Original article

Increased expression of SPARC and TIMP3 in epidermotropic melanoma metastasis

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Abstract

Primary cutaneous melanoma generally arises in the epidermis, followed by invasion into the dermis. Although infrequent, invasive melanoma cells can, alternatively, migrate to the intraepidermal area and form epidermotropic melanoma metastasis (EMM). In this study, we focused on this unique manner of metastasis. To identify the key molecules which affect EMM, gene expression in EMM was compared with that in common skin metastasis (CSM). PCR analysis was performed for genes affecting the extracellular matrix, cellular adhesion, and tumor metastasis on three EMM and three CSM samples as an initial screening. For molecules showing altered expression in the EMM, expression levels were further verified using real-time quantitative PCR (qPCR) and immunohistochemistry. Five molecules showed an expression difference in the initial screening. Among these, SPARC was preferentially expressed in EMM (p=0.01) by real-time qPCR. Another candidate molecule, TIMP3, was not statistically significant (p=0.07), but showed the tendency of higher expression. These results correlated negatively to expression of N-cadherin and β-catenin. The upregulation of SPARC and TIMP3 may disrupt the continuity of the canonical Wnt pathway. This pathway regulates adhesion activity of melanoma cells to localize within the dermis, which consequently promotes EMM. Our study highlights the potential role of SPARC and TIMP3 as key molecules in EMM, and analysis of EMM may contribute for understanding melanoma invasion between the epidermis and the dermis.

Key words:

epidermotropic melanoma metastasis (EMM)

beta catenin (β-catenin)

canonical Wnt pathway

neural cadherin (N-cadherin)

secreted protein acidic and rich in cysteine (SPARC)

tissue inhibitor of metalloproteinase-3 (TIMP3)

1. Introduction

Primary cutaneous melanoma generally occurs in the epidermis, and once extending to the dermis, it generally metastasizes to lymph nodes and other organs.[1] Epidermotropic metastasis is a rare form of skin metastasis, and epidermotropic melanoma metastasis (EMM) was histopathologically analyzed by Kornberg *et al*: 1) aggregates of atypical melanocytes in the dermis without inflammatory infiltrate and junctional activity; 2) elongated rete ridges surrounding tumor nests in the papillary dermis; 3) existence of tumor cells in the lumen; 4) epidermal component of atypical melanocytes which is equivalent or inside the lateral limits of the dermal component.[2] Furthermore, various features of EMM, such as pagetoid spreading of atypical melanocytes, dominant epidermal components, and connected lesions in the epidermis and the dermis have been reported.[3-5]

The mechanism underlying the spreading of melanoma cells into the epidermis remains unknown. A candidate factor is a cell adhesion molecule which affects a role in cell motility through cell-cell and cell-extracellular matrix (ECM) interactions. Normally, keratinocytes keep melanocytes from leaving the epidermis through epithelial cadherin (E-cadherin). However, during transition from the radial growth phase to the vertical growth phase, melanoma cells undergo epithelial to mesenchymal transition (EMT) that leads to loss of epithelial markers (*i.e.*, E-cadherin), changes in cell polarity and intercellular junctions, and increases mesenchymal markers (*i.e.*, neural cadherin or N-cadherin), all of which promote melanoma invasiveness.[6-8] As cadherins appear to affect the location of melanocytes within the skin,[9] a decrease in N-cadherin may repress metastasis by disturbing heterotypic adhesion of melanoma cells with other N-cadherin positive cells such as dermal fibroblasts and vascular endothelial cells. In addition, integrin alteration may promote migration of melanoma

cells into the epidermis through binding with ECM components. Although the mechanisms of melanoma metastasis to specific sites are still largely unclear, ECM, matrix metalloproteases (MMP), and several adhesion receptors are being intensively studied.[10-15] Vascular cell adhesion protein 1 (VCAM1) has been found to initiate melanoma metastasis to the lung,[10,14] and secreted protein acidic and rich in cysteine (SPARC) activates VCAM1 to enable the extravasation of melanoma cells into the lung parenchyma.[15] Activation of MMP-2 has also been correlated with increased melanoma malignancy in xenograft models.[13]

We speculate that key molecules of epidermotropism in EMM are also associated with invasion of melanoma cells from the epidermis to the dermis. In this study, we investigated the potential molecules which differentiate EMM from common skin metastasis (CSM) by performing gene expression profiling on EMM and CSM.

2. Material and methods

2.1 Subjects and tissues

Formalin-fixed paraffin-embedded (FFPE) tissues of metastatic melanoma were obtained from patients who consented in Shinshu University Hospital. Samples were pathologically categorized as three EMM tumors and three CSM tumors. Eleven primary melanoma, one normal skin, and one placenta were used as control samples for staining of SPARC and tissue inhibitor of metalloproteinase-3 (TIMP3). Hematoxylin and eosin (HE) glass slides were reviewed for histologic confirmation (Supplementary Figure S1) and to ensure adequate tumor was present within the samples. The study was approved by the Ethics Committee of the Shinshu University School of Medicine (approval numbers 358 and 430). All patients approved their participation in this research and provided written consent forms.

2.2 RNA extraction

Laser-assisted microdissection of EMM and CSM samples was performed on 10 μm histologic sections using a Zeiss PALM MicroBeam IV Laser-Captured Microdissection system (Carl Zeiss Microscopy GmbH, Göttingen, Germany) to prevent contamination of fibroblast from the dermis. Normal skin tissue was microdissected as a control. Next, RNA was extracted from the dissected cells using Absolutely RNA FFPE kit (Agilent, West Cedar Creek, TX, USA). The RNA extraction was done following the manufacturer's instructions. Using xylene, all samples were deparaffinized and treated with proteinase K to solubilize the fixed tissues and release the nucleic acids, followed by DNase treatment. Extracted RNA underwent quantity assessment using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Madison, WI, US) before conversion to cDNA using a reverse transcription system (Promega, Madison, WI, USA). Quality assessment was performed by conducting PCR for glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

2.3 Screening of mRNA expression using TaqMan® Array

Two types of 96-well Fast TaqMan® Arrays (Human Extracellular Matrix & Adhesion Molecules and Human Tumor Metastasis; Applied Biosystems, Foster City, CA, USA) were used to screen for expression of candidate molecules of epidermotropism on EMM and CSM. Normal skin was used as a control for each sample (conducted in duplicate). Each well consisted of 5 μl cDNA sample diluted in nuclease-free water and 5 μl TaqMan® Fast Advanced Master Mix (Applied Biosystems, Foster City, CA, USA). Final cDNA concentration per well was 25 ng per 10 μl reaction solution. Reactions were performed using a StepOnePlus™ Real-Time PCR System (Applied Biosystems). Thermal cycling conditions used were as follows: 50℃ for 2 min,

95℃ for 20 sec for 1 cycle followed by 95℃ for 1 sec, 60℃ for 20 sec for 40 cycles. 18S rRNA was used as internal controls for both arrays. Relative gene expression was expressed as fold change, using the $2^{-\Delta\Delta Ct}$ method. Each molecule was duplicated in an array, and we used two array plates for each sample. Thus, experiments were essentially performed in quadruplicate.

2.4 Real-time quantitative PCR

Real-time quantitative PCR (qPCR) experiments were performed in triplicate using TaqMan® Fast Advanced Master Mix (Applied Biosystems) according to the manufacturer's instructions on a StepOnePlus™ Real-Time PCR System (Applied Biosystems). The thermal cycling conditions were as followed: 50°C for 2 min, 95℃ for 20 s for 1 cycle followed by 95℃ for 1 s, 60℃ for 20 s for 40 cycles. The expression of GAPDH was analyzed as an endogenous control, and target gene expression was normalized to GAPDH in each sample. Relative gene expression is expressed as fold change, using the $2^{-\Delta\Delta Ct}$ method.

2.5 Immunohistochemistry (IHC)

Briefly, 4 μm thick FFPE sections from representative blocks were deparaffinized and rehydrated in graded alcohols and distilled water. A peroxidase block (DAKO, Glostrup, Denmark) was done for 5 min. For antigen retrieval, heating was performed in 10 mmol/L sodium citrate pH 6.0 (SPARC, TIMP3, beta catenin (β-catenin), human melano-black 45 (HMB45), and melanoma antigen recognized by T cells 1 (MART-1)) or pH 9.0 (E-cadherin, N-cadherin, and phosphatase and tensin homolog (PTEN))

(Muto Pure Chemicals. Co, Tokyo, Japan). The sections were incubated overnight at 4℃ with primary antibody (Supplementary Table S1), which was followed by incubation with a secondary antibody. The staining was developed with 3-amino-9-ethylcarbazole (AEC) substrate (DAKO) and counterstained with hematoxylin.

2.6 Evaluation of staining

Two histopathological image readers (M.T and Y.K) evaluated each panel of samples and semiquantitatively estimated the number of stained tumor cells. Each reader evaluated the images separately and was blinded to other opinions to minimize possible observer bias. In the case of disagreement, a consensus was reached by discussion among the evaluators.

The IHC was graded according to both staining intensity and frequency of tumor cells. Staining intensity was scored as 0 for no staining, 1 for weak staining, 2 for medium staining, and 3 for strong staining (Supplementary Figure S2). The frequency of staining was also scored as 0 for no staining of the tumor cells was observed, 1 for <5% of the tumor cells were stained, 2 for 5-50% of the tumor cells were stained, and 3 for >50% of the tumor cells were stained. Stained tumor cells presented cytoplasmic staining with or without nuclear expression. The total IHC score was determined as the sum of the intensity and frequency scores for tumor cells and categorized as low expression score (ranging between 0 and 2, absent of the stain also included in this group), moderate expression score (ranging between 3 and 4), and high expression score (ranging between 5 and 6) groups. Next, the data underwent manual tabulation and analysis procedure.

2.7 Statistical analysis

Statistical significance of TaqMan® Array and real-time qPCR levels between EMM and CSM was evaluated using Student's *t*-test. Differences were considered significant at $p<0.05$. Experiments were performed at least three times.

3. Results

3.1 Gene expression difference between EMM and CSM

The epidermotropism of metastatic melanoma may be related to the expression of genes involved in the extracellular matrix and cell adhesion. The selective distribution of metastases is controlled by several factors, including the pattern of vascular flow from the primary site, complementary adhesive contacts, and molecular interactions between the tumor cell and the stroma at the secondary site was reported previously.

To test this hypothesis, we performed TaqMan® arrays for human extracellular matrix and adhesion molecules using cDNA obtained from EMM and CSM samples. **This array targets genes involved in ECM structure and remodeling, defining connective tissue, cell adhesion, transmembrane inhibitors, basement membrane construction and collagen proteins.** The arrays showed higher expression of several genes in EMM (Figure 1, Supplementary Table S2): SPARC, TIMP3, collagen type I alpha I chain (COL1A1), collagen type XV alpha I chain (COL15A1), and connective tissue growth factor (CTGF). We excluded genes that amplified with threshold cycles ≥35. In addition, expression of metastatic genes was examined on EMM and CSM using the Human Tumor Metastasis Array. However, there was no meaningful difference found between EMM and CSM (data not shown).

3.2 Increased mRNA expression of SPARC and TIMP3 in EMM

Using the TaqMan® array, five candidate genes linked to epidermotropic metastasis were identified (Supplementary Table S3). To validate these findings, individual real-time qPCR was performed in three EMM, three CSM, and one normal skin sample as a control. SPARC was significantly increased in EMM, compared with CSM (Figure 2A, p=0.01). Although not statistically significant, TIMP3 tended to be highly expressed in EMM (Figure 2B, p=0.07). A statistically significant difference was not observed in the expression of COL15A1 (p=0.7), COL1A1 (p=0.2), and CTGF (p=0.7) between EMM and CSM (Figure 2C~E).

3.3 Increased protein expression of SPARC and TIMP3 in EMM

We next focused on SPARC and TIMP3 and examined their protein expression by IHC. IHC was performed in EMM (n=3), CSM (n=8), normal skin (n=1), and placenta (n=1) as external positive controls for both molecules. IHC of the melanoma markers HMB45 and MART-1 showed specific distribution within the tumor cells (Figure 3). The IHC staining patterns for SPARC and TIMP3 are illustrated in Figure 3. The SPARC protein was high expression in 66.7% of EMM samples (2/3) and 33.3% moderate expression (1/3) with a predominantly cytoplasmic pattern (Table 1, Figure 3A). Remarkably, SPARC was barely detected in CSM samples. Low expression of SPARC was presented on 87.5% (7/8) CSM samples (Table 1 and Figure 3B). In addition, TIMP3 expression was also increased in EMM when compared with CSM. High expression of TIMP3 was presented on 2 of 3 EMM samples, meanwhile only one CSM sample ((1/8) 12.5%) (CSM5) which presented high expression of TIMP3 (Table 1). No nuclear expression of was observed in SPARC and TIMP3 staining (Figure 3A-B) These findings were consistent with those obtained using real-time qPCR.

3.4 Absence of N-cadherin upregulation on EMM

As SPARC and TIMP3 were preferentially expressed in EMM, we next investigated association of these molecules with known pathways that involved migration of melanoma cells. EMT is one of the key factors in melanoma metastasis which enhances tumor cell invasion. Hallmarks of EMT are N-cadherin upregulation together with E-cadherin downregulation.[16] To examine this issue, we evaluated the expression levels of N-cadherin and E-cadherin in EMM and CSM samples (Table 2 and Figure 3). Interestingly, moderate to high membranous N-cadherin expression was observed in all CSM samples while low expression was in all EMM samples (Table 2). No nuclear expression of N-cadherin was observed (Figure 3A-B). E-cadherin expression was not different between EMM and CSM as two of three samples showed moderate to high expressions and one sample displayed low expression in both EMM and CSM samples (Table 2). These findings suggest that there is a difference in the presence of the N-cadherin between EMM and CSM.

3.5 Low expression of β-catenin on EMM

PTEN acts as a tumor suppressor molecule. The reduced or absent PTEN expression is followed by N-cadherin upregulation, which leads to progression of melanoma.[17] In this study, N-cadherin was expressed specifically in CSM, but not EMM (Table 2, Figure 3), and we performed PTEN staining using same samples. However, these analyses indicated that there was no difference in PTEN expression between EMM and CSM (Table 2).

Next, we examined β-catenin expression. β-catenin expression was moderate expression in one (33.3%) and low expression in two (66.7%) of three EMM samples

(Table 2, Figure 3A). Oppositely, it was high expression in two (66.7%) and moderate expression in one (33.3%) of three CSM samples (Table 2, Figure 3B). The tendency to highly express β-catenin was associated with N-cadherin expression.

3.6 Most primary melanoma lesion expressed TIMP3 but not SPARC

For evaluating roles of SPARC and TIMP3 during invasion to the dermis, we next examined SPARC and TIMP3 expression in primary cutaneous melanoma. Samples which extended to Clark's levels Ⅱ-Ⅳ were selected. While SPARC was expressed in 45.5% (5/11) of primary lesions, TIMP3 was expressed in all (11/11) primary melanoma lesions (Figure 4A). To investigate changes in expression levels of SPARC and TIMP3 during the invasion, we compared IHC expression between melanoma cells in the epidermis and in the dermis on the same sample. The dermis presented higher expression than epidermis in 36.3% (4/11) primary melanoma lesions and 9.1% (1/11) was higher expression in the epidermis than dermis for SPARC staining (Figure 4A~B). In 54.5% (6/11), there was no difference between tumor nests in the epidermis and the dermis. TIMP3 showed higher expression in 63.6% (7/11) of the epidermal lesions and 9% (1/11) of the dermal lesions, and 27.3% (3/11) showed no difference between the epidermal and dermal lesions (Figure 4A~B). These results suggest that TIMP3 might have more potential role on localization of melanoma cells within the epidermis.

In addition we also performed SPARC and TIMP3 staining on primary melanoma lesions of EMM1-3. We compared IHC expression between melanoma cells in the epidermis and in the dermis on the same sample. Primary lesion of EMM1 presented higher expression of SPARC in the epidermis than dermis, while TIMP3 was shown relatively similar between epidermis and dermis (moderate expression) (Figure 5A). In the opposite primary lesion of EMM2 had moderate expression of SPARC on the

dermis with low expression on the epidermis, but TIMP3 was consistent with primary EMM1 which shown similar expression level between epidermis and dermis (Figure 5B). Primary lesion of EMM3 was shown stronger expression of SPARC and TIMP3 on the epidermis than dermis (Figure 5C).

4. Discussion

In this study, we observed higher expression of SPARC and TIMP3 in EMM than CSM. SPARC, also known as osteonectin or BM-40, is a 43 kD glycoprotein that binds with high affinity to albumin. SPARC has roles on cell proliferation, survival, and migration suggesting that it may promote tumor progression. In addition, SPARC regulates the interactions of cells with ECM, leading to modulating cell adhesion.[18] Controversially, SPARC has been suggested to act as an antitumor factor in ovarian, prostate, and breast cancers.[19, 20] TIMP3 is a member of the tissue inhibitor of metalloproteinases (TIMP) family, an inhibitor of endogenous MMP. TIMP3 is a tumor suppressor in several cancers, with loss of expression correlating with poor prognosis.[21-23] A recent study reported a role for TIMP3 as a dominant negative regulator of angiogenesis in melanoma.[21] Several studies show that TIMP3 inhibits neovascularization with suppression of endothelial cell migration in tumor xenografts.[24-26]

It remains unclear how SPARC and TIMP3 relate the epidermotropism of melanoma cells. We attempted to connect our findings with the known cadherin switch phenomenon (i.e., loss of E-cadherin and up-regulation of N-cadherin) which plays a role in the invasion of melanoma cells from the epidermis to dermis.[7,27,28] Previously, it

was reported that SPARC reduced E-cadherin expression in melanoma cells, which led to promoting melanoma metastasis.[29] However, in the present study, E-cadherin expression was similar in both EMM and CSM, suggesting that E-cadherin expression is not affected by SPARC upregulation in this tumor type. Different from CSM, EMM did not express N-cadherin, which may open up a possibility that the lack of N-cadherin expression supports the presence of tumor cells in the epidermis. In melanoma, N-cadherin upregulation has been reported to promote adhesion of melanoma cells to dermal fibroblasts and vascular endothelial cells, and an N-cadherin-neutralizing antibody blocked melanoma cell migration to the dermis.[7] N-cadherin also plays a role in activation of canonical Wnt pathways via β-catenin nuclear localization.[30-32] Interestingly, our results showed TIMP3 expression in EMM samples was relatively correlated with N-cadherin expression and β-catenin expression in a negative manner, suggesting that TIMP3 inactivates β-catenin signaling via N-cadherin down-regulation in coordination with localization of melanoma cells within the epidermis.

Based on this result, we also speculate that the invasion mechanism in primary melanoma occurs via the TIMP3 and Wnt/β-catenin pathway. Despite the limited number of samples, TIMP3 expression tended to be higher in the epidermal than the dermal component, and was negatively correlated with β-catenin expression in primary melanoma. Excessive TIMP3 expression in melanoma cells may prevent translocation of a sufficient amount of β-catenin to the nucleus due to decreased β-catenin expression, which leads to the localization of melanoma cells within the epidermis. Indeed, TIMP3 has been reported to block β-catenin nuclear localization in the NRAS-driven melanoma murine model.[33]

Roles of SPARC in β-catenin nuclear localization during melanoma metastasis remains unknown. In adipocyte morphogenesis, SPARC-Integrin linked kinase

complex induces the accumulation and nuclear localization of β-catenin.[34, 35] This mechanism is contrary to findings reported in this work.

The present study showed novel findings of SPARC and TIMP3 expression in EMM, and proposed a potential mechanism of melanoma cell invasion. These molecules may be useful markers to predict migration of melanoma cells.

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Conflict of interests: None declared

All authors confirm that the material presented here has neither been published previously nor is it under consideration by another journal.

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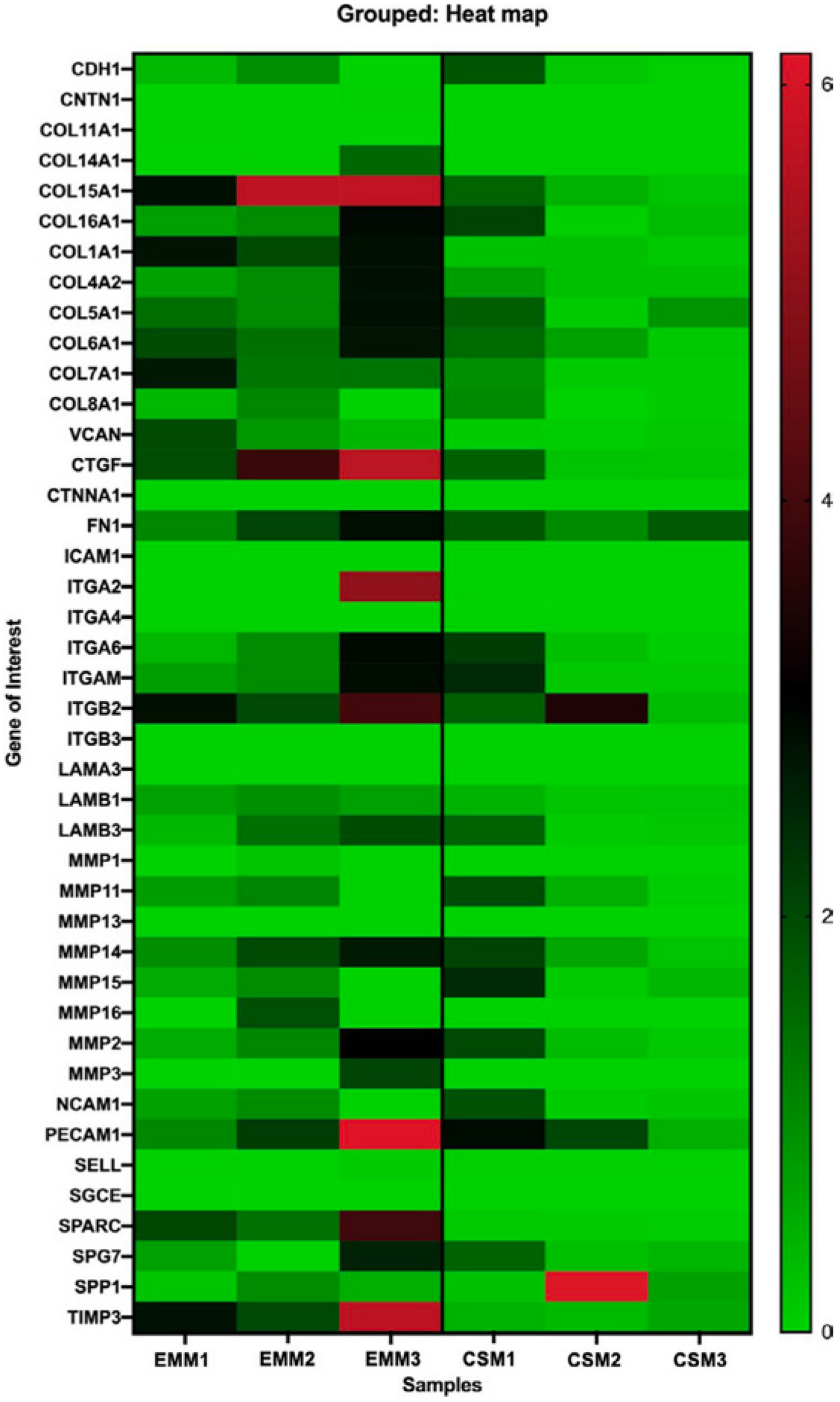
Figure legends

Figure 1. Heat map of TaqMan® array for human extracellular matrix and adhesion molecules in EMM and CSM. Relative gene expression was analyzed using microarray methods. Fold change values ($2^{-\Delta\Delta Ct}$) are calculated and shown with different colors in the heat map. Green to red represents downregulated to upregulated genes. EMM, epidermotropic melanoma metastasis; CSM, common skin metastasis.


Grouped: Heat map
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CNTN1
COL11A1
COL14A1
COL15A1
COL16A1
COL1A1
COL4A2
COL5A1
COL6A1
COL7A1
COL8A1
VCAN
CTGF
CTNNA1
FN1
ICAM1
ITGA2
ITGA4
ITGA6
ITGAM
ITGB2
ITGB3
LAMA3
LAMB1
LAMB3
MMP1
MMP11
MMP13
MMP14
MMP15
MMP16
MMP2
MMP3
NCAM1
PECAM1
SELL
SGCE
SPARC
SPG7
SPP1
TIMP3
Gene of Interest
EMM1
EMM2
EMM3
CSM1
CSM2
CSM3
Samples
6
4
2
0

Figure 2. Validation of mRNA levels in selected genes of interest by RT-qPCR.

(a) SPARC expression is significantly higher in EMM than CSM (*p = 0.01). (b) TIMP3 expression is increased but not statistically significantly higher in EMM than CSM (p = 0.07). (c–e)The expression of COL15A1, COL1A1, and CTGF shows no significant difference between EMM and CSM (p = 0.7, 0.2 and 0.7, respectively). COL15A1, collagen type XV alpha I chain; COL1A1, collagen type I alpha I chain; CSM, common skin metastasis; CTGF, connective tissue growth factor; EMM, epidermotropic melanoma metastasis; SPARC, secreted protein acidic and rich in cysteine; TIMP3, tissue inhibitor of metalloproteinase-3.

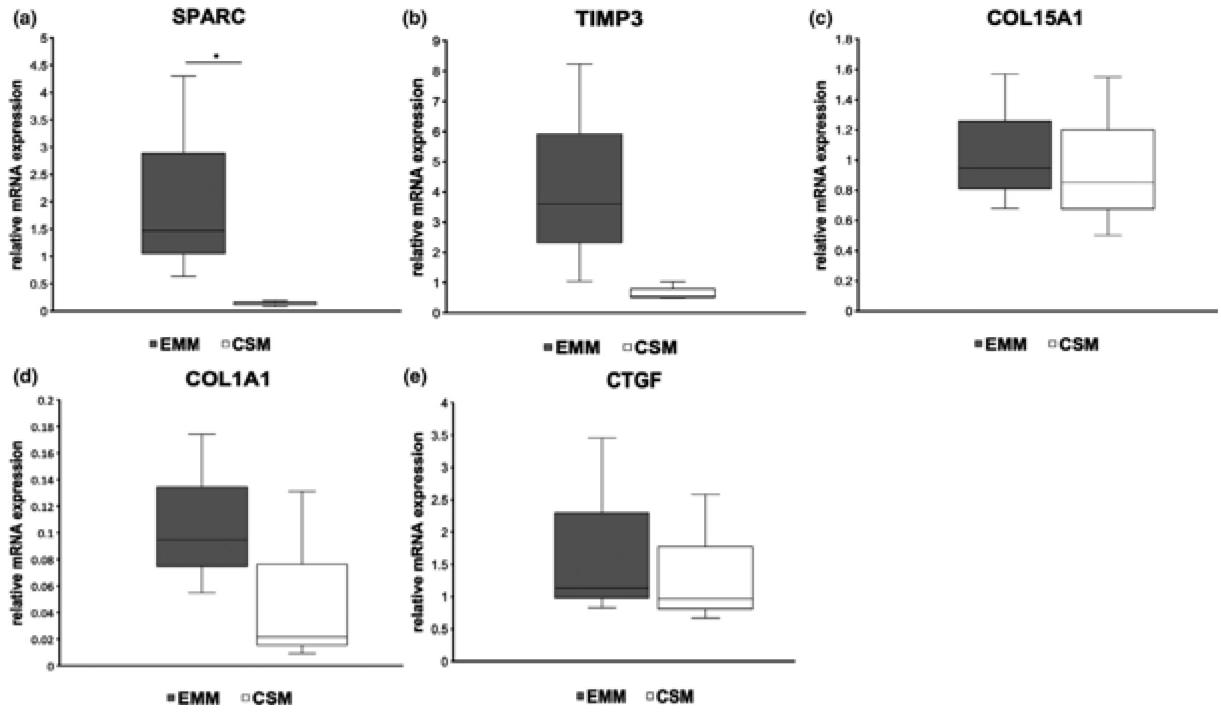

Figure 3. Immunohistochemical staining of SPARC, TIMP3, N-cadherin and β-catenin. (A) High expression of SPARC and TIMP3, and low expression of N-cadherin and β-catenin in EMM. (B) Low expression of SPARC and TIMP3, and high expression of N-cadherin and β-catenin in CSM. Hematoxylin and eosin (HE) and melanoma specific markers (HMB45 and MART-1) staining are also shown. The squares point to

the selected sites for captured image with higher magnification. Scale bars=500 μm. SPARC, secreted protein acidic and rich in cysteine; TIMP3, tissue inhibitor of metalloproteinase-3; N-cadherin, neural cadherin; β-catenin, beta catenin; EMM, epidermotropic melanoma metastasis; CSM, common skin metastasis; HMB45, human melano-black 45; MART-1, melanoma antigen recognized by T cells 1.

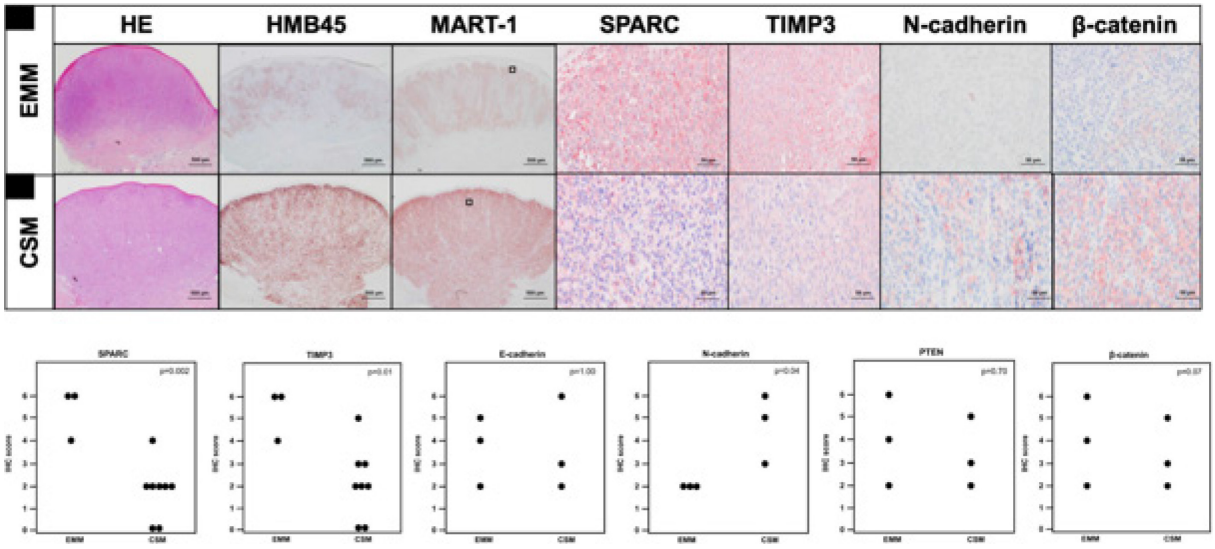

Figure 4. SPARC and TIMP3 expression levels in primary cutaneous melanoma. (A) Expression levels of SPARC and TIMP3 in primary cutaneous melanoma are shown. Each level is represented as follows: (dark green; high expression); (light green; moderate expression); and (grey; low expression). The expression was compared between tumor cells in the epidermis and dermis on the same sample. (B) Representative staining is shown. In case 4, SPARC is stronger in the dermal tumor cells, while TIMP3 is stronger in the epidermal tumor cells. Scale bars, 100 μm. SPARC, secreted protein acidic and rich in cysteine; TIMP3, tissue inhibitor of metalloproteinase-3.

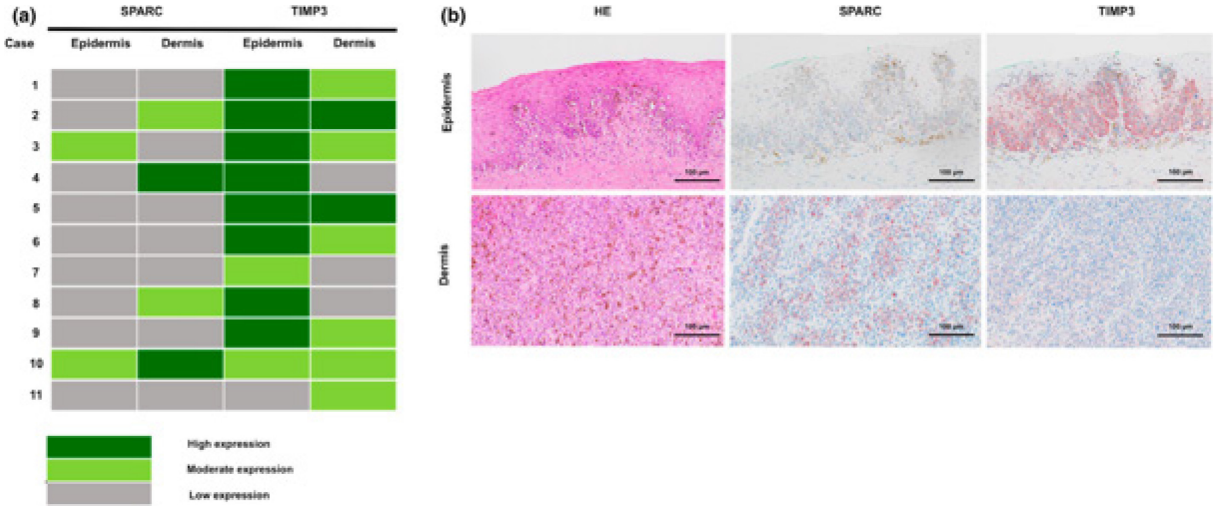

Figure 5. SPARC and TIMP3 expression levels in primary of epidermotropic melanoma metastasis (EMM) lesions. (A) Primary of EMM1 lesion, SPARC: high expression in the epidermis and moderate expression in the dermis, TIMP3: moderate expression in the epidermis and moderate expression in the dermis. (B) Primary of EMM2 lesion, SPARC: low expression in the epidermis and moderate expression in the dermis, TIMP3: low expression in the epidermis and low expression in the dermis. (C) Primary of EMM3 lesion, SPARC: high expression in the epidermis and low expression in the dermis, TIMP3: high expression in the epidermis and moderate expression in the dermis. Scale bars, 50 μm.

Appendix A. Supplementary data

Figure S1. (A) Histopathologic features of epidermotropic melanoma metastasis (EMM). The square points to the selected location for captured image with higher magnification. Scale bars, 500 μm. **(B)** EMM demonstrates thinning of the epidermis and the lateral extension of atypical melanocytes within the epidermis less than the

metastasis in the dermis. Scale bars, 100 μm. **(C)** A corresponding features of common skin metastasis. Scale bars, 1 mm. **(D)** Histopathologic features of a primary melanoma with the presence of inflammatory infiltrates. Scale bars, 100 μm.

Figure S2. The example of SPARC staining intensity scoring. (A) Placenta as positive control was used as reference for strong staining (score 3). **(B)** EMM1 showed strong staining intensity (score 3). **(C)** EMM2 showed medium staining intensity (score 2). **(D)** CSM3 showed weak staining intensity (score 1). **(E)** CSM 2 showed no staining (score 0). Scale bars, 50 μm.

Table S1. Antibodies for immunohistochemistry

Table S2. Table S2. The data value of TaqMan® array for human extracellular matrix and adhesion molecules in EMM and CSM.

Table S3. TaqMan Assay ID for real time-quantitative PCR