNA in breast cancer. The analysis identified altered miRNAs that were involved in (A) advanced malignant tumors, (B) invasive cancer and (C) metastasis. MiRNA was either significantly lower (green) or higher (red) in canine breast cancer (corresponding to Tab. 1) and then extrapolated to human structural homologues. Lines with arrows indicate miRNA that leads to activation; meanwhile, lines with blunt ends indicate miRNA that inhibits this biological process.

Common microRNAs deregulated in canine mammary cancer and human breast cancer

The list of common microRNAs deregulated in canine mammary and human breast cancer, separated by downregulated and upregulated transcripts, is presented as a Venn diagram in Figure 5B, which we can observe as a common signature of eight downregulated miRNAs and one overexpressed transcript.

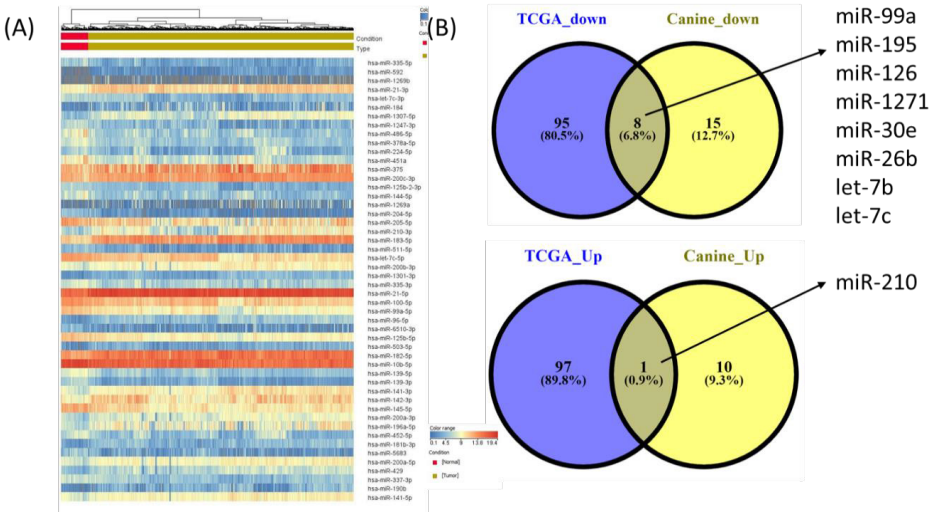

Fig. 5. Common and specific miRNA signature in human (TCGA cohort) and CMT. (A) Heatmap graphical representation of altered miRNA pattern in of human breast cancer-TCGA patient cohort differentially expressed miRNA with $p < 0.05$ and FC ≥ 1.5**;** (B) Venny diagram emphasis common and specific miRNA signature in human and canine breast cancer

One particular interest was exploring the signalling pathways related to common signature; this was done using miRPath ver.3. KEGG pathway and gene union analyses showed enriched pathways, as can be observed from Figure 6A. We selected to present a detailed view of the target genes related to TP53 (Fig. 6B)

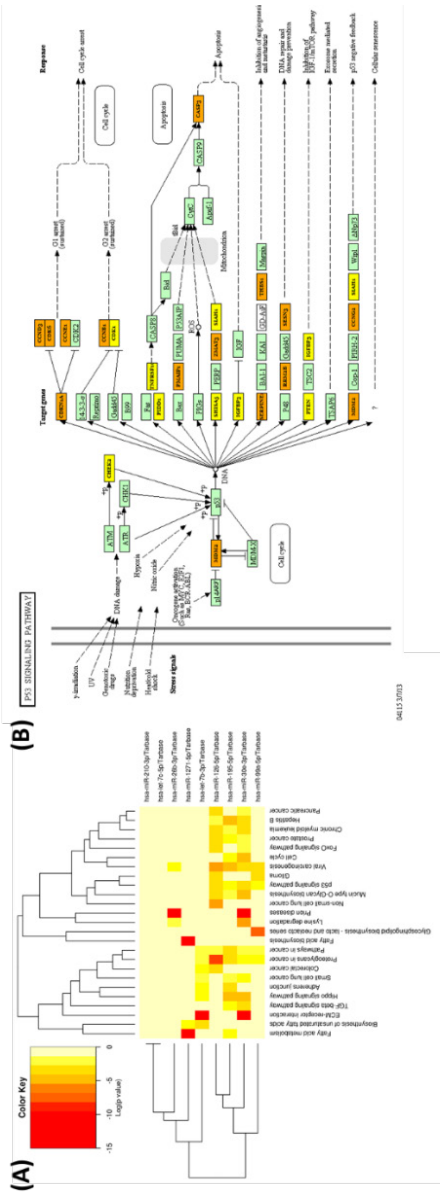

Fig. 6. Heatmaps for enrichment analysis of common miRNA signature using miRPath. (A) Heatmap for KEGG's pathway union analysis of common miRNA signature among human and canine breast cancer (B) implication of the common signature in TP53 signaling.

Our study presents valuable information related to the altered miRNA pattern in CMT. Therefore, the dysregulated miRNAs in canine breast cancer may have diagnostic and prognostic value [Fish *et al*. 2020]. This study offers valuable implications for both veterinary medicine and comparative oncology. miRNAs have been investigated as potential biomarkers for cancer detection, classification, and prediction of patient outcomes [Fish *et al.* 2020]. It's important to note that miRNA expression patterns can vary between different subtypes of canine breast cancer and individual tumors, as shown in the heatmap graphical representation in Figure 1. Additionally, the functional implications of dysregulated miRNAs in canine breast cancer are still being investigated. Further research is needed to elucidate the precise roles of these miRNAs in the development, progression, and potential therapeutic targeting of canine breast cancer.

The study successfully identified a common signature within another dataset, underscoring the robustness and reproducibility of the findings. This convergence of results across datasets enhances the confidence in the identified miRNA signature and its relevance to the context of breast cancer research and comparative oncology.

The dysregulation of numerous miRNAs is associated with the altered regulation of TP53 signaling pathways, indicating a complex interplay between commonly altered miRNA signatures for canine and human breast tumours and the TP53 signaling. These miRNAs can target various components of the TP53 pathway, influencing its activation or suppression and contributing to the intricate network of molecular events in cancer development and progression in both human and canine models [Alsaihati *et al*. 2021]. Understanding these miRNA-mediated regulatory mechanisms provides valuable insights into the molecular landscape of cancer and potential therapeutic strategies targeting TP53 signaling.

cfa-miR-125b is attributed to host cell resistance against canine influenza virus in canine models [Xie *et al*. 2021]. The alteration of this transcript might not be specific to CMT. The role of this transcript is unknown [Kim *et al*. 2023], but a previous study revealed overexpression of this transcript in CMT [Boggs *et al*. 2008].

Also, some transcripts show discrepancies in the expression levels between dogs and humans. This is the case of miR-181a, a fact sustained by a previous study on comparative oncology [Bulkowska *et al*. 2017]. The discrepancies in the expression levels can be caused by other target genes having a more substantial influence on canine mammary cancer than human breast cancer development, other genes than those reported in the literature about breast cancer [Bulkowska *et al*. 2017].

Identifying miR-210 as a common transcript in both canine breast cancer and human breast cancer datasets underscores its potential as a valuable biomarker with cross-species relevance. miR-210 is commonly upregulated in canines and human, particularly in more aggressive and metastatic forms of the disease [von Deetzen *et al*. 2014]. Elevated expression of miR-210 is associated with tumor progression, angiogenesis, and poor prognosis in the canine breast cancer [Qin *et al*. 2014, von Deetzen *et al*. 2014]. This finding suggests that miR-210 could serve as a diagnostic or

prognostic marker not only in canine breast cancer but also in its human counterpart. The shared molecular signature across species highlights the translational potential of miR-210, opening avenues for further exploration in both veterinary and human oncology [Bulkowska *et al*. 2017, Evangelista *et al*. 2021].

Conclusions

Our study provides comprehensive insights into the altered miRNA expression patterns in CMTs, shedding light on their potential contribution to the pathogenesis of these tumors. However, further research is necessary to fully elucidate the functional significance of specific dysregulated miRNAs and their potential as therapeutic targets in canine breast cancer. miR-210 emerges as a common biomarker in both canine and human breast cancer datasets, highlighting its potential diagnostic and prognostic value across species. These finding positions miR-210 as a promising indicator for breast cancer research in veterinary and human medicine.

It is essential to acknowledge the strengths and weaknesses of our study. One strength lies in the comprehensive analysis of miRNA expression patterns in CMTs, which has provided valuable insights into potential mechanisms underlying CMTs. Additionally, the integration of canine and human breast cancer datasets enhances the robustness and translational relevance of our findings. Despite these strengths, our study also has limitations. Firstly, the lack of functional validation of identified miRNAs limits our ability to infer causality or elucidate their precise roles in CMT pathogenesis. Additionally, while bioinformatics analyses have facilitated the identification of dysregulated miRNAs, experimental validation studies are necessary to confirm their functional significance. Therefore, our study contributes significantly to the understanding of miRNA involvement in CMT pathogenesis and highlights potential avenues for future research. By elucidating the molecular underpinnings of CMTs, our findings may inform the development of novel diagnostic and therapeutic strategies for this prevalent canine malignancy.

REFERENCES

1. ALSAIHATI B.A., K. L. HO K.L., WATSON J., FENG Y., WANG T., DOBBIN K.K., ZHAO S., 2021 - Canine tumor mutational burden is correlated with TP53 mutation across tumor types and breeds. ***Nature Communications*** 12(1), 4670.
2. BOGGS R.M., WRIGHT Z.M., STICKNEY M.J., PORTER W.W., MURPHY K.E., 2008 - MicroRNA expression in canine mammary cancer. ***Mammalian Genome*** 19(7-8), 561-569.
3. BULKOWSKA M., RYBICKA A., SENSES K.M., ULEWICZ K., WITT K., SZYMANSKA J., TACIAK B., KLOPFLEISCH R., HELLMÉN E., DOLKA I., GURE A.O., MUCHA J., MIKOW M., GIZINSKI S., KROL M., 2017 - MicroRNA expression patterns in canine mammary cancer show significant differences between metastatic and non-metastatic tumours. ***BMC Cancer*** 17(1), 728.
4. BURRAI G.P., GABRIELI A., POLINAS M., MURGIA C., BECCHERE M.P., DEMONTIS P., ANTUOFERMO E., 2023 - Canine mammary tumor histopathological image classification via computer-aided pathology: an available dataset for imaging analysis. ***Animals*** 13(9), 1563.

5. CASSALI G.D., 2013 - Comparative mammary oncology: canine model. ***BMC Proceedings*** 7(2), K6.
6. EVANGELISTA A.F., OLIVEIRA R.J., SILVA V.A.O., VIEIRA R.A.D.C., REIS R.M., MARQUES M.M.C., 2021 - Integrated analysis of mRNA and miRNA profiles revealed the role of miR-193 and miR-210 as potential regulatory biomarkers in different molecular subtypes of breast cancer. ***BMC Cancer*** 21(1), 76.
7. FISH E.J., MARTINEZ-ROMERO E.G., DEINNOCENTES P. KOEHLER J.W., PRASAD N., SMITH A.N., BIRD R.C., 2020 - Circulating microRNA as biomarkers of canine mammary carcinoma in dogs. ***Journal of Veterinary Internal Medicine*** 34(3), 1282-1290.
8. GIAMBRONE G., DI GIORGIO S., VULLO C., MARINO G., PULEIO R., MARIOTTI F., MAZZULLO G., SFACTERIA A., 2022 - Does TLS Exist in canine mammary gland tumours? Preliminary results in simple carcinomas. ***Veterinary Sciences*** 9(11), 628.
9. GRAIM K., GORENSHTEYN D., ROBINSON D.G., CARRIERO N.J., CAHILL J., CHAKRABARTI R., GOLDSCHMIDT M.H., DURHAM A.C., FUNK J., STOREY J.D., KRISTENSEN V.N., THEESFELD C.L., SORENMO K.U., TROYANSKAYA O.G., 2020 - Abstract 2504: Modeling molecular development of breast cancer in canine mammary tumors. ***Cancer Research*** 80(16_Supplement), 2504-2504.
10. GRAY M., MEEHAN J., MARTÍNEZ-PÉREZ C., KAY C., TURNBULL A.K., MORRISON L.R., PANG L.Y., ARGYLE D., 2020 - Naturally-Occurring canine mammary tumors as a translational model for human breast cancer. ***Frontiers in Oncology*** 10, 617.
11. INGLEBERT M., DETTWILER M., HAHN K., LETKO A., DROGEMULLER C., DOENCH J., BROWN A., MEMARI Y., DAVIES H.R., DEGASPERI A., NIK-ZAINAL S., ROTTENBERG S., 2022 - A living biobank of canine mammary tumor organoids as a comparative model for human breast cancer. ***Scientific Reports*** 12(1), 18051.
12. KIM E.P., JANG G., KIM J.W., KIM S.W., CHUNG H., YANG Y.J., KIM W.H., KIM G.A., 2023 - MicroRNA and Messenger RNA Expression Profiles in Canine Mammary Gland Tumor. ***International Journal of Molecular Sciences*** 24(3).
13. KLOPFLEISCH R., KLOSE P., WEISE C., BONDZIO A., MULTHAUP G., EINSPANIER R., GRUBER A.D., 2010 - Proteome of Metastatic canine mammary carcinomas: similarities to and differences from human breast cancer. ***Journal of Proteome Research*** 9(12), 6380-6391.
14. KWON J.Y., MOSKWA N., KANG W., FAN T.M., LEE C., 2023 - Canine as a Comparative and translational model for human mammary tumor. ***Journal of Breast Cancer*** 26(1), 1-13.
15. LEVI M., MUSCATELLO L.V., BRUNETTI B., BENAZZI C., PARENTI F., GOBBO F., AVALLONE G., BACCI B., ZAMBON E., VALENTI P., SARLI G., 2021 - High intrinsic expression of P-glycoprotein and breast cancer resistance protein in canine mammary carcinomas regardless of immunophenotype and outcome. ***Animals*** 11(3), 658.
16. OH J.H., CHO J.-Y., 2023 - Comparative oncology: overcoming human cancer through companion animal studies. ***Experimental & Molecular Medicine*** 55(4), 725-734.
17. PETRI B.J. KLINGE C.M., 2020 - Regulation of breast cancer metastasis signaling by miRNAs. ***Cancer and Metastasis Reviews*** 39(3), 837-886.
18. QIN, Q., FURONG W., BAOSHENG L., 2014 - Multiple functions of hypoxia-regulated miR-210 in cancer. ***Journal of Experimental & Clinical Cancer Research*** 33(1), 50.
19. SAHABI K., SELVARAJAH G.T., ABDULLAH R., CHEAH Y.K., TAN G.C., 2018 - Comparative aspects of microRNA expression in canine and human cancers. ***Journal of Veterinary Science*** 19(2), 162-171.

20. SORENMO K.U., KRISTIANSEN V.M., COFONE M.A., SHOFER F.S., BREEN A.-M., LANGELAND M., MONGIL C.M., GRONDAHL A.M., TEIGE J., GOLDSCHMIDT M.H., 2009 - Canine mammary gland tumours; a histological continuum from benign to malignant; clinical and histopathological evidence. ***Veterinary and Comparative Oncology*** 7(3), 162-172.
21. VALDIVIA G., ALONSO-DIEZ Á.,PÉREZ-ALENZA D., PEÑA L., 2021 - From conventional to precision therapy in canine mammary cancer: a comprehensive review. ***Frontiers in Veterinary Science*** 8, 623800.
22. VON DEETZEN M.C., SCHMECK B.T., GRUBER A.D., KLOPFLEISCH R., 2014 - Malignancy associated microRNA expression changes in canine mammary cancer of different malignancies. ***ISRN Veterinary Science*** 2014, 148597.
23. WAGNER S., WILLENBROCK S., NOLTE I., MURUA ESCOBAR H., 2013 - Comparison of non-coding RNAs in human and canine cancer. ***Frontiers in Genetics*** 4, 46.
24. XIE X., PANG M., LIANG S., LIN Y., ZHAO Y., QIU D., LIU J., DONG Y., LIU Y., 2021 - Cellular microRNAs influence replication of H3N2 canine influenza virus in infected cells. ***Veterinary Microbiology*** 257, 109083.