Fascin 1 is transiently expressed in mouse melanoblasts during development and promotes migration and proliferation

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SUMMARY

Fascins, a family of actin-bundling proteins, are expressed in a spatially and temporally restricted manner during development and often in cancer. Fascin 1 has a clear role in cell migration in vitro, but its role in vivo in mammals is not well understood. Here, we investigate the role of fascin 1 in the melanocyte lineage and in melanoma cells. Fascin 1 knockout causes hypopigmentation in adult mice owing to migration and cell cycle progression defects in melanoblasts, the melanocyte precursor cell. Study of live embryo skin explants reveals that E14.5 fascin 1-null melanoblasts migrate slower, and generate fewer and thinner pseudopods. By contrast, fascin 1 expression drives faster migration and lamellipodia protrusion in melanocytes in vitro. In addition, fascin 1 depletion retards melanoblast proliferation in vivo and melanoma cell growth in vitro. These data indicate that fascin 1 not only promotes cell migration in mouse melanoblasts but it also has a role in growth and cell cycle progression.

KEY WORDS: Fascin 1 (Fscn1), Melanoblast, Migration, Mouse

INTRODUCTION

Fascins are actin-bundling proteins involved in cytoskeletal dynamics and regulated by protein kinase C (Adams, 1997; Adams and Schwartz, 2000; Aratyn et al., 2007). Fascin 1 is enriched in filopodia (Adams, 1997; Adams and Schwartz, 2000; Aratyn et al., 2007) and in invadopodia (Li et al., 2010; Schoumacher et al., 2010). The expression level and actin-bundling activity of fascin 1 positively correlate with the length, number and lifetime of filopodia (Vignjevic et al., 2006; Hashimoto et al., 2007; Yamakita et al., 2009), as well as two-dimensional cell migration (Yamashiro et al., 1998). In addition, fascin 1 stabilizes invadopodia and protrusive pseudopods of mesenchymal-type cancer cells (Li et al., 2010). Fascin 1 expression is time and tissue specific during embryogenesis (De Arcangelis et al., 2004), and a high level of fascin 1 is often found in various motile embryonic cells, such as macrophage-like plasmacytocytes and border cells in Drosophila (Borghese et al., 2006; Zanet et al., 2009), and E10.5-12.5 hepatoblasts in rat (Hayashi et al., 2008).

Melanoblasts, which are melanocyte precursors, emigrate dorsolaterally from the neural tube after differentiating from neural crest cells. Mouse melanoblasts translocate from dermis to epidermis and accumulate at hair follicles before birth. Melanoblast differentiation, migration, proliferation and survival are crucial for early skin development and adult pigmentation (Baxter et al., 2004).

Gene mutations or alterations causing melanoblast deficiencies often result in a ‘white-spot’ phenotype and several genes encoding growth factors and respective ligands (Jordan and Jackson, 2000; Harris et al., 2008), transcription factors (Hornyak et al., 2001), secreted metalloproteinases (Silver et al., 2008), Rac1 and the Rac1 GEF pREX-1 (Li et al., 2011; Lindsay et al., 2011) affect melanoblast migration and survival (see also http://www.espcr.org/micemut/).

Here, we demonstrate that mice lacking fascin 1 (Yamakita et al., 2009) have pigmentation defects that result from melanoblast migration defects and delayed cell cycle progression. We validated these results in human melanoma cultured cells. Our studies suggest that fascin 1 is transiently upregulated when melanoblasts are migratory and proliferative during mouse embryogenesis, and that some melanoma cells may also depend on fascin for enhanced motility and normal cell cycle progression.

MATERIALS AND METHODS

Transgenic mice

All experiments were performed according to UK Home Office regulations. Fascin 1−/− mice in C57BL/6 background have been previously described (Yamakita et al., 2009). Z/EG (lacZ/EGFP) mice (Novak et al., 2000) and DCT (dopachrome tautomerase::lacZ) mice (Mackenzie et al., 1997; Pla et al., 2004; Takemoto et al., 2006) have been previously described. Tyrosinase Cre (Tyr::Cre) mice have been previously described (Delsma et al., 2003). Mouse genotyping was performed by the company Transnetyx (Memphis, TN, USA).

Retroviral constructs

Stable cell lines expressing GFP-tagged human fascin 1 were generated by retroviral infection using the modified Retro-X retroviral expression system (Clontech Laboratories). A HindIII restriction site, followed by a Kozak consensus translation initiation site was introduced to the 5′ end of the coding sequence of GFP-fascin 1 (Li et al., 2010) by PCR (5′- AGAAGTATATGTTGAGCAAGGCAGGAGCTGTTTC-3′). In the same reaction, a ClaI restriction site was introduced to the 3′ end (5′- AGAACTGGATCTAGTACTCCCAGAGCGGAGGCGGCTGTC-3′). The HindIII-ClaI DNA fragments containing human fascin 1 were subcloned into
mM MgCl₂, 0.01% sodium deoxycholate, 0.02% NP-40, 5 mM K₄Fe(CN)_6, minutes at room temperature and stained with X-gal staining solution [2 stage under Zeiss Stemi-2000 dissection microscope (EOS utility, Edmund Thermo Scientific or sifascin1-2: Hs-FSCN1 2HP siRNA from Qiagen) with protein-specific primary antibodies.

β-Galactosidase assay
Timed embryos were dissected in ice-old PBS and fixed in ice-cold 0.25% glutaraldehyde in PBS for 40 minutes. After 10 minutes of washing in ice-cold PBS, embryos were immersed in permeabilization solution (2 mM MgCl₂, 0.01% sodium deoxycholate, 0.02% NP-40) for 30 minutes at room temperature and stained with X-gal staining solution [2 mM MgCl₂, 0.01% sodium deoxycholate, 0.02% NP-40, 5 mM K₄Fe(CN)_6, 5 mM K₃Fe(CN)_6, and 1 mg/ml X-gal (Invitrogen, Carlsbad, CA, USA) in PBS (pH 7.4)] as described previously (Loughna and Henderson, 2007) for 48 hours at 4°C. Embryos were maintained in 10% formalin after washes with PBS. The pictures of the forelimbs, trunks and entire embryos were taken at the same magnification for embryos at the same developmental stage under Zeiss Stemi-2000 dissection microscope (EOS utility, Edmund Optics, NJ, USA).

For the quantification of the melanoblasts in the trunks, an area of 1000 x 500 pixels² was cropped and gridded with an area per point of 1000 pixels² (Image J). For melanoblast quantification in the forelimb, an area of 1100 x 450 pixels² was cropped to cover most forelimb skin and gridded with an area per point of 15,000 pixels². Cell number in sub-areas was counted for E13.5 and E15.5 compared between wild-type, fascin 1–/+ male and female mice and in the trunk area of embryos was carefully removed under a Zeiss Stemi-2000 dissection microscope (Edmund Optics, NJ).

Embryonic skins were immediately mounted and cultured in Greiner Lumax culture dish (Greiner Bio-One) as described previously (Mort et al., 2010; Li et al., 2011; Lindsay et al., 2011). Ex vivo time lapse images were captured on a Nikon AR or Olympus FX1000 (20× objective, 1024 x 1024) for 4 hours with an interval of 5 minutes per frame in a 37°C humid chamber of 5% CO₂. Cell tracks and speeds were determined with ImageJ manual tracking and chemotaxis tool plug-in. The criteria for pseudopod generation rate, long or short pseudopods and numbers were determined as reported previously (Li et al., 2011). Skin samples were fixed after image capture and detailed cell protrusion pictures were taken as z-series on an Olympus FX1000 confocal microscope at 1 μm intervals (60× objective, 1024 x 1024). The diameter of a cross-section through the middle of a pseudopod is expressed as pixels measured with the straight-line tool (ImageJ).

Cell cycle distribution
Human melanoma cell line MV3 in six-well plates was transfected with siRNAs (100 nM, sifascin1-1: on-target plus siRNA fascin1 oligo3 from Thermo Scientific or sifascin1-2: Hs-FSCN1 2HP siRNA from Qiagen) with Hiperfect transfection reagent (Qiagen) twice on two sequential days. Cells were re-seeded and the experiments were performed 48 hours post-transfection. Asynchronously growing cells were collected after 1 hour BrdU pulse (20 μM) to measure cell in S phase or challenged with 100 ng/ml nocodazole in presence of cumulative BrdU for 2 hours, 4 hours, 6 hours, 8 hours, 10 hours and 12 hours. This is a modified method of the nocodazole trapping experiment used previously (Gillespie and Walker, 2006). For BrdU/PI analysis, cells were treated with 2 N HCl (room temperature, 15 minutes) prior to 1 hour primary antibody incubation with mouse anti-BrdU antibody (1:40, DAKO). After washing with PBT, cells were then incubated in PBT with Alexa 488-conjugated anti-mouse antibody (1:60; Invitrogen) and resuspended in PBS with 250 μg/ml RNaseA and 10 μg/ml propidium iodide for 30 minutes prior to FACS analysis on BD FACS caliber using CellQuest software (BD). The results were analysed and plotted with FlowJo (Tree Star).
and detected with ECL chemiluminescence detection kits (Pierce). Primary antibodies are: rabbit anti-fascin 1 (Sigma-Aldrich), rabbit anti-phospho-AKT (Ser473) (Cell Signaling), rabbit anti-p44/42 MAPK (Erk1/2) (Cell Signaling), rabbit anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (Cell Signaling), rabbit anti-pY397FAK (Invitrogen) and rabbit anti-total FAK (Cell Signaling).

**BrdU incorporation**
Timed matings were set up and pregnant females were injected with 250 μl BrdU (10 mg/ml, BD Pharmingen) at E13.5. After 24 hours, mice were sacrificed and embryos were fixed for histology in 10% neutral-buffered formalin.

**Eosin staining**
X-gal-stained, formalin-fixed and paraffin-embedded fascin 1 DCT::lacZ embryos (E13.5) were transversely sectioned (10 μm depth). Eosin staining on the sections was performed in a standard fashion. Pictures were taken on Olympus BX51 FL microscope. DCT-positive cells in the epidermis and dermis (E13.5) were counted.

**Statistical analysis**
To test whether groups of large datasets (e.g. wild type versus −/−) come from a Gaussian distribution, the D’Agostino and Pearson omnibus normality test was performed. If the data failed to pass the normality test, a Mann-Whitney test was used to assess statistical significance. Otherwise, two-tailed, unpaired Student’s t-test was used to assess statistical significance (GraphPad Prism 5). To compare three groups of data (wild type, −/+ and −/−), Kruskal-Wallis one-way ANOVA test and Mann-Whitney test were used (GraphPad Prism 5). *P<0.05, **P<0.01 and ***P<0.001 are statistically significant. n.s. indicates not significant.

**RESULTS**

**Fascin 1-null mice have melanocyte deficient patches**
Fascin 1-null pups were weaned at less than Mendelian ratios (Yamakita et al., 2009), but otherwise were healthy and fertile. Fascin 1 was undetectable at the protein level in fascin 1−/− mice and reduced in fascin 1−/+ mice (Fig. 1A; supplementary material Fig. S1A). Fascin 1−/− mice in C57BL/6 background displayed a white patch near the midline of the abdomen, varying from a few white hairs or a thin white line (63% of 30 animals) to a big white patch (37% of 30 animals, Fig. 1B). White abdominal patches have been previously correlated with the absence of melanocytes (Baxter et al., 2004; Silver et al., 2008; Li et al., 2011; Lindsay et al., 2011) and suggest possible melanoblast migration and/or proliferation defects. Immunofluorescence staining of embryonic skin (E14.5) indicated that melanoblasts (DCT positive) had relatively high cytoplasmic fascin 1 staining, which appeared to be enriched in protrusions (Fig. 1C, yellow arrowheads). Fascin 1 is also expressed by dermal stromal cells (Fig. 1C, below the lower white dotted line marked with Ep (epidermis) and De (dermis)) and weakly expressed by basal keratinocytes (Fig. 1C, white arrowheads). Furthermore, fascin 1 mRNA was robustly expressed in E15.5 melanoblasts at levels around threefold higher than in keratinocytes (Lionel Larue, personal communication) (Colombo et al., 2012). Histological analysis revealed an absence of melanocytes in the white patch of abdominal skin from fascin 1−/− mouse (P14, hair follicle in anagen stage) whereas the black dorsal skin of fascin 1−/+ mice had normal...
melanocyte distribution (Fig. 1D,E). Fascin 1−/− mice also displayed reduced pigmentation in the toes and tail (Fig. 1F). Thus, fascin 1 is highly expressed in melanoblasts, but perhaps only transiently, as it was absent from melanocytes isolated and cultured from 1-day-old pups (M4, supplementary material Fig. S1B). By contrast, human melanocytes in culture express fascin 1 (Li et al., 2010), possibly reflecting that human melanocytes remain migratory in the adult and therefore may maintain fascin 1 expression, whereas mouse melanocytes reside in hair follicles and are not motile in the adult (Larue and Beermann, 2007), and may thus lose fascin 1 expression.

**Fascin 1 deficiency reduces melanoblast number during embryogenesis**

To further understand the pigmentation defect, we crossed fascin 1−/− mice with mice harbouring DCT::lacZ, which is a reporter for melanocyte lineage cells (Mackenzie et al., 1997). DCT::lacZ fascin 1−/− mice generally displayed large white patches on the abdomen (supplementary material Fig. S1C). We performed a β-galactosidase assay to determine the number and distribution of DCT::lacZ positive melanoblasts. At E11.5, when the melanoblasts have just emerged from the neural tube, the number and position of melanoblasts was indistinguishable between wild-type, fascin 1+/− and fascin 1−/− embryos, e.g. melanoblasts appeared to have emerged from the neural tube and were situated in the migration staging area adjacent to the neural tube (Fig. 2A,B). However, fascin 1−/− displayed fewer melanoblasts on the forelimbs and trunks at E13.5 (Fig. 2D,G) and E15.5 (Fig. 2E,F,H), and significantly fewer melanoblasts in the distal areas (Fig. 2C−H). In most cases, fascin 1−/− embryos had an intermediate phenotype. Thus, fascin 1 is not required for neural crest cell to melanoblast differentiation or for emigration from the neural tube; however, fascin 1 rapidly becomes important for cell numbers to increase and for migration in the dermis/epidermis.

**Fascin 1 deficiency delays melanoblast and melanoma cell cycle progression**

We examined whether fascin 1 contributed to proliferation in melanoblasts and melanoma cell lines that express high levels of fascin 1 (Li et al., 2010). Melanoblasts proliferate with a doubling rate of ~16 hours at E10.5-E15.5 (Luciani et al., 2011). No apoptotic melanoblasts were detected from co-stains of TUNEL assay to determine the number and distribution of DCT::lacZ positive melanoblasts. At E11.5, when the melanoblasts have just emerged from the neural tube, the number and position of melanoblasts was indistinguishable between wild-type, fascin 1+/− and fascin 1−/− embryos, e.g. melanoblasts appeared to have emerged from the neural tube and were situated in the migration staging area adjacent to the neural tube (Fig. 2A,B). However, fascin 1−/− displayed fewer melanoblasts on the forelimbs and trunks at E13.5 (Fig. 2D,G) and E15.5 (Fig. 2E,F,H), and significantly fewer melanoblasts in the distal areas (Fig. 2C−H). In most cases, fascin 1−/− embryos had an intermediate phenotype. Thus, fascin 1 is not required for neural crest cell to melanoblast differentiation or for emigration from the neural tube; however, fascin 1 rapidly becomes important for cell numbers to increase and for migration in the dermis/epidermis.

**Fig. 2. Loss of fascin 1 results in fewer melanoblasts by E13.5 and E15.5.** (A) Wild-type and fascin 1−/− embryo pictures and cropped areas used for quantitation (E11.5). (B) Cell number in dorsal-ventral part of E11.5 embryos (wild type, n=8; −/+; n=6; −/−; n=3). Kruskal-Wallis one-way ANOVA test and Mann-Whitney test, n.s., not significant. (C) Wild-type and fascin 1−/− pictures of whole embryos (E15.5) with cropped, gridded subareas for quantitation. (D,E) Melanoblast number in dorsal-ventral regions of E13.5 (D) and E15.5 (E) embryos. (F) Wild-type and fascin 1−/− pictures of the forelimbs with cropped, gridded subareas for quantitation. (G,H) Melanoblast number in the forelimbs of E13.5 (G) and E15.5 (H) embryos. Results are expressed as means±s.e.m. (four to six embryos for each genotype), Kruskal-Wallis one-way ANOVA tests were used to compare melanoblast numbers between wild type, −/+ and −/− in each subarea and Mann-Whitney tests were used to compare between two groups, *P<0.05; **P<0.01, n.s., not significant.

G1-phase cells and fewer S-phase cells (Fig. 3D). To further validate this, MV3 melanoma cells were trapped with nocodazole, a mitotic inhibitor. From the time of the nocodazole addition, we measured the cumulative BrdU incorporation as cells progressed through S phase before being blocked in M phase (Gillespie and Walker, 2006). We observed substantially fewer sifascin (fascin 1 siRNAs) cells in S phase, indicating that fascin depletion slowed progression through...
**Fascin 1 deficiency reduces melanoblast migration and pseudopod generation rate**

Fascin 1 (singed in Drosophila) is important for Drosophila haemocyte migration (Zanet et al., 2009), migration of mature dendritic cells to lymph nodes in mice (Yamakita et al., 2011) and for melanoma tumour cell invasion in 3D Matrigel/collagen matrix (Li et al., 2010). We investigated the migration of melanoblasts in live embryo skin to better understand the role of fascin in vivo. Mice carrying a Z/EG double reporter transgene (Novak et al., 2000) and Trr::Cre (Delmas et al., 2003) were crossed into the fascin 1−/− background to drive GFP expression in the melanoblast lineage. The skin from E14.5 embryonic trunk was imaged as previously described (Mort et al., 2010; Li et al., 2011). Melanoblasts migrate individually within the developing mouse embryo epidermis using two types of protrusions: short stubs and long pseudopods (Li et al., 2011). Melanoblasts in E14.5 wild-type and fascin 1−/− skin explants maintained a similar elongated cell shape with both long and short protrusions (Fig. 4A,G; supplementary material Movies 1, 2). Fascin 1−/− melanoblasts were slightly smaller than wild type (Fig. 4C). In addition, cell migration speed and euclidean distance travelled was significantly reduced for melanoblasts in fascin 1−/− explants compared with wild type (Fig. 4D-F). Around 30% of fascin 1−/− melanoblasts migrated with a speed slower than 0.5 μm/minute compared with fewer than 10% of melanoblasts in wild-type explants (Fig. 4B,E). However, directionality (the ratio between the euclidean distance and total distance travelled) was unaffected by fascin 1 loss (supplementary material Fig. S5A).

In addition, the rate of pseudopod generation in fascin 1−/− melanoblasts was modestly reduced (Fig. 4G,H) but the lifetime of pseudopods was increased (supplementary material Fig. S5B). These two parameters roughly balanced out so that at any one time, fascin 1−/− melanoblasts and wild type had a similar number of pseudopods (supplementary material Fig. S5C); however, this number was quite variable and ranged between zero and eight pseudopods. Fascin 1−/− melanoblasts also extended thinner protrusions with a smaller cross-sectional width than wild type (Fig. 4G,I). This difference may be due to the proportionally smaller area of the fascin 1−/− cells in embryo skin (Fig. 4C). Thus, fascin 1 is required for efficient melanoblast migration and formation of thick robust pseudopods. In vitro fascin 1 is important for filopodia extension (Vignjevic et al., 2006; Hashimoto et al., 2007) and may be important for melanoblasts to probe the local environment, including contact with keratinocytes and the BM (basement membrane). Although we found that loss of fascin1 reduced the rate of generation of pseudopods, in agreement with these in vitro studies, it is possible that very small filopodia will not be detected at the resolution we are capable of imaging explants, so we cannot comment on those. Overall, we find that fascin 1 is required for efficient pseudopod generation rates, and for normal pseudopod dynamics, but lack of fascin causes only subtle changes overall in pseudopod dynamics.

Mouse melanoblasts migrate across the developing epidermal BM from dermis to epidermis between E11.5 and E13.5. As fascin 1 is implicated in invadopodia formation and invasion of melanoma cells (Li et al., 2010), we examined the effect of fascin 1 deletion on BM crossing. However, the proportion of melanoblasts crossing into the epidermis of Dct::lacZ wild-type, fascin 1−/− and fascin 1−/− embryos was similar at E13.5 (supplementary material Fig. S6). Although it seems surprising that fascin 1 is not important for migration across the BM, these data agree with our previous study of Rac1 and N-WASP (Li et al., 2011) and further confirm that the relocation from dermis to epidermis probably does not require matrix degradation by invadopod-like structures.

**Fascin 1 regulates melanocyte migration and lamellipodial dynamics**

To further explore the migratory role of fascin 1, we established an early passage melanocyte cell line from 1-day-old mouse pups with a genetic deletion of Ink4a, which we term M4 (see Materials and methods). M4 represents line 4, as described in our recent study (Li et al., 2012). Fascin 1 protein was undetectable in M4 cells (supplementary material Fig. S1B), perhaps reflecting that murine melanocytes reside mainly in hair follicles and may have lost fascin expression as they are not migratory (Jordan and Jackson, 2000). Fascin 1 protein was not detected in other mouse melanocyte (with Ink4a−/−) or melanoma (mutant NRasQ61K+/o Ink4a−/− background) cell lines that we derived from 1-day-old pups (data not shown) (Ackermann et al., 2005). However, fascin 1 expression was observed in mouse melanoma cell lines B16F1 and B16F10 (supplementary material Fig. S1B) (Vignjevic et al., 2006; Kim et al., 2009), which indicates that the expression of fascin 1 might be dependent on signals from the microenvironment or on mutations other than loss of Ink4a and activation of NRas. Because many human and murine melanoma cell lines express fascin 1 (Li et al., 2010), we stably overexpressed GFP-tagged human fascin 1 in M4
melanocytes (M4F cells). M4 and M4F showed the same sizes in 2D cell culture (supplementary material Fig. S5D) and migrated randomly with similar directionality (supplementary material Fig. S5E). GFP-fascin 1 was cytoplasmic and also concentrated at filopodia and microspikes (Fig. 5A). Fascin 1 expression increased migration speed (Fig. 5B), which is consistent with other studies (Yamashiro et al., 1998; Hashimoto et al., 2005a). Melanocytes displayed broad fan-like lamellipodia towards the direction of migration on a 2D surface. Fascin 1-enriched actin bundles (filopodia and microspikes) are interspersed within the lamellar actin meshwork of motile melanoma cells (Fig. 5A; supplementary material Movie 3) (Pelosi et al., 2003; Vignjevic et al., 2006). We further analysed four parameters of lamellipodial protrusions: protrusion rate, protrusion frequency, protrusion persistence and distance as shown in the lamellipodial kymographs (Fig. 5C). Expression of fascin 1 increased the speed of advancement of lamellipodial protrusions and the generation frequency. Cells expressing GFP-fascin 1 protruded less persistently, although protrusion distance was not affected (Fig. 5D-G). Thus, fascin 1 expression increases melanocyte migration and promotes lamellipodial dynamics in a 2D environment.

DISCUSSION

We demonstrate an important role for fascin in the migration and cell cycle progression of mouse embryo melanoblasts that gives rise to a white abdominal patch on fascin 1-null animals. The white patch arises because melanoblasts have not populated the areas most distal to their sites of origin near the neural tube during embryogenesis and once they differentiate into melanocytes after birth, they are no longer migratory in the mouse skin. Evidence for this comes from showing that expression of Kit-L in the skin can allow repopulation of new hair follicles after birth (Luciani et al., 2011). The role of fascin in migration in 2D in vitro is well documented (Jayo and Parsons, 2010; Machesky and Li, 2010) and recent reports show a requirement in 3D matrix degradation (Li et al., 2010), as well as in the normal embryonic migration and inflammatory responses of Drosophila haemocytes (Zanet et al., 2009). We find that loss of fascin 1 impairs pseudopod generation rate and fascin 1-null cells generate thinner pseudopodia that are less dynamic (longer-lived), leading to slower migration. We were surprised that pseudopods generated by fascin 1 nulls had a slightly increased lifetime, but we also found that expressing fascin 1 in M4 melanocytes in culture decreased lamellipodial persistence, which also indicates that fascin 1 promotes dynamics. This agrees with studies showing increased lamellipodial dynamics of fascin 1-expressing Drosophila haemocytes (Zanet et al., 2009) and is interesting in light of a recent study showing that actin bundles containing fascin 1 are more susceptible to turnover by cofilin (Breitsprecher et al., 2011). Furthermore, fascin 1 can interact with LIMK, a cofilin regulatory kinase (Jayo et al., 2012) and thus fascin...
Mtorc1 regulation of transcription

interspersed in the lamellipodium. Scale bar: 5 μm. (melanocytes indicate that fascin 1-enriched filopodia (white arrows) are

P<0.05; n.s., not significant.

P and whiskers indicate maximum value to minimum value. Mann-Whitney plots, horizontal line indicates median, box indicates interquartile range

and for M4F cells from 144 protrusion events. In the box and whisker
cells. (speed generated from three independent experiments. Data are

densities along this pixel-wide line are recorded at 1-second intervals for

at a 90° angle across a lamellipodium in the direction of protrusion. Pixel

are analysed by drawing a one pixel wide line (white line in top right box)

have higher rates of lamellipodia protrusions. Lamellipodial kymographs

kymograph pictures of M4 (top) and M4F (bottom) showing M4F cells

generated from 98 M4 cells and 135 M4F cells. (DEVELOPMENT)

An interesting difference between the role of fascin 1 in Drosophila

haemocytes and fascin 1 in mouse melanoblasts seems to be that

fascin 1 loss in Drosophila haemocytes dramatically impairs directed

migration and guidance (Zanet et al., 2009). Drosophila embryos

mutant for fascin 1 showed an accumulation of haemocytes in the

anteroventral regions where they originate and a failure to disperse
during development or in response to wounding. By contrast, fascin

1-null melanoblasts were able to home to hair follicles and to reach

most areas of the mouse epidermis during embryogenesis. Fascin 1-

null haemocytes failed to polarize their actin cytoskeletons (Zanet et al., 2009), whereas melanoblasts devoid of fascin 1 showed relatively

normal shape and polarization of pseudopods. This indicates that the

requirements for fascin 1 in migration during development are cell
type specific, similar to requirements for Rac1, which vary depending

on the cell type and tissue context (Migeotte et al., 2010; Wang et al.,

2010; Li et al., 2011).

The role of fascin 1 in cell proliferation and cell cycle progression

is not entirely clear and has only previously been seen in tumour

tissues or tissue culture cells. Quantification of the cell numbers at
different stages of embryogenesis and subsequent staining for

markers of apoptosis and cell proliferation revealed that fascin-null

melanoblasts proliferate slower than normal cells and this

phenotype was recapitulated in fascin 1-expressing melanoma cell

lines in culture. The embryonic requirement for fascin 1 during

melanoblast proliferation might reflect a possible mechanism by

which cancer cells could gain not only a motility advantage but also

a proliferation advantage by expressing fascin 1. In our studies,

fewer fascin 1 knockout melanoblasts were BrdU positive (Fig. 3E),

indicating that cells lacking fascin 1 were proliferating slower than

controls. In agreement with our knockdown studies, enhanced

proliferation was seen in fascin 1-transfected colon carcinoma cells

(Jawhari et al., 2003) and oesophageal squamous carcinoma cells

(Xie et al., 2005; Xie et al., 2010). However, as highlighted by

Hashimoto et al. (Hashimoto et al., 2005b), there is some

controversy over whether high fascin 1 levels correlate with high or

low proliferation rates in tumours (Pelosi et al., 2003; Hashimoto et

al., 2005a). We did not see any significant changes in levels of

ERK/MAPK or FAK signalling by western blot detection of

phospho-epitopes in cell lysates from fascin knockdown melanoma

cells, so the mechanism of involvement of fascin 1 in cell

proliferation remains unknown. Although the role of fascin 1 in cell

cycle progression is not known, fascin 1 might indirectly affect

microtubule spindle dynamics and cell mitosis through the

interaction of a myosin X/fascin/actin and actin-microtubule

network (Woolner et al., 2008; Nagy and Rock, 2010).

In summary, fascin 1 loss results in hypopigmentation defects in

mice that arise from a reduced migration and proliferation of

melanoblasts during embryogenesis. Melanoblasts devoid of fascin

can still differentiate and migrate in the dermis and epidermis, but

with reduced efficiency. Melanoblasts and melanoma cells show

![Fig. 5. Fascin 1 overexpression increases melanocyte lamellipodium dynamics.](image)

(A) GFP fluorescence and transmitted light photo of M4F melanocytes indicate that fascin 1-enriched filopodia (white arrows) are interspersed in the lamellipodium. Scale bar: 5 μm. (B) Cell migration speed generated from three independent experiments. Data are generated from 98 M4 cells and 135 M4F cells. (C) Representative kymograph pictures of M4 (top) and M4F (bottom) showing M4F cells have higher rates of lamellipodia protrusions. Lamellipodial kymographs are analysed by drawing a one pixel wide line (white line in top right box) at a 90° angle across a lamellipodium in the direction of protrusion. Pixel densities along this pixel-wide line are recorded at 1-second intervals for a period of 300 seconds, resulting in a time-space image. Scale bars for time and space are shown in the top image. Within the kymograph, a steeper slope of a protrusion corresponds to higher rates for lamellipodia protrusion. Distance extended (d) was measured against lamellipodial persistence (p), defined as the amount of time that the cell spends persistently extending a lamellipod before pausing or entering a retraction phase. Speed of protrusion is shown as a broken line and represents the distance protruded (d) divided by the time of protrusion (p). Frequency is measured by counting the number of lamellipodia per minute. (D) Frequency of lamellipodial protrusion events in M4 and M4F cells. (E-G) Speed (E), persistence (F) and distance (G) of individual lamellipodial protrusions. Data for M4 cells are from 273 protrusion events and for M4F cells from 144 protrusion events. In the box and whisker plots, horizontal line indicates median, box indicates interquartile range and whiskers indicate maximum value to minimum value. Mann-Whitney test; ***P<0.001; *P<0.05; n.s., not significant.
proliferation defects when fascin is depleted, suggesting a dependence of these cells on fascin for normal cell cycle progression. Melanoma tumour cells undergo changes associated with de-differentiation towards a more migratory and embryonic state, and this is thought to aid metastatic dissemination. Fascin 1 expression benefits melanoma and melanocyte cells in migration, invasion and proliferation; such as expression, of fascin 1 likely provides advantages in tumours.

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