Fascin-1 re-expression promotes cell metastasis through epithelial-mesenchymal transition in canine mammary tumour CHMm cell

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Research Article

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Abstract

Background

Canine mammary tumour (CMT) is the most common tumour disease in female dogs. At the same time, the popular concept of preferring purebred dogs when choosing pets has affected the incidence of mammary tumours in dogs. In this study, we obtained a metastatic key protein, Fascin-1, by comparing the proteomics data of in situ tumour and metastatic cell lines from the same individual. However, the role of Fascin-1 in CMT cell line is still unclear.

Results

To investigated the effects and mechanism of re-expression Fascin-1 on the migration, adhesion and invasion using the CMT cell line CHMm in vitro. Firstly, the proteomics and Western blot methods were used to analyse the differential expression of Fascin-1 between CMT cell line CHMm and CHMp. The CHMm cells isolated from CMT abdominal metastases barely express Fascin-1. Then, the Fascin-1 re-expression cell line CHMm-OE was established using Lentivirus transduction. The CHMm-OE cells could enhance the abilities of cell migration, adhesion, and invasion, promote the formation of lamellipodia, and affect the protein expression related to metastasis and EMT proteins that proteomic points out. Finally, the differentially expressed proteins (DEPs) in CHMm and CHMm-OE cells were identified through proteomics, which involved the positive regulation of cell adhesion, epithelial cell differentiation, extracellular matrix, focal adhesion, dioxygenase activity, and cytoskeleton protein binding. Reactive oxygen species, IL-17, VEGF, and HIF-1 signalling pathway along with Fascin-1 might be involved in cell metastasis.

Conclusions

However, the Fascin-1 re-expressed could promote cell EMT and increase the formation of lamellipodia, resulting in the enhancement of CHMm cell migration, adhesion and invasion in vitro. This may be beneficial to improve the welfare of female dogs and improve the prognosis of CMT.

Background

The incidence of tumours in pet dogs has been on the rise due to the increasing of their population and longer lifespans [1]. CMT is the most common one in female dogs [2, 3], with over 50% of cases being malignant [4] and often metastatic [5]. CMT typically occurs in the mammary glands of the abdomen and inguinal regions of female dogs [6], and its occurrence and development are associated with dogs breed, age, gender, and neutering status [6–9]. CMT and human breast cancer (HBC) share great number of similarities in pathogenic factors, histological characteristics, molecular phenotypes, biological
behaviours, and modes of metastasis [10, 11], such as acquired chemotherapeutic resistance [12, 13]. CMT have been suggested as a model to study the pathogenesis and prognosis of HBC [14–16].

Fascin-1 is an actin binding protein (ABP) that is present in membrane folds, microspikes, and stress fibres [17]. It is a unique ABP found along the entire length of the filopodia [18]. Fascin-1 was first identified in sea urchins [19] and subsequently in HeLa cell lines [20]. Various human cancer studies have shown that Fascin-1 promotes [21–24] or inhibits [25, 26] cell migration and invasion when it is over-expressed or knocked down in vitro, and is related to the survival time in vivo [27]. Mechanically, high expression of Fascin-1 is correlated with the process of epithelial–mesenchymal transition (EMT) in HBC cells and endows them with the ability of migration and invasion [24, 25]. At the same time, the proliferation and EMT of epithelial cells in CMT are more frequent than HBC [28], which leads cells losing the original immunophenotype and complicates immunolabeling for them [29].

Metastatic tumour cells in vivo migrate through dense ECM by the highly dynamic filopodia formed by crosslinking Fascin-1 with F-actin and actin filament self-organization and membrane remodelling [30–32]. Filopodia formation has been shown to provide resistance to rigidity from the matrix and other cells, sense the environment around the cell, provide forward thrust for cell migration [33–37], and promote the survival of tumour cells at the site of metastasis [38].

The growth of filopodia is driven by G-actin at the tip, and it is elongate in the direction away from the cell body by the synergism of Formins and VASP [39–42], which promotes the establishment of lamellipodia and cell adhesion [34, 36]. In this process, Fascin-1 is very important for the growth of filopodia and the establish of lamellipodia. Once the cells reach secondary tissues and organs, Fascin-1 expression is down-regulated through a regulatory process to achieve settlement [43]. Therefore, the process of tumour metastasis, from the detachment of cancer cells from the primary tumour tissue to the invasion of the bloodstream and lymphatic vessels and the settlement in secondary tissues or organs, is closely related to the expression of Fascin-1 protein [44, 45]. The regulation of Fascin-1 expression can affects tumour cell metastasis by altering the number and strength of cell filopodia [42, 46–48].

Moreover, the role of Fascin-1 in tumour occurrence [49], colonization [50], resistance to anoikis and chemotherapy, and cancer stemness [51] is gradually defined. Therefore, Fascin-1 has been identified as one of the biomarkers for the clinical diagnosis and prognostication of human tumours.

Although, the role of Fascin-1 in human tumour development and metastasis is well established, whether Fascin-1 has the same effect on dogs’ tumours remains to be determined. Therefore, the function of Fascin-1 in CMT was investigated using a Fascin-1 re-expression cell line generated by transferring the gene of Fascin-1 into CHMm which is isolated from CMT abdominal metastases. We established a Fascin-1 re-expression cell line using CHMm isolated from CMT abdominal metastases to investigate the function of Fascin-1 in CMT. The aim of this project was to study the effect and mechanism of Fascin-1 on CHMm cell migration, adhesion, and invasion and provide theoretical foundations for CMT treatment with Fascin-1 as a potential therapeutic target.
Results

1 Difference of Fascin-1 expression between CHMm and CHMp cells

Comparison of the proteomic data of CHMp and CHMm cells revealed a total of 4734 credible proteins (Fig. 1A). With FC ≥ 2 and FC < 1/2, p < 0.05 set as the thresholds, 1857 DEPs were identified, of which 898 were up-regulated and 959 were down-regulated. It was found that the expression of Fascin-1, in the metastatic cell line CHMm was much lower than that in the in situ cell line CHMp (Fig. 1B), and its expression was verified by Western blot (Fig. 1C). The details of DEPs were summarized in supplementary material Table S3.

2 The re-expression of Fascin-1 in CHMm cells

The CHMm-OE cell line which re-expressed Fascin-1 was established. The expression of Fascin-1 in CHMm-OE cells was significantly higher than that in CHMm-Nt/CHMm cells both at mRNA (Fig. 2A) and protein (Fig. 2B) level (P < 0.001). GAPDH was used as the loading control. The cell viability assay results showed that Fascin-1 re-expression did not affect cell proliferation (Fig. 2C).

3 Migration, adhesion, and invasion in CMT cells

Comparison of the scratch area images in the cell monolayer created by scratching with pipette tip between 24 h and 0 h (Fig. 3A) revealed that Fascin-1 re-expression significantly increased cell migration ability. However, NP-G2-044 treatment prevented CHMm-OE cell migration (Fig. 3B). Fascin-1 re-expressed CHMm-OE cells showed stronger adhesion ability than CHMm/CHMm-Nt cell lines in unfavourable environment for adhesion. At the same time, NP-G2-044 significantly disrupted the adhesion of CHMm-OE (Fig. 3C). The Transwell test showed that, the invasive ability of CHMm-OE was significantly stronger than that of CHMm/CHMm-Nt (Fig. 3D, E). There was no significant difference in migration, adhesion, and invasion ability between CHMm-Nt and CHMm cell lines, CHMm-OE and CHMm-OE + DMSO groups. In conclusion, the migration, adhesion, and invasion ability of CHMm-OE are significantly enhanced by Fascin-1 and NP-G2-044 treatment reverted the level close to CHMm/CHMm-Nt.

4 Fascin-1 re-expression enhances the formation of filopodia and lamellipodia on the surface of CHMm cells

Vasodilator-stimulated phosphoprotein (VASP), a significant protein in actin dynamics, protects the polymerization of actin at the front end by promoting the formation of stress fibres. The localization of VASP allows us to determine the range and extension of lamellipodia.

Filopodia, thin and elongated protrusions on the extracellular membrane, were observed in CHMm cells (Fig. 4). In CHMm-OE cells, the number of VASP spots at the leading edge of lamellipodia decreased compared to CHMm cells. These results suggest that Fascin-1 can promote the rearrangement of the cell skeleton, resulting in an increase in the formation of filopodia and lamellipodia in CMT cells.
5 Differential expression of the proteins

A total of 8720 proteins with unique peptide sequences were identified in CHMm-OE vs CHMm. The sample cluster diagram is shown in Fig. 5A. The significance threshold was set as $\text{FC} \geq 1.2$ and $\text{FC} < 1/1.2$, $p < 0.05$ and 136 DEPs were identified in CHMm-OE when compared with CHMm, including 64 up-regulated proteins and 72 down-regulated proteins, which were drawn on the volcano map (Fig. 5B). The top 15 up- or down-regulated proteins in CHMm-OE vs CHMm are shown in Table 1.
Table 1
The top 15 up- or down-regulated proteins in CHMm-OE vs CHMm cells.

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<th>Gene Name</th>
<th>FoldChange</th>
<th>p-value</th>
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<td>TIMP1</td>
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6 Cluster analyses of differentially expressed proteins

To understand the functions of the DEPs and signalling pathways they are involved in, 136 proteins were annotated using GO, KEGG and GSEA, and the data patterns of the three biological replicate groups had a high similarity (Fig. 6A).

For GO analysis, predominantly enriched proteins in the biological process (BP) were associated with the following terms: muscle contraction, positive regulation of cell adhesion, glutathione metabolic process, and epithelial cell differentiation, among others. The predominantly enriched terms in the cellular component (CC) category were cytoskeleton, extracellular matrix, apical plasma membrane, mitochondrion, focal adhesion, etc. DEPs in the molecular function (MF) category were associated with the following terms: myosin binding, dioxygenase activity, galcium-dependent protein binding, glutathione transferase activity, etc. The top 10 significantly enriched terms in these three ontologies, biological processes, cellular components, and molecular functions were shown in Fig. 6A.

Further analysis of KEGG signalling pathways for these DEPs showed that they were involved in the IL-17 signalling pathway, the VEGF signalling pathway, the HIF-1 signalling pathway, reactive oxygen species, the platinum drug resistance, and the ECM-receptor interaction. the top 20 KEGG pathways in the list of DEPs were shown in Fig. 6B-D.
The change of proteins following Fascin-1 re-expression intervention was comprehensively analysed, which wielded GSEA (Fig. 6E-H). There were eight regulated gene sets incorporated mitochondrial inner membrane (GO:0005743), mitochondrial respiratory chain complex I (GO:0005747), structural molecule activity (GO:0005198), and mitochondrial matrix (GO:0005759). These results indicated that, the therapeutic effect of Fascin-1 on metastasis might be closely implicated in mitochondria and cytoskeleton regulation.

7 Fascin-1 re-expression changes the expression of tumour metastasis-related proteins

From the above-mentioned data, it was found that the expression of tissue inhibitor of matrix metalloproteinase 1 (TIMP1) and matrix metalloproteinase 3 (MMP3) in CHMm-OE cells were up-regulated, which might contribute to the enhancement of cell metastasis.

MMP3, TIMP1 and TIMP2 play critical roles in tumour invasion. The re-expression of Fascin-1 protein significantly increased their expression levels in CMT cells (Fig. 7). It was previously reported that Fascin-1 can enhance the cell migration and invasion in vivo by affecting the expression of MMPs [52]. Interestingly, the expression of MMP2 and MMP9 were not detected in the CHMm-OE cells, while their corresponding inhibitory proteins, TIMP1 and TIMP2 were upregulated as revealed by proteomic data and westerbloting.

8 Effect of Fascin-1 re-expression on the EMT-related protein expression

In HBC, EMT is a hallmark of tumour aggressiveness and associated with cell migration, invasion, and metastasis. Among them, Fascin-1 can change the expression level of EMT marker proteins (including mesenchymal vimentin and epithelial protein E- cadherin) [53–56], thus initiate EMT and promoting the migration and morphological changes of epithelial tumour cells. At the same time, in the proteomic data, the EMT-related protein N- cadherin (gene name CDH2) was up-regulated in CHMm-OE. Therefore, in order to further study the metastasis enhancement effect of the re-expression of Fasicn-1 in CHMm-OE, we detected the expression changes of the EMT-related protein and epithelial cell-related protein. As shown in Fig. 8, E-Cadherin, Cytokeratin 8 and Cytokeratin 18 were downregulated, while Vimentin, N-Cadherin, and Snail were upregulated in CHMm-OE cells.

Discussion

The CHMm, a metastatic mammary cancer cell line is derived from CMT abdominal metastases. In this study, we found that Fascin-1 protein was rarely expressed in CHMm cells and established a Fascin-1 re-expression CHMm-OE cell line. Compared with the CHMm cell line, the migration, adhesion, and invasion ability of CHMm-OE cells were enhanced, while the proliferation ability remained unchanged. These findings are consistent with the results of various studies related to Fascin-1 in human cancers [24–26] revealing that Fascin-1 plays the same role in migration, adhesion, and invasion of CMT as human cancer.
Cell migration is a complex process in which the cross-linking of F-actin with Fascin-1 provides support for filopodia and lamellipodia in the leading edge of the cell [32, 57] and cytoskeleton remodelling, which is one of the critical early events in cancer cells that acquire invasiveness [58]. And the binding ability of actin bundle to Ena/VASP is stronger than that of filamentous F-actin [59, 60]. Moreover, the EVH1 domain of the VASP protein binds to Fascin-1, can strengthen the binding between Ena/VASP and F-actin and promoting F-actin extension [42, 61–63]. Meanwhile, Fascin-1 in the cytoplasm plays a significant role in stabilizing the tension of stressed fibres [31], and promotes the turnover of cell focal adhesions together with Cofilin [46].

MMPs are proteases for tumour cells to decompose basement membrane and ECM, and they are a common secreted protein in metastatic tumours [64]. The noncovalent complex formed by TIMPs and MMPs can maintain the integrity of cell connections, reduce tumour metastasis and improve prognosis by reducing ECM degradation [65, 66]. However, the imbalance of MMPs/TIMPs may affect the invasion and metastasis of tumours [67]. The enhanced invasive ability of CHMm-OE may be caused by this imbalance.

EMT, as mentioned by proteomic results, is one of the key mechanisms regulating the pathogenesis of cancer [68] and plays a role in tumour progression and metastasis [69]. The transcription factor Snail can cause the loss of E-cadherin and tight junction proteins but up-regulate fibronectin and other biomarkers [70, 71]. This study found that Fascin-1 re-expression led to a significant decrease in E-cadherin and an increase in vimentin, Snail, and N-cadherin expression levels, which was consistent with previous studies [24, 25]. At the same time, Snail enhances cell membrane fluidity [53] and plays a role in tumour progression and metastasis [69], and its high expression is related to the poor prognosis of tumour patients [72]. Taken together, Fascin-1 is involved in the EMT process of mammary tumours in CMT and promotes these cell migration and invasion. The changes in this process are also consistent with previous reports in CMT [54].

Migrasome is located at the end and bifurcation of retraction fibres in the tail of migrating cells [73]. As the cell metastasis, an unequal number of small vesicles inside the migrasome are released into the ECM, and absorbed by neighbouring cells to enhance the migration ability of receiving cells [74–76]. At the same time, re-expression of Fascin-1 can enhance the ability and motility of migrating cells to sense the surrounding microenvironment at the leading edge. TSPAN4, a migrasome-related protein, which is up-regulated by re-expression of Fascin-1, may achieve collective migration of cells through the migrasome. This may provide clues for the performance of CHMm-OE in cell scratch assay.

At the same time, KEGG analysis showed the change of HIF-1 signalling pathway, and the key protein Hypoxia-inducible factor-1 (HIF-1), a regulatory factors for cancer cells to overcome environmental stress [77], and promoted EMT process by binding with Snail [78, 79].

Other enriched signal pathways, such as the IL-17 signalling pathway [80] and the VEGF signalling pathway [81, 82], can promote HBC metastasis and the EMT process. At the same time, the circulating
HBC cells can produce reactive oxygen species under hydrodynamic stress and promote cell migration and the EMT process through ERK/MAPK [83] and PI3K/Akt [84] signalling pathways [85, 86].

The results of GSEA suggest that after the re-expression of Fascin-1, there are many mitochondrial gene sets in DEPs enrichment analysis. Study of Lin et.al. show that Fascin-1 enhances mitochondrial DNA homeostasis by mediating mitochondrial F-actin cross-linking, which increases the biogenesis of mitochondrial respiratory chain complex I [87], giving tumour cells the survival advantage in the tumour environment with nutritional deficiency. Meanwhile, migrasome can maintain mitochondrial homeostasis by disposing damaged mitochondria in migrating cells [88].

In summary, our study on Fascin-1 in CMT provides new insights on cancer treatment from the comparative medicine perspective.

**Conclusion**

This study demonstrated that Fascin-1 re-expression in CHMm cell line increased cell migration, adhesion, and invasion abilities promoting the occurrence of EMT and did not significantly affect cell proliferation.

**Methods**

1 **Cell culture**

   All cells were maintained in Dulbecco's modified Eagle's medium with high glucose (DMEM, Gibco, USA) that was supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic at 37°C in a 5% CO2 incubator. Cells in the logarithmic growth phase were harvested for further analysis digested when they were in the logarithmic growth phase for all experiments.

   CMT cell lines CHMm and CHMp have been preserved in our laboratory, which were presented by Professor Liu Yun from College of Animal Medicine, Northeast Agricultural University, China. The HEK-293T cell line was purchased from the Cell Resource Centre of Peking Union Medical College (Beijing).

2 **Construction of Fascin-1 Re-expression cell line**

   The synthesized Fascin-1 gene was cloned into pUC57 vector (Sangon, China) and digested with XbaI and BamH I. The Fascin-1 gene fragment was then ligated to pLVX-IRES-neo (TaKaRa, USA) and the ligated product was transformed into DH5α (Sangon, China). Bacterial clones with pLVX-IRES-neo plasmid containing Fascin-1 gene insert were selected and cultured at 37°C with shaking at 225 rpm for 16 h. Endotoxin-free plasmids were extracted (OMEGA, USA), for subsequent cell transfection. The pLVX-IRES-neo empty vector was also used for transfection as a negative control. The lentivirus was packaged in the HEK-293T cell line using the Lenti-X Bicistronic Expression System (TaKaRa, USA).
CHMm cells were seeded at 2×10^5 cells/well in a six-well plate. An appropriate amount of virus supernatant and Polybrene were added when the cells were adhered. After 24 h of transduction, the cells passed normally in a complete medium. 48 h later, an appropriate amount of transduced cells were seeded with 20% density in a 10 cm cell culture dish and selected with G418. While all cells in the control dish were died, the remaining dish was cultured in a complete medium and passaged normally or preserved by freezing. Changes in the Fascin-1 gene and protein expression were identified in the transfected cell line. CHMm cells transfected with the pLVX-IRES-neo empty lentivirus and the pLVX-IRES-neo/Fascin-1 plasmid lentivirus was named CHMm-Nt and CHMm-OE cells, respectively.

3 Cell viability Assay

Cells were inoculated into 96-well plate with an initial cell density of 5×10^3 cells/well. Every 24 h, CCK-8 (BOSTER, China) was added to the cells, and the plate was further incubated at 37°C for 30 min, then the absorbance at 450 nm (with a reference wavelength of 600 nm) was measured using Infinite® E Plex (Infinite, Austria).

4 Cell migration assay

Cells were seeded at 4×10^5 cells/well in a six-well plate. When the cell density reached 70–80%, the medium was replaced with a serum-free medium, and the cells were starved for 16 h. After starvation, a line was scratched in the well using a 200 µL pipette tip, and the cells were washed with PBS to remove floating cells. Then, 50 µM NP-G2-044 was added (IC90) to the medium containing 2% FBS for the CHMm-OE + NP-G2-044 group, and an equal amount of DMSO was added for the CHMm-OE + DMSO group. After three hours incubation, the cells were photographed using an Olympus microscope. After further 24 h incubation, the cells were washed and photographed. All the pictures were analysed using ImageJ software (v. 1.53). The percentage of wound healing was calculated as follows: (0 h wound area – 24 h wound area) / 0 h wound area.

5 Cell adhesion assay

Matrix gel (Corning, USA) was coated on 96-well plates at a dilution of 1:8 with a DMEM medium and allowed to gel formation overnight in the cell incubator. Three hours before harvesting the cells, 50 µM NP-G2-044 was added to the cells in the CHMm-OE + NP-G2-044 group, and an equal amount of DMSO was added to the cells in the CHMm-OE + DMSO group. Cells were then resuspended in a medium containing 2% FBS and seeded at a concentration of 2×10^4 cells/well and incubated for 6 h for adhesion. Cells were washed with PBS to remove un-adhesion cells, and a blank medium with 10% CCK-8 was added to each well and incubated at 37°C for 1 h. The absorbance at 450 nm (with a reference wavelength of 600 nm) was measured using a microplate reader.

6 Cell invasion assay

Matrix gel was diluted 1:8 with blank medium and coated onto Transwell upper chambers overnight at 37°C. NP-G2-044 and DMSO were added in the same way as the cell adhesion assay. The lower chamber
was filled with 500 µL medium containing 20% FBS. The transwell was gently inserted into the plate well. Cells were resuspended in DMEM medium and seeded onto upper chamber with 2×104 cells density, and the chambers were placed back into the 24-well plates for further incubation.

After 24 h, the upper chamber cells were gently wiped off with a sterile cotton swab, fixed with 4% paraformaldehyde for 15 min, and stained with 0.1% crystal violet for 30 min. After washing with PBS, the membrane was photographed under a microscope and cell invasion ability was evaluated based on the number of cells that passed through the membrane.

7 Immunocytochemistry and Immunofluorescence assay

Cells (1 × 104 cells) were cultured in a 30 mm Glass bottom dish for 24 h, fixed with 4% paraformaldehyde, blocked with 5% BSA for 1 h, and then incubated overnight with primary antibodies. Cells were washed three times with PBST and probed with fluorescent-conjugated secondary antibodies (1:100) for 1 h. F-actin was subsequently stained with Alexa Fluor™ 680 Phalloidin (Invitrogen, USA) for 1 h at room temperature. The nuclear was counterstained with Hoechst 33342 (Solarbio, China) Images were captured with a Leica TCS SP8 confocal microscope (Leica, Germany).

8 Tandem mass tags (TMT) quantitative proteome analysis

Two groups of cells, CHMp vs CHMm and CHMm-OE vs CHMm, were collected and TMT based quantitative proteome analysis was performed by Shanghai Luming Biotechnology Co., Ltd. ProteomeDiscovererTM 2.4.1.15 (ThermoFisher Scientific, USA) was used for protein identification and quantification using the UniProt Canis lupus familiaris database (Organism ID 9615). DEPs were screened from the listed protein candidates according to the criteria of Score Sequest HT > 0 and unique peptide ≥ 1. Foldchange (FC) ≥ 1.2 or FC < 1/1.2 and Student's t-test p-value < 0.05 were set as the threshold for screening the proteins with significant differences. Gene ontology (GO), Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway analysis, and Gene Set Enrichment Analysis (GSEA) were used to investigate the biological function of these DEPs. p-value < 0.05 was considered statistically significant.

9 Quantitative reverse-transcription PCR (RT-qPCR)

Total RNA was extracted from cells using RNAiso Plus (TaKaRa, USA) according to the manufacturer's instruction, and reverse transcription was performed using the Primer-ScriptTM RT reagent Kit with gDNA Eraser (TaKaRa, USA). 1 µL of 10-fold diluted cDNA sample, along with 0.8 µL of upstream and downstream primers (10 µM), 7.4 µL of deionized water, and 10 µL of 2×SYBR Green Low ROX RT-qPCR Master Mix (Selleck, China) were used for each PCR reaction. GAPDH was used as an internal control, and RT-qPCR was performed using a two-step amplification method. The primer sequence is shown in Supplementary Table S1.

10 Western blot
Cells were lysed in RIPA buffer (Solarbio, China) for 40 min and the supernatant was collected after centrifugation (13,000 ×g). The protein concentration was measured using the BCA assay (BOSTER, China). The denatured proteins were separated by 8–12% SDS-PAGE gel electrophoresis, and subsequently transferred to a PVDF membrane. According to the size of protein, the PVDF membrane was cut and bound to antibody respectively. The membrane was blocked with 5% skim milk at room temperature for 2 h and incubated with the primary antibody (Supplementary Table S2) overnight at 4°C. The membrane were washed with TBST buffer and further incubated with a secondary antibody for an hour at room temperature. The target protein was visualized using an enhanced chemiluminescence reagent (MeilunBio, China) and the membrane was imaged using the Chemi Doc XRS + imaging system (BioRad, USA).

11 Data Analysis

All data were expressed as Mean ± SD, and statistical analysis was performed using GraphPad PrismTM 8 software with One-way ANOVA and Two-way ANOVA. P < 0.05 indicated a significant difference, while P < 0.01 indicated an extremely significant difference.

Abbreviations

CMT: canine mammary tumour
DEPs: differentially expressed proteins
HBC: human breast cancer
ABP: actin binding protein
EMT: epithelial–mesenchymal transition
FBS: fatal bovine serum
VASP: Vasodilator-stimulated phosphoprotein
RT-qPCR: quantitative reverse-transcription PCR
TIMP1: matrix metalloproteinase 1
MMP3: matrix metalloproteinase 3
HIF-1: Hypoxia-inducible factor-1

Declarations

Ethics approval and consent to participate
Not applicable

Consent for publication

Not applicable

Availability of data and materials

All the data generated or analysed during this study are contained in this published article and its supplementary documents, and the data set analysed during the current study can be obtained in the database. (After receiving the article, share it and attach the search number)

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

XW and YZ are responsible for the implementation of all the experiments. XW writes the manuscript and modifies it. HQL provides experimental funds. AH, XZZ, JHG XLC, WY, NS, PPS, ZBZ, HZY and KHF put forward valuable opinions for the writing and revision of the article.

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References


**Figures**

**Figure 1**

The expression difference of Fascin-1 protein between CHMm and CHMp cell lines. (A) Analysis of the proteomic data of CHMm and CHMp cell lines revealed 4734 credible protein data. (B) FC ≥ 2 and FC < 1/2, p < 0.05 were set as the thresholds to screen the data, 1857 DEPs in CHMm vs. CHMp were obtained, and the volcano map was drawn. (C) The Fascin-1 protein was detected by Western blot. *** means p < 0.001. Full-length blots are presented in Additional File 1.
Figure 2

Functional characterization of the CHMm-OE cell line. (A) The mRNA expression of Fascin-1 was up-regulated in CHMm-OE cells. (B) Western blot analysis showed that Fascin-1 protein expression was up-regulated in CHMm-OE cells. Full-length blots are presented in Additional File 2. (C) There was no significant difference in cell proliferation between the three cell lines. Significant differences from the CHMm were identified at * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.
Figure 3

Migration, adhesion, and invasion in CMT cells. (A) Under that objective lens of 10x, images in the scratch area and healing area after 24 h. (B) Comparison of migrated distance. (C) Light absorption value of successfully adhered cells after CCK-8 treatment for 1 hour. (D) Photos of invasion assay that under that objective lens of 20x. (E) Comparison of the number of migration cells. The scale bars in A and E
represent 100 μm and 50 μm respectively. Significant differences from the CHMm were identified at * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

**Figure 4**

The re-expression of Fascin-1 increased the filopodia and lamellipodia in CHMm-OE cells. Immunocytochemical staining is used as follows: VASP (AF448-green), Phalloidin (AF®680-red), and Hoechst 33342 (blue). The arrow indicates filopodia, and the triangle indicates plate lamellipodia. The scale of filopodia and lamellipodia in CHMm-OE is larger than that of the CHMm cell line.
Figure 5

Proteomic analysis of CHMm and CHMm-OE cell lines. (A) In the thermogram, the abscissa is the comparison group, and the ordinate is the number of DEPs. Different colours display up and down. (B) In the volcano map, red represents significantly differentially up-regulated proteins (FC ≥ 1.2, p < 0.05), green represents significantly down-regulated proteins (FC ≤ 1/1.2, p < 0.05), and the darker colour means a more significant difference. Grey represents non-differentially or non-statistically significant proteins.
Figure 6

Proteomic analysis of DEPs in CHMm-OE vs CHMm by GO, KEGG and GSEA. (A) Top-10 of three Ontologies in GO analysis. (B-D) The Top-20 terms of total (B), of up-regulated (C) and of down-regulated (D). (E-H) Gene set enrichment analysis results by Gene Set Enrichment Analysis (GSEA): a graphical view of the normalized enrichment score (NES) for a gene set between CHMm-OE and CHMm.
Figure 7

Changes in key protein expression during tumour invasion. (A) Change of MMP3, TIMP 1 and TIMP2 mRNA expression in CMT cells as assessed by RT-qPCR. (B) Representative image of western blot analysis. (C) The results of densitometric analysis of the western blot showed the protein expression levels of MMP3, TIMP 1 and TIMP2 in CHMm-OE versus CHMm/CHMm-Nt. GAPDH was blotted to ensure equal loading. Full-length blots are presented in Additional File 3. The significant differences at the expression level in the CHMm-OE were observed at * p < 0.05, ** p < 0.01, *** p < 0.001.
Figure 8

Fascin-1 promotes the EMT process in CMT cells. Changes in EMT marker mRNA expression in CMT cells assessed by RT-qPCR(A). (B) Western blot analysis showing the expression of Cytokeratin 8/18, E-cadherin, N-cadherin, Snail and Vimentin in CHMm/CHMm-Nt and CHMm-OE. Full-length blots are presented in Additional File 4. (C) Quantification of the relative expression of band intensity in the experiment. Three independent replicates were determined by ImageJ (V. 1.53), and the results are shown in B. GAPDH was blotted to ensure equal loading. At the mRNA and protein levels, the expression of Cytokeratin 8/18 and E-cadherin is down-regulated following the re-expression of Fascin, while the expression of N-cadherin, Vimentin, and Snail is up-regulated. The significant differences between CHMm-OE and CHMm/CHMm-Nt were indicated at * p < 0.05, ** p < 0.01, *** p < 0.001.

Supplementary Files
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- AdditionalFile3.pptx
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