LIDIA HILDEBRAND PULZ

Isolation and characterization of Cancer Associated Fibroblasts from canine Mast Cell Tumors and its influence on the malignancy of neoplastic cells: a combined molecular, pathologic and *in vitro* approach

São Paulo
2019

LIDIA HILDEBRAND PULZ

Isolation and characterization of Cancer Associated Fibroblasts from canine Mast Cell Tumors and its influence on the malignancy of neoplastic cells: a combined molecular, pathologic and *in vitro* approach

Thesis submitted to the Postgraduate Program in Experimental and Comparative Pathology of the School of Veterinary Medicine and Animal Science of the University of São Paulo to obtain the Doctor's degree in Sciences.

Department:
Pathology

Area:
Experimental and Comparative Pathology

Advisor:
Prof. Ricardo De Francisco Strefezzi, Ph.D.

São Paulo
2019

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO

(Biblioteca Virginie Buff D'Ápice da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo)

T. 3756
FMVZ

Pulz, Lidia Hildebrand
Isolation and characterization of Cancer Associated Fibroblasts from Canine Mast Cell Tumors and its influence on the malignancy of neoplastic cells: a combined molecular, pathologic and *in vitro* approach / Lidia Hildebrand Pulz. – 2019.
112 f. : il.

Título traduzido: Isolamento e caracterização de Fibroblastos Associados ao Câncer provenientes de Mastocitomas Caninos e sua influência sobre a malignidade de células neoplásicas: uma abordagem molecular, patológica e *in vitro*.

Tese (Doutorado) – Universidade de São Paulo. Faculdade de Medicina Veterinária e Zootecnia. Departamento de Patologia, São Paulo, 2019.

Programa de Pós-Graduação: Patologia Experimental e Comparada.
Área de concentração: Patologia Experimental e Comparada.
Orientador: Prof. Dr. Ricardo De Francisco Strefezzi.

1. RNA-Seq. 2. Cão. 3. Co-cultivo. 4. Miofibroblastos. 5. Matriz extracelular. I. Título.

Ficha catalográfica elaborada pela bibliotecária Maria Aparecida Laet, CRB 5673-8, da FMVZ/USP.

RESUMO

PULZ, L. H. **Isolamento e caracterização de Fibroblastos Associados ao Câncer provenientes de Mastocitomas caninos e sua influência sobre a malignidade de células neoplásicas:** uma abordagem molecular, patológica e *in vitro*. 2019. 112 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2019.

Os mastocitomas cutâneos (MCTs) são neoplasias comuns em cães e são considerados potencialmente malignos. Diversas pesquisas tentaram identificar biomarcadores para melhor predizer o comportamento biológico deste tumor. Além disso, estudos envolvendo o cultivo primário de MCTs caninos podem ser uma ferramenta valiosa para a análise das propriedades funcionais das células. O objetivo deste estudo foi identificar vias moleculares ligadas a características de malignidade histopatológicas, menor tempo de sobrevida e pior prognóstico associados ao MCT. Além disso, objetivamos investigar o comportamento de mastócitos *in vitro* obtidos a partir de MCTs de diferentes graus histopatológicos e determinar o tipo de interação com os fibroblastos estromais. Realizamos análises de expressão gênica em MCTs únicos obtidos de 15 cães e identificamos dois subtipos distintos de tumor – alto risco e baixo risco - associados a diferenças nos graus histológicos, tempos de sobrevida, índices Ki67 e ocorrência de morte devido a doença. Análises comparativas de perfis de sequência de RNA revelaram 71 genes diferencialmente expressos entre MCTs de alto e baixo risco. Ademais, examinamos redes de co-expressão gênica para explorar as funções biológicas dos genes identificados. A construção da rede revelou 63 módulos gênicos, dos quais 4 foram significativamente associados ao grupo mais agressivo. Dois dos módulos gênicos positivamente correlacionados com MCTs de alto risco também foram associados à proliferação celular e à matriz extracelular. No topo do módulo de matriz extracelular, foram identificados genes com funções diretamente relacionadas àqueles de fibroblastos associados ao câncer (CAFs). Análises imuno-histoquímicas também revelaram um maior número de CAFs em MCTs de alto risco. Os experimentos de cultivo foram feitos imediatamente após a ressecção cirúrgica e as células foram cultivadas em DMEM-F12 completo por 4 a 7 passagens. Os mastócitos foram evidenciados por coloração com Romanowsky e azul de toluidina com perda progressiva de seus grânulos em cultivo. A confirmação de fibroblastos como células aderentes foi feita por qRT-PCR para o gene da Proteína Específica de Fibroblastos 1 (FSP1). A caracterização dos fibroblastos

cultivados como miofibroblastos foi realizada por imunofluorescência para α-actina de músculo liso (SMA) e vimentina. A percentagem de células viáveis no sobrenadante foi determinada a cada passagem. Durante as 4-10 semanas de cultivo sem adição de fatores de crescimento ou citocinas, a população de mastócitos vivos diminuiu progressivamente e os fibroblastos e miofibroblastos continuam a crescer até a senescência. Amostras de MCTs de alto grau foram viáveis por períodos mais curtos ($P = 0.0442$) e menor número de passagens ($P < 0.0001$). Examinamos também os efeitos a curto prazo dos fibroblastos estromais na viabilidade dos mastócitos neoplásicos em diferentes condições de culturas. O contato célula-célula foi a melhor condição em que maior proporção de mastócitos neoplásicos permaneceu viável em comparação com todas as outras condições, isto é, utilizando-se de inserto e no cultivo de mastócitos isolados ($P<0.05$). Verificamos também que os mastócitos neoplásicos não ficam viáveis por mais de 4 dias na ausência de fibroblastos ou de seus fatores solúveis. Estes resultados indicam uma importante interação entre os mastócitos e os fibroblastos, que podem ocorrer no microambiente tumoral.

Palavras-chave: RNA-Seq. Cão. Co-cultivo. Miofibroblastos. Matriz extracelular.

ABSTRACT

PULZ, L. H. **Isolation and characterization of Cancer Associated Fibroblasts from canine Mast Cell Tumors and its influence on the malignancy of neoplastic cells:** a combined molecular, pathologic and *in vitro* approach. 2019. 112 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2019.

Mast cell tumours (MCTs) are common neoplasms in dogs and are considered potentially malignant. Several researches have attempted to identify biomarkers to better predict biological behavior for this tumor. In addition, studies with primary culture of canine MCTs coud be a valuable tool for the analysis of the cells functional properties. The objetive of this study was to identify molecular pathways connected to histopathological malignancies, shorter survival time and poor prognoses associated with MCTs. Moreover, we aimed to investigate the *in vitro* behavior of mast cells obtained from canine cutaneous MCTs of different histopathological grades and the type of interaction with the stromal fibroblasts. We performed genome-wide gene expression analyses on tissues obtained from 15 dogs with single MCTs, and identified two distinct tumour subtypes - high-risk and low-risk - associated with differences in histological grades, survival times, Ki67 indices, and occurrence of death due the disease. Comparative analyses of RNA sequence profiles revealed 71 genes that were differentially expressed between high and low-risk MCTs. In addition to these analyses, we examined gene co-expression networks to explore the biological functions of the identified genes. The network construction revealed 63 gene modules, 4 of which were significantly associated with the more aggressive tumour group. Two of the gene modules positively correlated with high-risk MCTs were also associated with cell proliferation and extracellular matrix-related terms. At the top of the extracellular matrix module category, genes with functions directly related to those of cancer-associated fibroblasts (CAFs) were identified. Immunohistochemical analyses also revealed a greater number of CAFs in high-risk MCTs. Culture experiments were made immediately after surgical resection and cells were cultured in complete DMEM-F12 for 4 to 7 passages. Mast cells was stained with Romanowsky and toluidine blue and showed progressive loss of their granules in culture. The presence of fibroblasts as adherent cells was confirmed by use of qRT-PCR for Fibroblast-specific Protein 1 (FSP1) gene. The characterization of cultured fibroblasts as myofibroblasts was performed by immunofluorescence for α-smooth

muscle-actin (SMA) and vimentin. The percentage of viable cells in the supernatant was determined in each passage. During 4–10 weeks of culture without any addition of growth factors or cytokines, living mast cell population decreased progressively and adherent fibroblasts and myofibroblasts continue to grow until senescence. High-grade MCTs samples were viable for shorter periods in culture (P=0.0442) and lower number of passages (P<0.0001). We also have examined the short-term effects of stroma fibroblasts on neoplastic mast cells in different cultures conditions. The cell-cell contact co-culture was the best condition in which canine neoplastic mast cells remained viable in highest proportion during the experiment compared to all other conditions, i.e. with the transwell condition and mast cells isolated cultures (P<0.05). We also found that isolated neoplastic mast cells are not viable for more than 4 days in the absence of fibroblasts or their soluble factors. These results indicate an important interaction between mast cells and fibroblasts, which may also occur in the tumor microenvirnomental setting.

Keywords**:** RNA-Seq. Dog. Co-culture. Myofibroblast. Extracellular matrix.

1. GENERAL INTRODUCTION

In view of the variable biologic behavior of canine cutaneous mast cell tumors (MCT), development of appropriate prognosis and treatment for individual affected dogs can be very difficult. Advances in human molecular medicine have sought to understand the molecular underpinnings of cancer development, metastatic pattern, and response to treatment. In canine oncology, over the past decades, much evidence has emerged indicating that a substantial part of the variability in tumor behavior is genetically determined, with age, breed, tumor location, environmental conditions, and concurrent therapy playing important contributory roles.

A profile of the gene variations of canine MCTs could guide more precise prognosis, and even allow the selection of drugs or treatment protocols that could minimize harmful side effects or ensure more successful outcomes. Additionally, molecular studies could indicate individual's susceptibility to diseases, allowing veterinarians and owners to design a plan for prevention and early diagnosis.

Another point to consider is the success in the treatment of several human cancers related to the development of targeted therapies. However, in order to develop more effective therapies for canine MCTs, it is necessary to learn about the cells that comprise the tumor, their characteristics, functionality, and interactions with the tumor microenvironment. Little is known about the different mast cell populations in dogs and the requirements for canine neoplastic mast cells culture remain poorly defined.

The objective of this work was to approach the canine cutaneous MCT in several aspects: *in vitro* characteristics, cell interactions and molecular signatures of malignancy. In the present research, we have characterized different RNA expression profiles using high-throughput sequencing (HTS) and compared with survival time and significant prognostic markers for canine cutaneous MCTs, including histologic grading and proliferation activity. In addition, *in vitro* studies have provided information for the understanding of the dynamics of neoplastic mast cell growth, suggesting an important role for cancer-associated fibroblasts in this disease.

2. GENERAL CONCLUSION

The aim of this study was to investigate mechanisms to understand the canine MCT malignant behavior beyond morphological characteristics. First, we found differentially expressed genes in high risk tumors related to the extracellular matrix. Changes in gene expression that mediate invasion and metastasis may not reflect histopathological alterations that could be evaluated in the microscopic examination. The differences in gene expression displayed by the cutaneous MCTs that had high grade of malignancy, high proliferation and short survival time appear to reflect the requirements of the extracelular stimuli. The avaiable literature particularly documents the connection between stromal cells as CAFs and poor prognoses, and this was also verified in the presente study. In addition, to highlight the importance of this tumoral compartment, we identified selected emerging genes affecting the malignant MCTs phenotype.

Then, we demonstrated the interaction between tumor cells and stromal fibroblasts *in vitro.* It seems clear that stromal fibroblasts and CAFs can influence neoplastic mast cell activity in culture condition. Future studies, are necessary to investigate several stroma-specific factors associated with canine mast cell tumor and elucidate the specific mechanisms of action of these molecules.

Cell migration is facilitated by reduced cell-cell and cell-ECM adhesion and cell softening achieved through cytoskeletal reorganisation. Some of these altered features mediate canine cutaneous MCT invasion and metastasis and may be potential targets for novel therapies directed toward the microenvironment. Furthermore, controlled studies are necessary to assess the real effect of chemotherapy protocols. Thus, *in vitro* experimental investigations will be required to demonstrate the effects of antineoplastic drugs and/or to clarify the role of strmoal cells derived molecules (e.g. on cell adhesion, deformability and motility) on neoplastic canine mast cells. The need for an improved understanding of the progression and treatment responses of MCTs has pushed for increased relevance of canine MCT *in vitro* tumor models.

Finally, we believe that further studies are necessary to identity stromal cell derived factors and their mechanisms of action to provide new tools for the establishment of more accurate prognosis or to predict tumor response to chemotherapy.

3. REFERENCES

ABADIE, J. J.; AMARDEILH, M. A.; DELVERDIER, M. E. Immunohistochemical detection of proliferating cell nuclear antigen and Ki-67 in mast cell tumors from dogs. **Journal of the American Veterinary Medical Association**, v. 215, n. 11, p. 1629-1634, 1999.

ABE, M.; KUROSAWA, M.; ISHIKAWA, O.; MIYACHI, Y.; KIDO, H. Mast cell tryptase stimulates both human dermal fibroblast proliferation and type I collagen production. **Clinical and experimental allergy: journal of the British Society for Allergy and Clinical Immunology**, v. 28, n. 12, p. 1509-1517, 1998.

ABEL, M.; VLIAGOFTIS, H. Mast cell-fibroblast interactions induce matrix metalloproteinase-9 release from fibroblasts: role for IgE-mediated mast cell activation. **The Journal of Immunology**, v. 180, n. 5, p. 3543-3550, 2008.

AKIRI, G.; SABO, E.; DAFNI, H.; VADASZ, Z.; KARTVELISHVILY, Y.; GAN, N.; KESSLER, O.; COHEN, T.; RESNICK, M.; NEEMAN, M.; NEUFELD, G. Lysyl oxidase-related protein-1 promotes tumor fibrosis and tumor progression in vivo. **Cancer Research**, v. 63, n. 7, p. 1657-1666, 2003.

ALBERTS, J.; JOHNSON, B.; LEWIS, A. et al. The Extracellular Matrix of Animals. In: ALBERTS, B.; JOHNSON, A.; LEWIS, J.; RAFF, M.; ROBERTS, K.; WALTER, P. **Molecular Biology of the Cell**. 4th edition. New York: Garland Science; 2002; Bookshelf ID: NBK26810.

ALLINEN, M.; BEROUKHIM, R.; CAI, L.; BRENNAN, C.; LAHTI-DOMENICI, J.; HUANG, H.; PORTER, D.; HU, M.; CHIN, L.; RICHARDSON, A.; SCHNITT, S.; SELLERS, W. R.; POLYAK, K. Molecular characterization of the tumor microenvironment in breast cancer. **Cancer Cell**, v. 6, n. 1, p. 17-32, 2004.

AMAGAI, Y.; TANAKA, A.; MATSUDA, A.; OIDA, K.; JUNG, K.; MATSUDA, H. The phosphoinositide 3-kinase pathway is crucial for the growth of canine mast cell tumors. **Journal of Veterinary Medical Science**, v. 75, n. 6, p. 791-794, 2013.

AMAGAI, Y.; TANAKA, A.; OHMORI, K.; MATSUDA, H. Establishment of a novel high-affinity IgE receptor-positive canine mast cell line with wild-type c-kit receptors. **Biochemical and Biophysical Research Communications**, v. 366, n. 3, p. 857-861, 2008.

ANDERS, S.; PYL, P. T.; HUBER, W. HTSeq—a Python framework to work with high-throughput sequencing data. **Bioinformatics**, v. 31, n. 2, p. 166-169, 2015.

ARTUC, M.; STECKELINGS, U. M.; HENZ, B. M. Mast cell–fibroblast interactions: human mast cells as source and inducers of fibroblast and epithelial growth factors. **Journal of Investigative Dermatology**, v. 118, n. 3, p. 391-395, 2002.

AU, S. R.; AU, K.; SAGGERS, G. C.; KARNE, N.; EHRLICH, H. P. Rat mast cells communicate with fibroblasts via gap junction intercellular communications. **Journal of Cellular Biochemistry**, v. 100, n. 5, p. 1170-1177, 2007.

BAGGIOLINI, M.; WALZ, A.; KUNKEL, S. L. Neutrophil-activating peptide-1/interleukin 8, a novel cytokine that activates neutrophils. **The Journal of Clinical Investigation**, v. 84, n. 4, p. 1045-1049, 1989.

BARRY-HAMILTON, V.; SPANGLER, R.; MARSHALL, D.; MCCAULEY, S.; RODRIGUEZ, H. M.; OYASU, M. et al. Allosteric inhibition of lysyl oxidase–like-2 impedes the development of a pathologic microenvironment. **Nature Medicine**, v. 16, n. 9, p. 1009, 2010.

BARSKY, S. H.; GREEN, W. R.; GROTENDORST, G. R.; LIOTTA, L. A. Desmoplastic breast carcinoma as a source of human myofibroblasts. **The American Journal of Pathology**, v. 115, n. 3, p. 329, 1984.

BAUER, A. L.; JACKSON, T. L.; JIANG, Y. Topography of extracellular matrix mediates vascular morphogenesis and migration speeds in angiogenesis. **PLoS Computational Biology**, v. 5, n. 7, p. e1000445, 2009.

BHATIA, S. N.; BALIS, U. J.; YARMUSH, M. L.; TONER, M. Effect of cell–cell interactions in preservation of cellular phenotype: cocultivation of hepatocytes and nonparenchymal cells. **The FASEB Journal**, v. 13, n. 14, p. 1883-1900, 1999.

BHOWMICK, N. A.; NEILSON, E. G.; MOSES, H. L. Stromal fibroblasts in cancer initiation and progression. **Nature**, v. 432, n. 7015, p. 332, 2004.

BISCHOFF, S. C. Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data. **Nature Reviews Immunology**, v. 7, n. 2, p. 93, 2007.

BLACKWOOD, L.; MURPHY, S.; BURACCO, P.; DE VOS, J. P.; DE FORNEL-THIBAUD, P.; HIRSCHBERGER, J. et al. European consensus document on mast cell tumours in dogs and cats. **Veterinary and comparative oncology**, v. 10, n. 3, p. e1-e29, 2012.

BOERKAMP, K. M.; VAN DER KOOIJ, M.; VAN STEENBEEK, F. G.; VAN WOLFEREN, M. E.; GROOT KOERKAMP, M. J. A.; VAN LEENEN, D. et al. Gene expression profiling of histiocytic sarcomas in a canine model: the predisposed flatcoated retriever dog. **PLoS One**, v. 8, n. 8, p. e71094, 2013.

BOSTOCK, D. E. Neoplasms of the skin and subcutaneous tissues in dogs and cats. **British Veterinary Journal**, v. 142, n. 1, p. 1-19, 1986.

BOWMAN, P. D.; MEEK, R. L.; DANIEL, C. W. Aging of human fibroblasts in vitro: correlations between DNA synthetic ability and cell size. **Experimental Cell Research**, v. 93, n. 1, p. 184-190, 1975.

BRAZÍS, P.; TORRES, R.; QUERALT, M.; MORA, F. D.; FERRER, L.; PUIGDEMONT, A. Evaluation of cell-surface IgE receptors on the canine mastocytoma cell line C2 maintained in continuous culture. **American Journal of Veterinary Research**, v. 63, n. 5, p. 763-766, 2002.

BUTTERFIELD, J. H.; WEILER, D.; DEWALD, G.; GLEICH, G. J. Establishment of an immature mast cell line from a patient with mast cell leukemia. **Leukemia Research**, v. 12, n. 4, p. 345-355, 1988.

CAIRNS, J. A.; WALLS, A. F. Mast cell tryptase stimulates the synthesis of type I collagen in human lung fibroblasts. **The Journal of Clinical Investigation**, v. 99, n. 6, p. 1313-1321, 1997.

CALONICO, L. D.; PHILLIPS, M. J.; MCDONALD, D. M.; GOLD, W. M. An ultrastructural analysis of dog mastocytoma cells and normal mast cells. **The Anatomical Record**, v. 212, n. 4, p. 399-407, 1985.

CATALANO, V.; TURDO, A.; DI FRANCO, S.; DIELI, F.; TODARO, M.; STASSI, G. Tumor and its microenvironment: a synergistic interplay. **Seminars in Cancer Biology**, v. 23, p. 522-532, 2013.

CAUGHEY, G. H.; LAZARUS, S. C.; VIRO, N. F.; GOLD, W. M.; NADEL, J. A. Tryptase and chymase: comparison of extraction and release in two dog mastocytoma lines. **Immunology**, v. 63, n. 2, p. 339, 1988.

CHARLES, A. C.; NAUS, C. C.; ZHU, D.; KIDDER, G. M.; DIRKSEN, E. R.; SANDERSON, M. J. Intercellular calcium signaling via gap junctions in glioma cells. **The Journal of Cell Biology**, v. 118, n. 1, p. 195-201, 1992.

CHIAVEGATO, A.; BOCHATON-PIALLAT, M. L.; D'AMORE, E.; SARTORE, S.; GABBIANI, G. Expression of myosin heavy chain isoforms in mammary epithelial cells and in myofibroblasts from different fibrotic settings during neoplasia. **Virchows Archiv**, v. 426, n. 1, p. 77-86, 1995.

COHEN, D.; REIF J. S.; BRODEY R. S.; KEISER H. Epidemiological analysis of the most prevalent sites and types of canine neoplasia observed in a veterinary hospital. **Cancer Research**, v. 34, n. 11, p. 2859-2868, 1974.

CONTI, P.; BOUCHER, W.; LETOURNEAU, R.; FELICIANI, C.; REALE, M.; BARBACANE, R. C. et al. Monocyte chemotactic protein-1 provokes mast cell aggregation and [3H] 5HT release. **Immunology**, v. 86, n. 3, p. 434, 1995.

DAYTON, E. T.; CAULFIELD, J. P.; HEIN, A.; AUSTEN, K. F.; STEVENS, R. L. Regulation of the growth rate of mouse fibroblasts by IL-3-activated mouse bone marrow-derived mast cells. **The Journal of Immunology**, v. 142, n. 12, p. 4307-4313, 1989.

DEMORA, F.; GARCIA, G.; PUIGDEMONT, A.; ARBOIX, M.; FERRER, L. Skin mast cell releasability in dogs with atopic dermtitis. **Inflammation Research**, v. 45, n. 8, p. 424-427, 1996.

DEVINNEY, R.; GOLD, W. M. Establishment of two dog mastocytoma cell lines in continuous culture. **Studies**, v. 9, p. 10, 1990.

DIMRI, G. P.; LEE, X.; BASILE, G.; ACOSTA, M.; SCOTT, G.; ROSKELLEY, C.; MEDRANO, E. E.; LINSKENS, M.; RUBELJ, I.; PEREIRA-SMITH, O.; PEACOCKE, M.; CAMPISI, J. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. **Proceedings of the National Academy of Sciences**, v. 92, n. 20, p. 9363-9367, 1995.

DOBSON, J. M.; SAMUEL, S.; MILSTEIN, H.; ROGERS, K.; WOOD, J. L. N. Canine neoplasia in the UK: estimates of incidence rates from a population of insured dogs. **Journal of Small Animal Practice**, v. 43, n. 6, p. 240-246, 2002.

DREXLER, H. G.; MACLEOD, R. A. F. Editorial: Malignant hemoatopoietic cell lines: in vitro models for the study of mast cell leukemia. **Leukemia Research**, v. 27, p. 671–676, 2003.

DURNING, P.; SCHOR, S. L. Fibroblasts from patients with breast cancer show abnormal migratory behaviour in vitro. **The Lancet**, v. 324, n. 8408, p. 890-892, 1984.

DVORAK, A. M.; FURITSU, T.; KISSELL-RAINVILLE, S.; ISHIZAKA, T. Ultrastructural identification of human mast cells resembling skin mast cells stimulated to develop in long-term human cord blood mononuclear cells cultured with 3T3 murine skin fibroblasts. **Journal of Leukocyte Biology**, v. 51, n. 6, p. 557-569, 1992.

DVORAK, A. M.; SCHLEIMER, R. P.; SCHULMAN, E. S.; LICHTENSTEIN, L. M. Human mast cells use conservation and condensation mechanisms during recovery from degranulation. In vitro studies with mast cells purified from human lungs. **Laboratory Investigation; A Journal of Technical Methods and Pathology**, v. 54, n. 6, p. 663-678, 1986.

DVORAK, H. F. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. New England Journal of Medicine. V. 315, p. 1650–59, 1986.

EL GHALBZOURI, A.; PONEC, M. Diffusible factors released by fibroblasts support epidermal morphogenesis and deposition of basement membrane components. **Wound Repair and Regeneration**, v. 12, n. 3, p. 359-367, 2004.

EREZ, N.; TRUITT, M.; OLSON, P.; HANAHAN, D. Cancer-associated fibroblasts are activated in incipient neoplasia to orchestrate tumor-promoting inflammation in an NF-κB-dependent manner. **Cancer Cell**, v. 17, n. 2, p. 135-147, 2010.

ERKAN, M.; MICHALSKI, C. W.; RIEDER, S.; REISER-ERKAN, C.; ABIATARI, I.; KOLB, A.; GIESE, N. A.; ESPOSITO, I.; FRIESS, H.; KLEEF, J. The activated stroma index is a novel and independent prognostic marker in pancreatic ductal adenocarcinoma. **Clinical Gastroenterology and Hepatology**, v. 6, n. 10, p. 1155-1161, 2008.

ERLER, J. T.; BENNEWITH, K. L.; NICOLAU, M.; DORNHÖFER, N.; KONG, C.; LE, Q. T.; CHI, J. T.; JEFFREY, S. S.; GIACCIA, A. J. Lysyl oxidase is essential for hypoxia-induced metastasis. **Nature.** v. 27, n.440 (7088), p. 1222-6, 2006

FINNIE, J. W.; BOSTOCK, D. E. Skin neoplasia in dogs. **Australian Veterinary Journal**, v. 55, n. 12, p. 602-604, 1979.

FITZGERALD, S. M.; LEE, S. A.; HALL, H. K.; CHI, D. S.; KRISHNASWAMY, G. Human lung fibroblasts express interleukin-6 in response to signaling after mast cell contact. **American Journal of Respiratory Cell and Molecular Biology**, v. 30, n. 4, p. 585-593, 2004.

FOLEY, T. T.; EHRLICH, H. P. Through gap junction communications, co-cultured mast cells and fibroblasts generate fibroblast activities allied with hypertrophic scarring. **Plastic and reconstructive surgery**, v. 131, n. 5, p. 1036-1044, 2013.

FONG, S. F.; DIETZSCH, E.; FONG, K. S.; HOLLOSI, P.; ASUNCION, L.; HE, Q.; PARKER, M. I.; CSISZAR, K. Lysyl oxidase-like 2 expression is increased in colon and esophageal tumors and associated with less differentiated colon tumors. **Genes, Chromosomes and Cancer**, v. 46, n. 7, p. 644-655, 2007.

FOWLES, J. S.; DENTON, C. L.; GUSTAFSON, D. L. Comparative analysis of MAPK and PI3K/AKT pathway activation and inhibition in human and canine melanoma. **Veterinary and Comparative Oncology**, v. 13, n. 3, p. 288-304, 2015.

FRANTZ, A. M.; SARVER, A. L.; ITO, D.; PHANG, T. L.; KARIMPOUR-FARD, A.; SCOTT, M. C. et al. Molecular profiling reveals prognostically significant subtypes of canine lymphoma. **Veterinary Pathology**, v. 50, n. 4, p. 693-703, 2013.

FREIJE, W. A.; CASTRO-VARGAS, F. E.; FANG, Z.; HORVATH, S.; CLOUGHESY, T.; LIAU, L. M.; MISCHEL, O. S.; NELSON, S. F. Gene expression profiling of gliomas strongly predicts survival. **Cancer Research**, v. 64, n. 18, p. 6503-6510, 2004.

FUNK, W. D.; WANG, C. K.; SHELTON, D. N.; HARLEY, C. B.; PAGON, G. D.; HOEFFLER, W. K. Telomerase expression restores dermal integrity to in vitro-aged fibroblasts in a reconstituted skin model. **Experimental Cell Research**, v. 258, n. 2, p. 270-278, 2000.

FURITSU, T.; TSUJIMURA, T.; TONO, T.; IKEDA, H.; KITAYAMA, H.; KOSHIMIZU, U.; SUGIHARA, H.; BUTTERFIELD, J. H.; ASHMAN, L. K.; KANAYAMA, Y.; MATSUZAWA, Y.; KITAMURA, Y.; KANAKURA, Y. Identification of mutations in the coding sequence of the proto-oncogene c-kit in a human mast cell leukemia cell line causing ligand-independent activation of c-kit product. **The Journal of Clinical Investigation**, v. 92, n. 4, p. 1736-1744, 1993.

GABBIANI G. The myofibroblast in wound healing and fibrocontractive diseases. **The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland**, v. 200, n. 4, p. 500-503, 2003.

GALLI, S. J. The mast cell: a versatile effector cell for a challenging world. **International archives of allergy and immunology**, v. 113, n. 1-3, p. 14-22, 1997.

GARCIA, G.; BRAZIS, P.; MAJO, N.; FERRER, L.; DE MORA, F.; PUIGDEMONT, A. Comparative morphofunctional study of dispersed mature canine cutaneous mast cells and BR cells, a poorly differentiated mast cell line from a dog subcutaneous mastocytoma. **Veterinary Immunology and Immunopathology**, v. 62, n. 4, p. 323-337, 1998.

GIANTIN, M.; BARATTO, C.; MARCONATO, L.; VASCELLARI, M.; MUTINELLI, F.; DACASTO, M. et al. Transcriptomic analysis identified up-regulation of a solute carrier transporter and UDP glucuronosyltransferases in dogs with aggressive cutaneous mast cell tumours. **The Veterinary Journal**, v. 212, p. 36-43, 2016.

GIANTIN, M.; GRANATO, A.; BARATTO, C.; MARCONATO, L.; VASCELLARI, M.; MORELLO, E. M.; VERCELLI, A.; MUTINELLI, F.; DACASTO, M. Global gene expression analysis of canine cutaneous mast cell tumor: could molecular profiling be useful for subtype classification and prognostication?. **PloS One**, v. 9, n. 4, p. e95481, 2014.

GIULIANO, A.; DOS SANTOS HORTA, R.; CONSTANTINO-CASAS, F.; HOATHER, T.; DOBSON, J. Expression of Fibroblast Activating Protein and Correlation with Histological Grade, Mitotic Index and Ki67 Expression in Canine Mast Cell Tumours. **Journal of comparative pathology**, v. 156, n. 1, p. 14-20, 2017.

GIULIANO, A.; DOS SANTOS HORTA, R.; CONSTANTINO-CASAS, F.; HOATHER, T.; DOBSON, J. Expression of Fibroblast Activating Protein and Correlation with Histological Grade, Mitotic Index and Ki67 Expression in Canine Mast Cell Tumours. **Journal of Comparative Pathology**, v. 156, n. 1, p. 14-20, 2017.

GOETZL, E. J.; PHILLIPS, M. J.; GOLD, W. M. Stimulus specificity of the generation of leukotrienes by dog mastocytoma cells. **Journal of Experimental Medicine**, v. 158, n. 3, p. 731-737, 1983.

GORDEN, B. H.; KIM, J. H.; SARVER, A. L.; FRANTZ, A. M.; BREEN, M.; LINDBLAD-TOH, K. et al. Identification of three molecular and functional subtypes in canine hemangiosarcoma through gene expression profiling and progenitor cell characterization. **The American Journal of Pathology**, v. 184, n. 4, p. 985-995, 2014.

GRUBER, B. L. Mast cells: accessory cells which potentiate fibrosis. **International Reviews of Immunology**, v. 12, n. 2-4, p. 259-279, 1995.

GRUBER, B. L.; KEW, R. R.; JELASKA, A.; MARCHESE, M. J.; GARLICK, J.; REN S. et al. Human mast cells activate fibroblasts: tryptase is a fibrogenic factor stimulating collagen messenger ribonucleic acid synthesis and fibroblast chemotaxis. **The Journal of Immunology**, v. 158, n. 5, p. 2310-2317, 1997.

GRÜNTZIG, K.; GRAF, R.; BOO, G.; GUSCETTI, F.; HÄSSIG, M.; AXHAUSEN, K. W.; FABRIKANT, S.; WELLE M.; MEIER, D.; FOLKERS, G.; POSPISCHIL, A. Swiss Canine Cancer Registry 1955-2008: occurrence of the most common tumour diagnoses and influence of age, breed, body size, sex and neutering status on tumour development. **Journal of Comparative Pathology**, v. 155, n. 2-3, p. 156-170, 2016.

GUECK, T.; SEIDEL, A.; FUHRMANN, H. Effects of essential fatty acids on mediators of mast cells in culture. **Prostaglandins, Leukotrienes and Essential Fatty acids**, v. 68, n. 5, p. 317-322, 2003.

GYOTOKU, E.; MORITA, E.; KAMEYOSHI, Y.; HIRAGUN, T.; YAMAMOTO, S.; HIDE, M. The IL-6 family cytokines, interleukin-6, interleukin-11, oncostatin M, and leukemia inhibitory factor, enhance mast cell growth through fibroblast-dependent pathway in mice. **Archives of Dermatological Research**, v. 293, n. 10, p. 508-514, 2001.

HEINRICH, P. C.; BEHRMANN, I.; MULLER-NEWEN, G.; SCHAPER, F.; GRAEVE, L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. **Biochemical Journal**, v. 334, n. 2, p. 297-314, 1998.

HOLLE, A. W.; YOUNG, J. L.; SPATZ, J. P. In vitro cancer cell–ECM interactions inform in vivo cancer treatment. **Advanced drug delivery reviews**, v. 97, p. 270-279, 2016.

HOLLOSI, P.; YAKUSHIJI, J. K.; FONG, K. S.; CSISZAR, K.; FONG, S. F. Lysyl oxidase-like 2 promotes migration in noninvasive breast cancer cells but not in normal breast epithelial cells. **International Journal of Cancer**, v. 125, n. 2, p. 318-327, 2009.

HORTON, M. A.; O'BRIEN, H. A. Characterization of human mast cells in long-term culture. **Blood**, v. 62, n. 6, p. 1251-1260, 1983.

HOTTENDORF, G. H.; NIELSEN, S. W. Pathologic report of 29 necropsies on dogs with mastocytoma. **Pathologia Veterinaria**, v. 5, n. 2, p. 102-121, 1968.

HUGO, H. J.; LEBRET, S.; TOMASKOVIC-CROOK, E.; AHMED, N.; BLICK, T.; NEWGREEN, D. F. et al. Contribution of fibroblast and mast cell (afferent) and tumor (efferent) IL-6 effects within the tumor microenvironment. **Cancer Microenvironment**, v. 5, n. 1, p. 83-93, 2012.

HWANG, R. F.; MOORE, T.; ARUMUGAM, T.; RAMACHANDRAN, V.; AMOS, K. D.; RIVERA, A.; JI, B.; EVANS, D. B.; LOGSDON, C. D. Cancer-associated stromal fibroblasts promote pancreatic tumor progression. **Cancer research**, v. 68, n. 3, p. 918-926, 2008.

INAMURA, N.; MEKORI, Y. A.; BHATTACHARYYA, S. P.; BIANCHINE, P. J.; METCALFE, D. D. Induction and enhancement of FcεRI-dependent mast cell degranulation following coculture with activated T cells: dependency on ICAM-1-and leukocyte function-associated antigen (LFA)-1-mediated heterotypic aggregation. **The Journal of Immunology**, v. 160, n. 8, p. 4026-4033, 1998.

ISHIGURO, T.; KADOSAWA, T.; MORI, K.; TAKAGI, S.; OKUMURA, M.; FUJINAGA, T. Establishment and characterization of a new canine mast cell tumor cell line. **Journal of Veterinary Medical Science**, v. 63, n. 9, p. 1031-1034, 2001.

ITO, A.; NAKAJIMA, S.; SASAGURI, Y.; NAGASE, H.; MORI, Y. Co-culture of human breast adenocarcinoma MCF-7 cells and human dermal fibroblasts enhances the production of matrix metalloproteinases 1, 2 and 3 in fibroblasts. **British Journal of Cancer**, v. 71, n. 5, p. 1039, 1995.

KAGAN, H. M.; LI, W. Lysyl oxidase: properties, specificity, and biological roles inside and outside of the cell. **Journal of Cellular Biochemistry**, v. 88, n. 4, p. 660-672, 2003.

KAJI, H.; CAMCI-UNAL, G.; LANGER, R.; KHADEMHOSSEINI, A. Engineering systems for the generation of patterned co-cultures for controlling cell–cell interactions. **Biochimica et Biophysica Acta (BBA)-General Subjects**, v. 1810, n. 3, p. 239-250, 2011.

KALLURI, R.; ZEISBERG, M. Fibroblasts in cancer. **Nature Reviews Cancer**, v. 6, n. 5, p. 392, 2006.

KAMBE, M.; KAMBE, N.; OSKERITZIAN, C. A.; SCHECHTER, N.; SCHWARTZ, L. B. IL-6 attenuates apoptosis, while neither IL-6 nor IL-10 affect the numbers or protease phenotype of fetal liver-derived human mast cells. **Clinical & Experimental Allergy**, v. 31, n. 7, p. 1077-1085, 2001.

KIM, J.; HAYWARD, R. C. Mimicking dynamic in vivo environments with stimuli-responsive materials for cell culture. **Trends in Biotechnology**, v. 30, n. 8, p. 426-439, 2012.

KIRSCHMANN, D. A.; SEFTOR, E. A.; FONG, S. F.; NIEVA, D. R. C.; SULLIVAN, C. M.; EDWARDS, E. M.; SOMMER, P.; CSISZAR, K.; HENDRIX, M. J. C. A molecular role for lysyl oxidase in breast cancer invasion. **Cancer Research**, v. 62, n. 15, p. 4478-4483, 2002.

KIUPEL, M.; WEBSTER, J. D.; BAILEY, K. L.; BEST, S.; DELAY, J.; DETRISAC, C. J. et al. Proposal of a 2-tier histologic grading system for canine cutaneous mast cell tumors to more accurately predict biological behavior. **Veterinary pathology**, v. 48, n. 1, p. 147-155, 2011.

KLOBUKOWSKA, H. J.; MUNDAY, J. S. High numbers of stromal cancer-associated fibroblasts are associated with a shorter survival time in cats with oral squamous cell carcinoma. **Veterinary Pathology**, v. 53, n. 6, p. 1124-1130, 2016.

KLOPFLEISCH, R.; LENZE, D.; HUMMEL, M.; GRUBER A. D. Metastatic canine mammary carcinomas can be identified by a gene expression profile that partly overlaps with human breast cancer profiles. **BMC Cancer**, v. 10, n. 1, p. 618, 2010.

KLOPFLEISCH, R.; VON EULER, H.; SARLI, G.; PINHO, S. S.; GÄRTNER, F.; GRUBER, A. D. Molecular carcinogenesis of canine mammary tumors: news from an old disease. **Veterinary Pathology**, v. 48, n. 1, p. 98-116, 2011.

KOHN, R. R. Aging and cell division. **Science**, v. 188, n. 4185, p. 203-204, 1975.

KOJIMA, Y.; ACAR, A.; EATON, E. N.; MELLODY, K. T.; SCHEEL, C.; BEN-PORATH, I. et al. Autocrine TGF-β and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. **Proceedings of the National Academy of Sciences**, v. 107, n. 46, p. 20009-20014, 2010.

LANGDON, S. P. Introduction to Cancer Cell Culture: basic Principles of Cancer Cell Culture. In: LANGDON, S. P. **Cancer Cell Culture:** Methods and Protocols. Volume 88. Nova York: Human Press Inc, 2003.

LANGFELDER, P.; HORVATH, S. WGCNA: an R package for weighted correlation network analysis. **BMC Bioinformatics**, v. 9, n. 1, p. 559, 2008.

LANGFELDER, P.; ZHANG, B.; HORVATH, S. Defining clusters from a hierarchical cluster tree: the Dynamic Tree Cut package for R. **Bioinformatics**, v. 24, n. 5, p. 719-720, 2007.

LAZARUS, S. C.; DEVINNEY, R.; MCCABE, L. J.; FINKBEINER, W. E.; ELIAS, D. J.; GOLD, W. M. Isolated canine mastocytoma cells: propagation and characterization of two cell lines. **American Journal of Physiology-Cell Physiology**, v. 251, n. 6, p. C935-C944, 1986.

LEE, H. N.; KIM, C. H.; SONG, G. G.; CHO, S. W. Effects of IL-3 and SCF on histamine production kinetics and cell phenotype in rat bone marrow-derived mast cells. **Immune Network**, v. 10, n. 1, p. 15-25, 2010.

LEVI-SCHAFFER, F.; AUSTEN, K. F.; CAULFIELD, J. P.; HEIN, A.; GRAVALLESE, P. M.; STEVENS, R. L. Co-culture of human lung-derived mast cells with mouse 3T3 fibroblasts: morphology and IgE-mediated release of histamine, prostaglandin D2, and leukotrienes. **The Journal of Immunology**, v. 139, n. 2, p. 494-500, 1987.

LEVI-SCHAFFER, F.; AUSTEN, K. F.; GRAVALLESE, P. M.; STEVENS, R. L. Coculture of interleukin 3-dependent mouse mast cells with fibroblasts results in a phenotypic change of the mast cells. **Proceedings of the National Academy of Sciences**, v. 83, n. 17, p. 6485-6488, 1986.

LEVI-SCHAFFER, F.; RUBINCHIK E. Activated mast cells are fibrogenic for 3T3 fibroblasts. **Journal of Investigative Dermatology**, v. 104, n. 6, p. 999-1003, 1995.

LEVI-SCHAFFER, F.; SEGAL, V.; SHALIT, M. Effects of interleukins on connective tissue type mast cells co-cultured with fibroblasts. **Immunology**, v. 72, n. 2, p. 174, 1991.

LI, H.; HANDSAKER, B.; WYSOKER, A.; FENNELL, T.; RUAN, J.; HOMER, N.; ABECASIS, G.; DURBIN, R. The sequence alignment/map format and SAMtools. **Bioinformatics**, v. 25, n. 16, p. 2078-2079, 2009.

LIN, T. Y.; RUSH, L. J.; LONDON, C. A. Generation and characterization of bone marrow-derived cultured canine mast cells. **Veterinary Immunology and Immunopathology**, v. 113, n. 1-2, p. 37-52, 2006.

LIU, D.; XIONG, H.; ELLIS, A. E.; NORTHRUP, N. C.; DOBBIN, K. K.; SHIN, D. M. et al. Canine spontaneous head and neck squamous cell carcinomas represent their human counterparts at the molecular level. **PLoS Genetics**, v. 11, n. 6, p. e1005277, 2015.

LIU, D.; XIONG, H.; ELLIS, A. E.; NORTHRUP, N. C.; RODRIGUEZ, C. O.; O'REGAN, R. M. et al. Molecular homology and difference between spontaneous canine mammary cancer and human breast cancer. **Cancer Research**, 2014.

LIVAK, K. J.; SCHMITTGEN, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. **Methods**, v. 25, n. 4, p. 402-408, 2001.

LONDON, C. A.; SEGUIN, B. Mast cell tumors in the dog. **The Veterinary clinics of North America. Small animal practice**, v. 33, n. 3, p. 473-89, v, 2003.

MARGULIS, A.; NOCKA, K. H.; BRENNAN, A. M.; DENG, B.; FLEMING, M.; GOLDMAN, S. J.; KASAIAN, M. T. Mast cell-dependent contraction of human airway smooth muscle cell-containing collagen gels: influence of cytokines, matrix metalloproteases, and serine proteases. **The Journal of Immunology**, p. jimmunol. 0803951, 2009.

MARQUIS, B. J.; HAYNES, C. L. The effects of co-culture of fibroblasts on mast cell exocytotic release characteristics as evaluated by carbon-fiber microelectrode amperometry. **Biophysical Chemistry**, v. 137, n. 1, p. 63-69, 2008.

MARTIN, G. M.; SPRAGUE, C. A.; EPSTEIN, C. J. Replicative life-span of cultivated human cells. **Lab. Invest**, v. 23, n. 1, p. 86-92, 1970.

MATSUDA, A.; TANAKA, A.; AMAGAI, Y.; OHMORI, K.; NISHIKAWA, S.; XIA, Y. et al. Glucocorticoid sensitivity depends on expression levels of glucocorticoid receptors in canine neoplastic mast cells. **Veterinary Immunology and Immunopathology**, v. 144, n. 3-4, p. 321-328, 2011.

MATSUSHIMA, K.; BALDWIN, E. T.; MUKAIDA, N. Interleukin-8 and MCAF: novel leukocyte recruitment and activating cytokines. In: KISHIMOTO T. **Interleukins: molecular biology and immunology**. Karger Publishers, 1992. p. 236-265.

MICKE, P.; OSTMAN, A. Tumour-stroma interaction: cancer-associated fibroblasts as novel targets in anti-cancer therapy?. **Lung Cancer**, v. 45, p. S163-S175, 2004.

MIKI, Y.; ONO, K.; HATA, S.; SUZUKI, T.; KUMAMOTO, H.; SASANO, H. The advantages of co-culture over mono cell culture in simulating in vivo environment. **The Journal of Steroid Biochemistry and Molecular Biology**, v. 131, n. 3-5, p. 68-75, 2012.

MILLAR, H. J.; NEMETH, J. A.; MCCABE, F. L.; PIKOUNIS, B.; WICKSTROM, E. Circulating Human Interleukin-8 as an Indicator of Cancer Progression in a Nude Rat Orthotopic Human Non–Small Cell Lung Carcinoma Model. **Cancer Epidemiology and Prevention Biomarkers**, v. 17, n. 8, p. 2180-2187, 2008.

MISCHEL, P. S.; NELSON, S. F.; CLOUGHESY, T. F. Molecular analysis of glioblastoma: pathway profiling and its implications for patient therapy. **Cancer Biology & Therapy**, v. 2, n. 3, p. 242-247, 2003.

MONTIER, Y.; LORENTZ, A.; KRÄMER, S.; SELLGE, G.; SCHOCK, M.; BAUER, M. et al. Central role of IL-6 and MMP-1 for cross talk between human intestinal mast cells and human intestinal fibroblasts. **Immunobiology**, v. 217, n. 9, p. 912-919, 2012.

MOONEY, M.; BOND, J.; MONKS, N.; EUGSTER, E.; CHERBA, D.; BERLINSKI, P. et al. Comparative RNA-Seq and microarray analysis of gene expression changes in B-cell lymphomas of Canis familiaris. **PloS One**, v. 8, n. 4, p. e61088, 2013.

MOYER, K. E.; SAGGERS, G. C.; EHRLICH, H. P. Mast cells promote fibroblast populated collagen lattice contraction through gap junction intercellular communication. **Wound Repair and Regeneration**, v. 12, n. 3, p. 269-275, 2004.

MUDALIAR, M. A. V.; HAGGART, R. D.; MIELE, G.; SELLAR, G.; TAN, K. A. L.; GOODLAD, J. R. et al. Comparative gene expression profiling identifies common molecular signatures of NF-κB activation in canine and human diffuse large B cell lymphoma (DLBCL). **PloS One**, v. 8, n. 9, p. e72591, 2013.

MUELLER, M. M.; FUSENIG, N. E. Friends or foes—bipolar effects of the tumour stroma in cancer. **Nature Reviews Cancer**, v. 4, n. 11, p. 839, 2004.

NABESHIMA, Y.; HIRAGUN, T.; MORITA, E.; MIHARA, S.; KAMEYOSHI, Y.; HIDE, M. IL-4 modulates the histamine content of mast cells in a mast cell/fibroblast co-culture through a Stat6 signaling pathway in fibroblasts. **FEBS Letters**, v. 579, n. 29, p. 6653-6658, 2005.

NAGAMINE, M. K.; SANCHES, D. S.; PINELLO, K. C.; TORRES, L. N.; MENNECIER, G.; LATORRE, A. O.; FUKUMASU, H.; DAGLI, M. L. Z. In vitro inhibitory effect of trichostatin A on canine grade 3 mast cell tumor. **Veterinary Research Communications**, v. 35, n. 6, p. 391-399, 2011.

O'DONOGHUE, L. E.; PTITSYN, A A.; KAMSTOCK, D. A.; SIEBERT, J.; THOMAS, R. S.; DUVAL, D. L. Expression profiling in canine osteosarcoma: identification of biomarkers and pathways associated with outcome. **BMC Cancer**, v. 10, n. 1, p. 506, 2010.

OGAWA, M.; NAKAHATA, T.; LEARY, A. G.; STERK, A. R.; ISHIZAKA, K.; ISHIZAKA, T. Suspension culture of human mast cells/basophils from umbilical cord blood mononuclear cells. **Proceedings of the National Academy of Sciences**, v. 80, n. 14, p. 4494-4498, 1983.

OHASHI, E.; MIYAJIMA, N.; NAKAGAWA, T.; TAKAHASHI, T.; KAGECHIKA, H.; MOCHIZUKI, M.; NISHIMURA, R.; SASAKI, N. Retinoids induce growth inhibition and apoptosis in mast cell tumor cell lines. **Journal of Veterinary Medical Science**, v. 68, n. 8, p. 797-802, 2006.

OHMORI, K.; KAWARAI, S.; YASUDA, N.; TANAKA, A.; MATSUDA, H.; NISHIMURA, R.; SASAKI, N.; TSUJIMOTO, H.; MASUDA, K. Identification of c-kit mutations-independent neoplastic cell proliferation of canine mast cells. **Veterinary Immunology and Immunopathology**, v. 126, n. 1-2, p. 43-53, 2008.

ORIMO, A.; GUPTA, P. B.; SGROI, D. C.; ARENZANA-SEISDEDOS, F.; DELAUNAY, T.; NAEEM, R.; CAREY, V. J.; RICHARDSON, A. L.; WEINBERG, R. A. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. **Cell**, v. 121, n. 3, p. 335-348, 2005.

ORIMO, A.; WEINBERG, R. A. Stromal fibroblasts in cancer: a novel tumor-promoting cell type. **Cell Cycle**, v. 5, n. 15, p. 1597-1601, 2006.

ÖSTMAN, A.; AUGSTEN, M. Cancer-associated fibroblasts and tumor growth–bystanders turning into key players. **Current opinion in genetics & development**, v. 19, n. 1, p. 67-73, 2009.

OZSOLAK, F.; MILOS, P. M. RNA sequencing: advances, challenges and opportunities. **Nature Reviews Genetics**, v. 12, n. 2, p. 87, 2011.

PANG, L. Y.; GATENBY, E. L.; KAMIDA, A.; WHITELAW, B. A.; HUPP, T. R.; ARGYLE, D. J. Global gene expression analysis of canine osteosarcoma stem cells reveals a novel role for COX-2 in tumour initiation. **PLoS One**, v. 9, n. 1, p. e83144, 2014.

PATHAK, A.; KUMAR, S. Independent regulation of tumor cell migration by matrix stiffness and confinement. **Proceedings of the National Academy of Sciences**, v. 109, n. 26, p. 10334-10339, 2012.

PATNAIK, A. K.; EHLER, W. J.; MACEWEN, E. G. Canine cutaneous mast cell tumor: morphologic grading and survival time in 83 dogs. **Veterinary Pathology**, v. 21, n. 5, p. 469-474, 1984.

PAYNE, S. L.; HENDRIX, M. J.; KIRSCHMANN, D. A. Paradoxical roles for lysyl oxidases in cancer—a prospect. **Journal of Cellular Biochemistry**, v. 101, n. 6, p. 1338-1354, 2007.

PENG, L.; RAN, Y. L.; HU, H.; YU, L.; LIU, Q.; ZHOU, Z.; SUN, Y. M.; SUN, L. C.; PAN, J.; SUN, L. X.; ZHAO, P.; YANG, Z. H. Secreted LOXL2 is a novel therapeutic target that promotes gastric cancer metastasis via the Src/FAK pathway. **Carcinogenesis**, v. 30, n. 10, p. 1660-1669, 2009.

PIETRAS, K.; ÖSTMAN, A. Hallmarks of cancer: interactions with the tumor stroma. **Experimental Cell Research**, v. 316, n. 8, p. 1324-1331, 2010.

PINELLO, K. C.; NAGAMINE, M.; SILVA, T. C.; MATSUZAKI, P.; CAETANO, H. V.; TORRES L. N.; FUKUMASU, H.; DAGLI, M. L. Z. In vitro chemosensitivity of canine mast cell tumors grades II and III to all-trans-retinoic acid (ATRA). **Veterinary Research Communications**, v. 33, n. 6, p. 581-588, 2009.

PRIESTER, W. A. Skin tumors in domestic animals. Data from 12 United States and Canadian colleges of veterinary medicine. **Journal of the National Cancer Institute**, v. 50, n. 2, p. 457-466, 1973.

PULZ L. H. **Canine cutaneous Mast Cells Tumor: molecular characterization, culture and intercellular interactions between mast cells and stromal fibroblasts.** Data de depósito. Total de páginas/folhas. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo-SP, 15 de março de 2019.

PULZ, L. H.; BARRA, C. N.; KLEEB, S. R.; XAVIER, J. G.; CATÃO-DIAS, J. L.; SOBRAL, R. A.; FUKUMASU, H.; STREFEZZI, R. F. Increased expression of Tissue Inhibitor of Metalloproteinase-1 correlates with improved outcome in canine cutaneous mast cell tumours. **Veterinary And Comparative Oncology**, v. 15, n. 2, p. 606-614, 2017.

QIAN, L. W.; MIZUMOTO, K.; MAEHARA, N.; OHUCHIDA, K.; INADOME, N.; SAIMURA, M. et al. Co-cultivation of pancreatic cancer cells with orthotopic tumor-derived fibroblasts: fibroblasts stimulate tumor cell invasion via HGF secretion whereas cancer cells exert a minor regulative effect on fibroblasts HGF production. **Cancer Letters**, v. 190, n. 1, p. 105-112, 2003.

RAIZMAN, M. B.; AUSTEN, K. F.; KATZ, H. R. Mast cell heterogeneity. Differential synthesis and expression of glycosphingolipids by mouse serosal mast cells as compared to IL-3-dependent bone marrow culture-derived mast cells before or after coculture with 3T3 fibroblasts. **The Journal of Immunology**, v. 145, n. 5, p. 1463-1468, 1990.

RAIZMAN, M. B.; AUSTEN, K. F.; KATZ, H. R. Mast cell heterogeneity. Differential synthesis and expression of glycosphingolipids by mouse serosal mast cells as compared to IL-3-dependent bone marrow culture-derived mast cells before or after coculture with 3T3 fibroblasts. **The Journal of Immunology**, v. 145, n. 5, p. 1463-1468, 1990.

RAMSEY, S. A.; XU, T.; GOODALL, C.; RHODES, A. C.; KASHYAP, A.; HE, J. et al. Cross-species analysis of the canine and human bladder cancer transcriptome and exome. **Genes, Chromosomes and Cancer**, v. 56, n. 4, p. 328-343, 2017.

RAO, P. S.; FRIEDMAN, M. M.; ATKINS, F. M.; METCALFE, D. D. Phagocytosis of mast cell granules by cultured fibroblasts. **The Journal of Immunology**, v. 130, n. 1, p. 341-349, 1983.

RENNICK, D.; HUNTE, B.; HOLLAND, G.; THOMPSON-SNIPES, L. Cofactors are essential for stem cell factor-dependent growth and maturation of mast cell progenitors: comparative effects of interleukin-3 (IL-3), IL-4, IL-10, and fibroblasts. **Blood**, v. 85, n. 1, p. 57-65, 1995.

ROBINSON, M. D.; MCCARTHY, D. J.; SMYTH, G. K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. **Bioinformatics**, v. 26, n. 1, p. 139-140, 2010.

ROFSTAD, E. K.; HALSØR, E. F. Vascular endothelial growth factor, interleukin 8, platelet-derived endothelial cell growth factor, and basic fibroblast growth factor promote angiogenesis and metastasis in human melanoma xenografts. **Cancer Research**, v. 60, n. 17, p. 4932-4938, 2000.

RONNOV-JESSEN, L.; PETERSEN, O. W.; BISSELL, M. J. Cellular changes involved in conversion of normal to malignant breast: importance of the stromal reaction. **Physiological reviews**, v. 76, n. 1, p. 69-125, 1996.

ROSSI, G. L.; DI COMITE, V.; OLIVIERI, D. Mast cell cultures: bench to bedside. **Clinical & Experimental Allergy**, v. 28, n. 10, p. 1182-1190, 1998.

SABATTINI, S.; SCARPA, F.; BERLATO, D.; BETTINI, G. Histologic grading of canine mast cell tumor: is 2 better than 3?. **Veterinary Pathology**, v. 52, n. 1, p. 70-73, 2015.

SALOMON, D.; SAURAT, J. H.; MEDA, P. Cell-to-cell communication within intact human skin. **The Journal of clinical investigation**, v. 82, n. 1, p. 248-254, 1988.

SAMOSZUK, M.; TAN, J.; CHORN, G. Clonogenic growth of human breast cancer cells co-cultured in direct contact with serum-activated fibroblasts. **Breast Cancer Research**, v. 7, n. 3, p. R274, 2005.

SCASE, T. J.; EDWARDS, D.; MILLER, J.; HENLEY, W.; SMITH, K.; BLUNDEN, A. et al. Canine mast cell tumors: correlation of apoptosis and proliferation markers with prognosis. **Journal of Veterinary Internal Medicine**, v. 20, n. 1, p. 151-158, 2006.

SCHALL, T. J.; BACON, K. B. Chemokines, leukocyte trafficking, and inflammation. **Current Opinion in Immunology**, v. 6, n. 6, p. 865-873, 1994.

SCHNEIDER, E. L.; MITSUI, Y. The relationship between in vitro cellular aging and in vivo human age. **Proceedings of the National Academy of Sciences**, v. 73, n. 10, p. 3584-3588, 1976.

SCHOR, S. L.; SCHOR, A. M.; GREY, A. M.; RUSHTON, G. Foetal and cancer patient fibroblasts produce an autocrine migration-stimulating factor not made by normal adult cells**. Journal of Cell Science**, v. 90, n. 3, p. 391-399, 1988.

SCHULTZ, G. S.; WYSOCKI, A. Interactions between extracellular matrix and growth factors in wound healing. **Wound Repair and Regeneration**, v. 17, n. 2, p. 153-162, 2009.

SCHVARTZ, I.; SEGER, D.; SHALTIEL, S. Vitronectin. **The International Journal of Biochemistry & Cell Biology**, v. 31, n. 5, p. 539-544, 1999.

SELVARAJAH, G. T.; KIRPENSTEIJN, J.; VAN WOLFEREN, M. E.; RAO, N. A. S.; FIETEN, H.; MOL, J. A.Gene expression profiling of canine osteosarcoma reveals genes associated with short and long survival times. **Molecular Cancer**, v. 8, n. 1, p. 72, 2009.

SHAHZAD, M. M.; AREVALO, J. M.; ARMAIZ-PENA, G. N.; LU, C.; STONE, R. L.; MORENO-SMITH, M.; NISHIMURA, M.; LEE, J. W.; JENNINGS, N. B.; BOTTSFORD-MILLER, J. et al. Stress effects on FOSB and interleukin-8 (IL8) driven ovarian cancer growth and metastasis. **Journal of Biological Chemistry**, p. jbc-M110. 109579, 2010.

SHOOP, S. J.; MARLOW, S.; CHURCH, D. B.; ENGLISH, K.; MCGREEVY, P. D.; STELL, A. J. et al. Prevalence and risk factors for mast cell tumours in dogs in England. **Canine Genetics and Epidemiology**, v. 2, n. 1, p. 1, 2015.

SKÖLD, C. M.; OHKUNI, Y.; LIU, X. D.; NUMEROF, R.; RENNARD, S. I. Co-cultured human mast cells stimulate fibroblast-mediated contraction of collagen gels. **Inflammation**, v. 25, n. 1, p. 47-51, 2001. SOLL, A. H.; LEWIN, K.; BEAVEN, M. A. Isolation of histamine-containing cells from canine fundic mucosa. **Gastroenterology**, v. 77, n. 6, p. 1283-1290, 1979.

SOLL, A. H.; TOOMEY, M.; CULP, D.; SHANAHAN, F.; BEAVEN, M. A. Modulation of histamine release from canine fundic mucosal mast cells. **American Journal of Physiology-Gastrointestinal and Liver Physiology**, v. 254, n. 1, p. G40-G48, 1988.

SPIEKSTRA, S. W.; BREETVELD, M.; RUSTEMEYER, T.; SCHEPER, R. J.; GIBBS, S. Wound-healing factors secreted by epidermal keratinocytes and dermal fibroblasts in skin substitutes. **Wound Repair and Regeneration**, v. 15, n. 5, p. 708-717, 2007.

SPRAY, D. C.; BENNETT, M. V. L. Physiology and pharmacology of gap junctions. **Annual Review of Physiology**, v. 47, n. 1, p. 281-303, 1985.

STREFEZZI, R. D. F.; KLEEB, S. R.; XAVIER, J. G.; DIAS, J. L. C. Avaliação da proliferação celular como indicador prognóstico para mastocitomas cutâneos caninos. **Pesquisa Veterinária Brasileira**, v. 30, n. 7, p. 559-565, 2010.

STROBER, W. Trypan blue exclusion test of cell viability. **Current Protocols in Immunology**, v. 21, n. 1, p. A. 3B. 1-A. 3B. 2, 1997.

SUBRAMANIAN, A.; TAMAYO, P.; MOOTHA, V. K.; MUKHERJEE, S.; EBERT, B. L.; GILLETTE, M. A.; PAULOVICH, A.; POMEROY, S. L.; GOLUB, T. R.; LANDER, E. S.; MESIROV, J. P. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. **Proceedings of the National Academy of Sciences**, v. 102, n. 43, p. 15545-15550, 2005.

SUN, X.; MAO, Y.; WANG, J.; ZU, L.; HAO, M.; CHENG, G. et al. IL-6 secreted by cancer-associated fibroblasts induces tamoxifen resistance in luminal breast cancer. **Oncogene**, 2014.

SUZUKI, S.; SATO, M.; SENOO, H.; ISHIKAWA, K. Direct cell–cell interaction enhances pro-MMP-2 production and activation in co-culture of laryngeal cancer cells and fibroblasts: involvement of EMMPRIN and MT1-MMP. **Experimental Cell Research**, v. 293, n. 2, p. 259-266, 2004.

TAKAHASHI, T.; KITANI, S.; NAGASE, M.; MOCHIZUKI, M.; NISHIMURA, R.; MORITA, Y.; SASAKI, N. IgG-mediated histamine release from canine mastocytoma-derived cells. **International Archives of Allergy and Immunology**, v. 125, n. 3, p. 228-235, 2001.

TAKAHASHI, T.; OHASHI, E.; NAKAGAWA, T.; MOCHIZUKI, M.; NISHIMURA, R.; SASAKI, N. Role of β1 integrins in adhesion of canine mastocytoma cells to extracellular matrix proteins. **Journal of Veterinary Medical Science**, v. 69, n. 5, p. 495-499, 2007.

TAMBURINI, B. A.; PHANG, T. L.; FOSMIRE, S. P.; SCOTT, M. C.; TRAPP, S. C.; DUCKETT, M. M. et al. Gene expression profiling identifies inflammation and angiogenesis as distinguishing features of canine hemangiosarcoma. **BMC Cancer**, v. 10, n. 1, p. 619, 2010.

TERMEI, R.; LASCHINGER, C.; LEE, W.; MCCULLOCH, C. A. Intercellular interactions between mast cells and fibroblasts promote pro-inflammatory signaling. **Experimental cell research**, v. 319, n. 12, p. 1839-1851, 2013.

TERTIAN, G.; YUNG, Y-P.; GUY-GRAND, D.; MOORE, M. A. Long-term in vitro culture of murine mast cells. I. Description of a growth factor-dependent culture technique. **The Journal of Immunology**, v. 127, n. 2, p. 788-794, 1981.

THIERY, J. P.; ACLOQUE, H.; HUANG, R. Y.; NIETO, M. A. Epithelial-mesenchymal transitions in development and disease. **Cell**, v. 139, n. 5, p. 871-890, 2009.

THOMAS, R.; BORST, L.; ROTROFF, D.; MOTSINGER-REIF, A.; LINDBLAD-TOH, K.; MODIANO, J. F. et al. Genomic profiling reveals extensive heterogeneity in somatic DNA copy number aberrations of canine hemangiosarcoma. **Chromosome Research**, v. 22, n. 3, p. 305-319, 2014.

TRAUTMANN, A.; FEUNERSTEIN, B.; ERNST, N.; BRÖCKER, E.B.; KLEIN, C. E. Heterotypic cell-cell adhesion of human mast cells to fibroblasts. **Archives of dermatological research**, v. 289, n. 4, p. 194-203, 1997.

TRAUTMANN, A.; KROHNE, G.; BRÖCKER, E. B.; KLEIN, C. E. Human mast cells augment fibroblast proliferation by heterotypic cell-cell adhesion and action of IL-4. **The Journal of Immunology**, v. 160, n. 10, p. 5053-5057, 1998.

TSAI, M.; TAKEISHI, T.; THOMPSON, H.; LANGLEY, K. E.; ZSEBO, K. M.; METCALFE, D. D., et al. Induction of mast cell proliferation, maturation, and heparin synthesis by the rat c-kit ligand, stem cell factor. **Proceedings of the National Academy of Sciences**, v. 88, n. 14, p. 6382-6386, 1991.

TSUJINO, T.; SESHIMO, I.; YAMAMOTO, H.; NGAN, C. Y.; EZUMI, K.; TAKEMASA, I.; IKEDA, M.; SEKIMOTO, M.; MATSUURA, N.; MONDEN, M. Stromal myofibroblasts predict disease recurrence for colorectal cancer. **Clinical Cancer Research**, v. 13, n. 7, p. 2082-2090, 2007.

TSUKADA, T.; MCNUTT, M. A.; ROSS, R.; GOWN, A. M. HHF35, a muscle actin-specific monoclonal antibody. II. Reactivity in normal, reactive, and neoplastic human tissues. **The American Journal of Pathology**, v. 127, n. 2, p. 389, 1987.

UVA, P.; AURISICCHIO, L.; WATTERS, J.; LOBODA, A.; KULKARNI, A.; CASTLE J. et al. Comparative expression pathway analysis of human and canine mammary tumors. **BMC Genomics**, v. 10, n. 1, p. 135, 2009.

VAIL, D. M. Mast cell tumors. In: WITHROW S. J, MACEWEN E. G. **Small Animal Clinical Oncology**. Philadelphia: W.B. Saunders, 1996. p. 192–210.

VALENTI, M. T.; AZZARELLO, G.; BALDUCCI, E.; SARTORE, S.; SANDRI, M.; MANCONI, R. et al. Conditioned medium from MCF-7 cell line induces myofibroblast differentiation, decreased cell proliferation, and increased apoptosis in cultured normal fibroblasts but not in fibroblasts from malignant breast tissue. **The Histochemical Journal**, v. 33, n. 9-10, p. 499-509, 2001.

VASCELLARI, M.; GIANTIN, M.; CAPELLO, K.; CARMINATO, A.; MORELLO, E. M.; VERCELLI,

A. et al. Expression of Ki67, BCL-2, and COX-2 in canine cutaneous mast cell tumors: association with grading and prognosis. **Veterinary Pathology**, v. 50, n. 1, p. 110-121, 2013.

VÉGRAN, F.; BOIDOT, R.; MICHIELS, C.; SONVEAUX, P.; FERON, O. Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF-kappaB/IL-8 pathway that drives tumor angiogenesis. **Cancer Research**, p.2828, 2011.

WALKER, M. A.; HARLEY, R. A.; LEROY, E. C. Inhibition of fibrosis in TSK mice by blocking mast cell degranulation. **The Journal of Rheumatology**, v. 14, n. 2, p. 299-301, 1987.

WEBSTER, J. D.; YUZBASIYAN-GURKAN, V.; KANEENE, J. B.; MILLER, R.; RESAU, J. H.; KIUPEL, M. The role of c-KIT in tumorigenesis: evaluation in canine cutaneous mast cell tumors. **Neoplasia**, v. 8, n. 2, p. 104-111, 2006.

WEBSTER, J. D.; YUZBASIYAN-GURKAN, V.; MILLER, R. A.; KANEENE, J. B.; KIUPEL, M. Cellular proliferation in canine cutaneous mast cell tumors: associations with c-KIT and its role in prognostication. **Veterinary Pathology**, v. 44, n. 3, p. 298-308, 2007.

WEI L, Y.; LEE, J. J.; YEH, C. Y.; YANG, C. J.; KOK, S. H.; KO, J. Y. et al. Reciprocal activation of cancer-associated fibroblasts and oral squamous carcinoma cells through CXCL1. **Oral Oncology**, v. 88, p. 115-123, 2019.

WELLE, M. M.; BLEY, C. R.; HOWARD, J.; RÜFENACHT, S. Canine mast cell tumours: a review of the pathogenesis, clinical features, pathology and treatment. **Veterinary Dermatology**, v. 19, n. 6, p. 321-339, 2008.

WERNER, S.; KRIEG, T.; SMOLA, H. Keratinocyte–fibroblast interactions in wound healing. **Journal of Investigative Dermatology**, v. 127, n. 5, p. 998-1008, 2007.

WIGMORE, S. J.; WALSH, T. S.; LEE, A.; ROSS, J. A. Pro-inflammatory cytokine release and mediation of the acute phase protein response in fulminant hepatic failure. **Intensive Care Medicine**, v. 24, n. 3, p. 224-229, 1998.

WILLIAMS, W. J.; LARSON, E.; PHILLIPS, T. L. A neoplastic connective tissue mast cell capable of continuous growth in tissue culture. **The Journal of Cell Biology**, v. 6, n. 3, p. 361-368, 1959.

XIE, K.Interleukin-8 and human cancer biology. **Cytokine & Growth Factor Reviews**, v. 12, n. 4, p. 375-391, 2001.

YAMADA, N.; MATSUSHIMA, H.; TAGAYA, Y.; SHIMADA, S.; KATZ, S. I. Generation of a large number of connective tissue type mast cells by culture of murine fetal skin cells. **Journal of Investigative Dermatology**, v. 121, n. 6, p. 1425-1432, 2003.

YAMASHITA, M.; OGAWA, T.; ZHANG, X.; HANAMURA, N.; KASHIKURA, Y.; TAKAMURA, M.; YONEDA, M.; SHIRAISHI, T. Role of stromal myofibroblasts in invasive breast cancer: stromal expression of alpha-smooth muscle actin correlates with worse clinical outcome. **Breast Cancer**, v. 19, n. 2, p. 170-176, 2012.

YE, J.; COULOURIS, G.; ZARETSKAYA, I.; CUTCUTACHE, I.; ROZEN, S.; MADDEN, T. L. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. **BMC Bioinformatics**, v. 13, n. 1, p. 134, 2012.

ZHANG, B.; HORVATH, S. A general framework for weighted gene co-expression network analysis. **Statistical Applications in Genetics and Molecular Biology**, v. 4, n. 1, 2005.

ZHANG, Q.; OH, C. K.; MESSADI, D. V.; DUONG, H. S.; KELLY, A. P.; SOO, C. et al. Hypoxia-induced HIF-1 α accumulation is augmented in a co-culture of keloid fibroblasts and human mast cells: involvement of ERK1/2 and PI-3K/Akt. **Experimental Cell Research**, v. 312, n. 2, p. 145-155, 2006.