Impaired wound healing in embryonic and adult mice lacking vimentin

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SUMMARY

It is generally assumed that the vimentin intermediate filament network present in most mesenchymally-derived cells is in part responsible for the strength and integrity of these cells, and necessary for any tissue movements that require the generation of significant tractional forces. Surprisingly, we have shown that transgenic KO mice deficient for vimentin are apparently able to undergo embryonic development absolutely normally and go onto develop into adulthood and breed without showing any obvious phenotype. However, fibroblasts derived from these mice are mechanically weak and severely disabled in their capacity to migrate and to contract a 3-D collagen network. To assess whether these functions are necessary for more challenging tissue movements such as those driving in vivo tissue repair processes, we have analysed wound healing ability in wild-type versus vimentin-deficient embryos and adult mice. Wounds in vimentin-deficient adult animals showed delayed migration of fibroblasts into the wound site and subsequently retarded contraction that correlated with a delayed appearance of myofibroblasts at the wound site. Wounds made to vimentin-deficient embryos also failed to heal during the 24 hour culture period it takes for wild-type embryos to fully heal an equivalent wound. By DiI marking the wound mesenchyme and following its fate during the healing process we showed that this impaired healing is almost entirely due to a failure of mesenchymal contraction at the embryonic wound site. These observations reveal an in vivo phenotype for the vimentin-deficient mouse, and challenge the dogma that key morphogenetic events occurring during development require generation of significant tractional forces by mesenchymal cells.

Key words: Wound healing, Vimentin, Granulation tissue formation, Myofibroblast, Morphogenesis

INTRODUCTION

Intermediate filaments are one of the three major cytoskeletal systems of eukaryotic cells and have classically been considered as providers of an intracellular scaffold that supplies cell strength and tissue integrity (Fuchs, 1994). Transgenic knockout mice null for several of the adult keratin genes have severe skin fragility (Lloyd et al., 1995; Porter et al., 1996), and it is now clear that many of the human skin blistering diseases, for example epidermolysis bullosa simplex, are due to simple point mutations in one or other of these epidermal-specific intermediate filament genes (Chan et al., 1994; Rugg et al., 1994; Lane et al., 1992). In cells of mesenchymal origin, the major structural component of intermediate filaments is vimentin and it is presumed that in these cells, vimentin plays a similar role to that played by keratins in epithelia. During embryonic development vimentin is first expressed in the mouse at about E8.5 in the cells of the parietal endoderm and also in those cells that delaminate through the primitive streak to become primary mesoderm (Franke et al., 1982; Lane et al., 1983). Later expression is seen in early pre-differentiated cell types during development of the neural and myogenic tissue before being replaced by more specialised intermediate filament networks such as GFAP and desmin (Cochard and Paulin, 1984; Fürst et al., 1989). In adult tissues vimentin is the only intermediate filament expressed by various cell types, in particular fibroblasts, endothelial cells and cells of the haemopoetic lineages. Curiously, given this extensive pattern of expression and the severe phenotypes revealed in transgenic mice null for several of the keratins, mice null for vimentin exhibit no obviously abnormal phenotype. These mice appear to develop and reproduce entirely normally and even detailed studies of tissues such as the lens, in which the sole intermediate filament expressed is vimentin, reveal no differences in structure or function from wild-type counterparts (Colucci-Guyon et al., 1994). Moreover, these observations cannot simply be explained by genetic redundancy since no other intermediate filament gene appears to be upregulated in fibroblasts in order to compensate for the lack of vimentin (Colucci-Guyon et al., 1994). Recently, deficiencies in the modulation of vascular tuning (Terzi et al., 1997) and in the mechanotransduction of shear stress (Henrion et al., 1997), as well as a cerebellar defect and impaired motor

coordination (Colucci-Guyon et al., 1999) have been described in vimentin null mice.

In vitro studies of fibroblasts from vimentin null embryos are significantly more revealing of cell defects in the absence of vimentin (Eckes et al., 1998). Vimentin deficient fibroblasts in tissue culture have an unusual actin cytoskeletal architecture with fewer, but thicker actin bundles and correspondingly larger, but more sparse focal adhesions. Magnetic twisting cytometry experiments to test cell strength and stiffness reveal that vimentin deficient fibroblasts are less resistent to twisting, i.e. less stiff, by comparison to wild-type cells (Eckes et al., 1998). Moreover, cell behaviour assays show that vimentin deficient fibroblasts crawl more slowly to fill in a tissue culture 'scratch' wound and contract a collagen gel more slowly than do their wild-type counterparts (Eckes et al., 1998). Because of these observations and because in vivo tissue repair processes demand a concerted motile and contractile effort by various cell types, we have chosen to investigate the wound healing capacity of vimentin deficient mice both at embryonic stages and as adults.

In wild-type adult tissues, wound healing is normally accompanied by an inflammatory response and by conversion of fibroblasts into a specialised contractile cell, the myofibroblast (Gabbiani et al., 1971; reviewed by Martin, 1997). By contrast, during embryonic tissue repair, mesenchymal contraction occurs in the absence of an inflammatory influx and without cells converting into myofibroblasts (Estes et al., 1994; Hopkinson-Woolley et al., 1994; McCluskey and Martin, 1995). Here we show that in both embryonic and adult situations, vimentin is necessary for the connective-tissue component of repair, which fails, or is severely retarded, in mice null for vimentin.

MATERIALS AND METHODS

For this study we used mice homozygous for the targetted Vim mutation as previously described (Colucci-Guyon et al., 1994), and wild-type mice of the same parental strain to serve as controls. The Vim +/− mice were originally obtained by crossing a chimaeric male (generated from a C57Bl/6 blastocyst injected with the mutant ES cells) with (C57Bl/6 × DBA2)F1 females. The colony was maintained by brother-sister or first cousin matings between heterozygous mice to produce Vim +/+, Vim +/−, and Vim −/− mice. To generate vimentin deficient embryos, we crossed male and female Vim −/− mice, and the resulting litters were staged according to the rule: midday on the day when the copulatory plug was first apparent was designated E0.5. For genotype confirmation, embryo and adult tissues were analysed by PCR as described by Colucci-Guyon et al. (1994) and Eckes et al. (1998).

Adult wounding study

Wild-type and vimentin-deficient female mice aged 3 months were anaesthetised with Avertin and paired 6 mm diameter, full-thickness, excisional wounds were made through the skin on the back of each mouse. Our protocol was essentially as described by Frank et al. (1996). Wound tissue and surrounding wound margin skin were harvested from mice at 3, 5, 7 and 14 days post-wounding and either fixed in 4% paraformaldehyde in PBS prior to paraffin-embedding and sectioning at 4-6 µm or snap-frozen in liquid nitrogen prior to embedding in TissueTek (Sakura Finetek Europe). Paraffin sections were stained with Haematoxylin and Eosin, before viewing and photography using a Zeiss Axioskop. The extent of wound closure was quantified by measuring the area of wound remaining open at the time of harvest and calculating this as a percentage of the previously recorded '0 hr' wound area for that specimen.

Counts of cell number and proliferative index

Numbers of fibroblasts in vimentin deficient versus wild-type wounds at days 5 and 7 (two biopsies for each genotype) were counted from Haematoxylin and Eosin-stained paraffin-embedded sections. At least fifteen fields of a defined size (1200 µm^2) were randomly selected throughout the granulation tissue. In parallel we incubated acetone fixed 6 µm cryosections from 5 day and 7 day wounds (two biopsies each of wild-type and vimentin deficient wounds) with an antibody which recognises the murine equivalent of Ki-67 (TEC-3, Dianova) at a dilution of 1 in 50, in order to reveal proliferating cells. After several rinses in PBS, bound antibody was detected using a 1:100 Cy3-conjugated donkey anti-rat secondary antibody (Dianova). These sections were counter stained with the nuclear marker, DAPI (Sigma) and viewed under a Nikon E800 microscope in order to quantitate the proliferation index i.e. the percentage of double labelled cells. At least 300 DAPI-stained nuclei in the granulation tissue of each section were scored.

Immunohistochemistry for myofibroblasts

Immunohistochemistry to reveal α-smooth muscle actin localisation and thus presence of myofibroblasts (Darby et al., 1990) was performed as described by Desmouliere et al. (1993) on sections through the healing wound at 3, 5, 7 and 14 days after wounding. Specimens were deparaffinized and rehydrated, and endogenous peroxidase was quenched with 1% H_2O_2 in methanol for 20 minutes at RT, followed by several rinses in PBS. Sections were then incubated for 45 minutes at room temperature with a 1 in 30 dilution (in 1% BSA in 0.05 M Tris-Cl, pH 7.6) of biotin-coupled anti-αSM-1, an antibody raised against the N-terminal peptide of α-smooth muscle actin (Skalli et al., 1986). After several further rinses in PBS, bound antibody was detected using 1:100 HRP-conjugated streptavidin (Vector) and 0.4 mg/ml of the chromogen, amino-ethyl-carbazol (AEC). Sections were counterstained with Hematoxylin.

Embryo wounding and culture

Embryonic day 11.5 progeny from +/+ × +/+ and −/− × −/− crosses were dissected from the uterus in Tyrode's saline using watchmaker's forceps and prepared for open yolk sac whole-embryo culture using the protocol of Martin and Cockroft (1999). The deciduum was trimmed away and the yolk sac cut almost all the way around where it abuts the placenta. Embryos were then delivered out of their yolk sac using watchmaker's forceps and the amniotic membranes peeled back to expose the embryo. The left hind limb bud was amputated using iridectomy scissors to expose a clean ovoid wound stump (the limb tissue so removed was transferred to lysis buffer in preparation for confirmatory PCR genotyping of the embryo). The wounded embryo was then transferred to a 50 ml Falcon tube containing 4 ml of a 1:3 mix of normal rat serum and Tyrode's saline. The tubes were gassed with 95% O_2/5% CO_2 before culturing and again after 12 hours in culture. Tubes containing embryos were then rotated at 30 rpm in an incubator at 37°C. Previous studies of ours have shown that embryos cultured in this way maintain a healthy heart beat and keep developmental pace with their peers in utero throughout the 24 hour period it takes to heal these wounds in wild-type embryos (McCluskey and Martin, 1995).

Scanning electron microscopy (SEM) of embryo wounds

After 0, 12 and 24 hours of culture a number of embryos were harvested and prepared for scanning electron microscopy (SEM) by fixing overnight at 4°C in 0.5 strength Karnovsky's fixative (Karnovsky, 1965). After rinsing in 0.1 M cacodylate buffer, specimens were dehydrated through a graded series of alcohols and critical point dried in the standard fashion. The specimens were finally mounted on stubs, sputtercoated with gold and viewed using a JEOL – JSM 35C scanning electron microscope.

DiI labelling of embryonic wound mesenchyme

To measure the extent of mesenchymal contraction occuring at the wounded site, small groups of mesenchymal cells at the wound perimeter were labelled with the lipophilic fluorescent dye DiI (Molecular Probes) (stock 0.5% DiI in absolute ethanol, diluted 1: 9 in 0.3 M sucrose solution on the day of use). Using a capillary needle of approximate diameter 15 μm controlled by mouth pipette, we placed 5-8 dots of DiI on the wounded mesenchyme close to the cut edge of the epithelium, thus marking the area of mesenchyme exposed by the wound. After 24 hours of culture the extent of mesenchymal contraction was estimated by measuring the area now bounded by the DiI labelled cells. Since each DiI dot marks a group of cells and this group spreads somewhat during healing, we determined DiI-delineated mesenchymal areas by joining the points at the centre (rather than the leading edge) of each of the six or so DiI wound marks, in order to get a measure of the average (rather than the maximal) mesenchymal movement. Embryos were fixed in ice-cold 4% paraformaldehyde in PBS for 2 hours and then mounted in Citiflour/PBS solution in a slide chamber and sealed beneath a coverslip. Wounds were visualised and photographed using the rhodamine filter block on a Leitz DiaPlan fluorescent microscope. The areas of exposed mesenchyme and contracted mesenchyme (from SEM and DiI-marked specimens, respectively) were traced from digitised images and measured as a proportion of mean initial wound area using standard NIH Image software.

RESULTS

Adult wound healing is delayed in vimentin deficient mice, as is the appearance of the wound granulation tissue

To test whether tissue repair capacity is altered in the absence of vimentin, we made circular, full thickness wounds on the backs of vimentin deficient mice and compared rates of healing with identically treated wild-type mice. Standardly, a 6 mm diameter wound of this sort rapidly contracts and is fully healed in just over a week (Frank et al., 1996). Our experiments mirrored this and showed the wound area remaining open in wild-type mice after 3, 5 and 7 days of healing to be 43%, 18% and 14% of initial wound area, respectively (ns=8, 4 and 4 – see Fig. 1A and C). Histology revealed a build up of inflammatory cells by 3 days, and a dense granulation tissue by 5 days (Fig. 2C). Macroscopically, after two weeks, all wounds were closed with eschars (scabs) discarded (Fig. 2E). By contrast, wounds on the back of vimentin deficient mice were only 50%, 54% and 27% closed (ns=6, 6 and 4 – see Fig. 1B and C) after 3, 5 and 7 days, respectively. However, as with wild-type mice, all wounds appeared fully closed by 2 weeks (Fig. 2F). The clearest difference between vimentin deficient wounds and wild-type equivalents was at 5 days, suggesting that the null animals were suffering a lag phase before wound contraction commenced. Accordingly, at 3 days post lesioning, histology through these wounds revealed a reduction in the extent of connective-tissue filling the wound defect, with vimentin deficient wounds having smaller wedges of ingrowing granulation tissue (Fig. 2B). By 5 days, wild-type wounds have generally established a thick, cell-rich granulation tissue, but in vimentin deficient wounds the equivalent tissue is still thin and far less cellular (compare Fig. 2C and D). We quantified numbers of cells per unit area in vimentin deficient, versus wild-type wounds at 5 days post wounding and observed that cell density is almost halved in the vimentin deficient wound connective tissue (17 ± 1 cells/1200 μm^2) by comparison to wild-type granulation tissue (32 ± 5 cells/1200 μm^2). By 7 days this difference is significantly reduced with cell counts in vimentin deficient wounds at 27 ± 3/1200 μm^2 versus 30 ± 5/1200 μm^2 for wild-
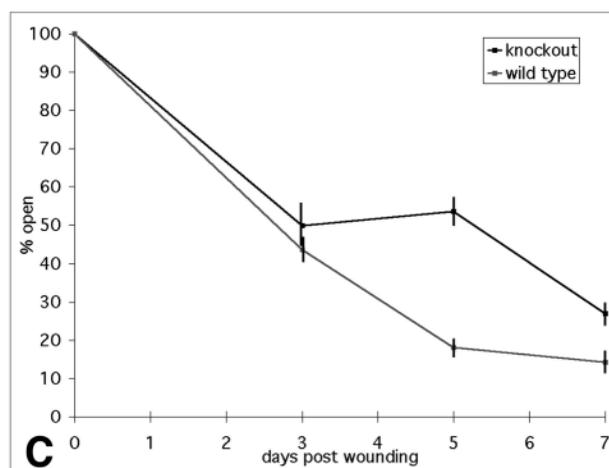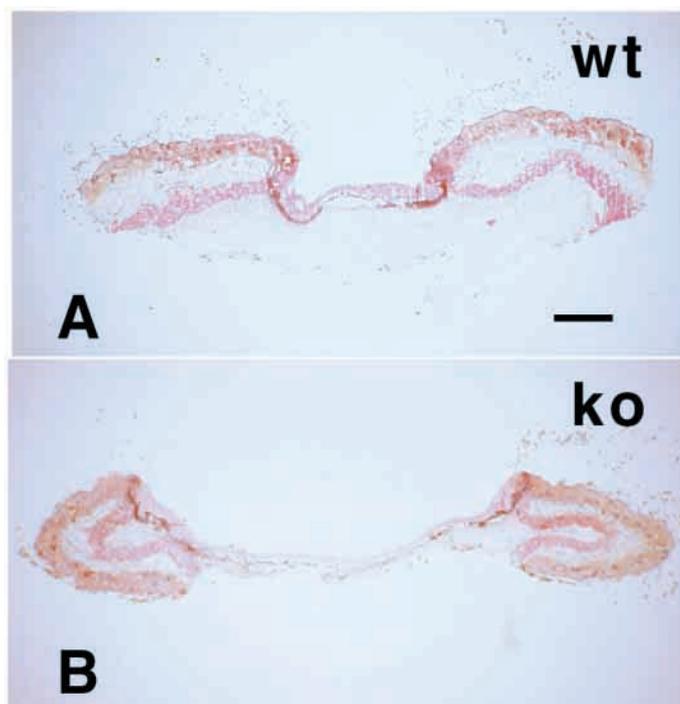

Fig. 1. Contraction kinetics of adult wild-type and vimentin deficient wounds. (A) Wax section through the mid-point of a 3 day wild-type wound with wound edges beginning to draw together. (B) Similar section through a 3 day vimentin deficient wound where the contraction responsible for drawing the wound edges together has failed. (C) Graphical representation of wound closure in wild-type and vimentin deficient adult mice. Grey and black points indicate area measurements during closure of wild-type wound and vimentin deficient wounds respectively. Error bars are s.e.m.s. Bar, 500 μm (A,B).
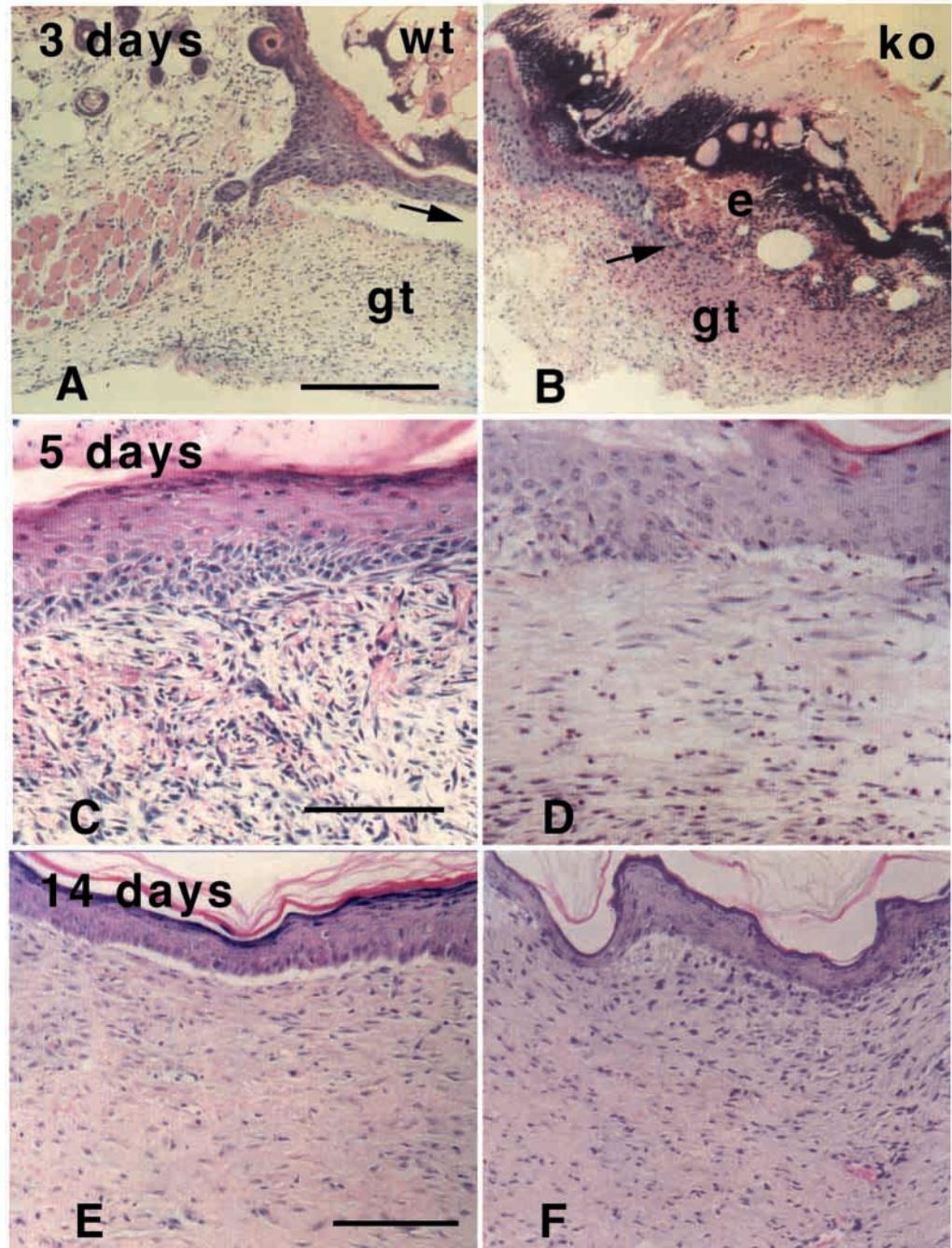
Fig. 2. Morphology of adult wild-type and vimentin-deficient wounds. (A) Wax histology of a wild-type wound at 3 days. Re-epithelialisation is proceeding (arrow) across a broad band of granulation tissue (gt). (B) In an equivalent vimentin deficient wound the leading epithelial tongue (arrow) migrates between eschar (e) and a reduced granulation tissue (gt). (C) By 5 days post wounding in the wild-type mouse, a cell-dense granulation tissue has established beneath the repairing epithelium. (D) By contrast, in an equivalent region of a vimentin deficient wound, the wound connective-tissue is far less cellular, suggesting a lag phase in the establishment of granulation tissue. (E) By 14 days in the wild-type mouse, wounds have fully closed and cell density in the wound connective tissue has returned to the levels of unwounded dermis. (F) At 14 days, vimentin deficient wounds are healed also, but wound connective tissue still has elevated cellularity, suggesting that these wounds still lag behind their wild-type equivalents. Bars: 100 µm (A,B); 50 µm (C,D and E,F).

type wound connective tissue. At 14 days post lesioning, wound closure is complete in both genotypes with regressing cell numbers in the wild type, but still with a thinner granulation tissue of slightly elevated cellularity in vimentin deficient animals (compare Fig. 2E and F). In order to test whether the reduced cell numbers at vimentin deficient wound sites might reflect reduced proliferative capacity of vimentin null fibroblasts as suggested by in vitro studies of Gillard et al. (1998) we calculated proliferative indices as a ratio of Ki67 positive cells over DAPI staining nuclei and found no significant difference at either 5 or 7 days post wounding. The proliferative index for wild-type wound connective tissue at 5 days was 18%±1% versus 18%±3% for vimentin deficient wounds, whilst by 7 days post wounding, cell division had

dropped considerably in both genotypes, such that proliferative indices were down to only 6%±2% (for wild-type mice) and 8%±2% (for vimentin deficient mice).

A delay in myofibroblast transformation may explain why wound contraction is retarded

Since myofibroblasts are considered to play a major role in wound contraction, we wondered whether impaired healing in vimentin deficient mice might be due to an incapacity for vimentin deficient fibroblasts to transform into myofibroblasts. However, using an α-smooth muscle actin antibody to reveal myofibroblasts, we showed that this transformation indeed occurs in vimentin deficient granulation tissue but that myofibroblast appearance is somewhat delayed by comparison
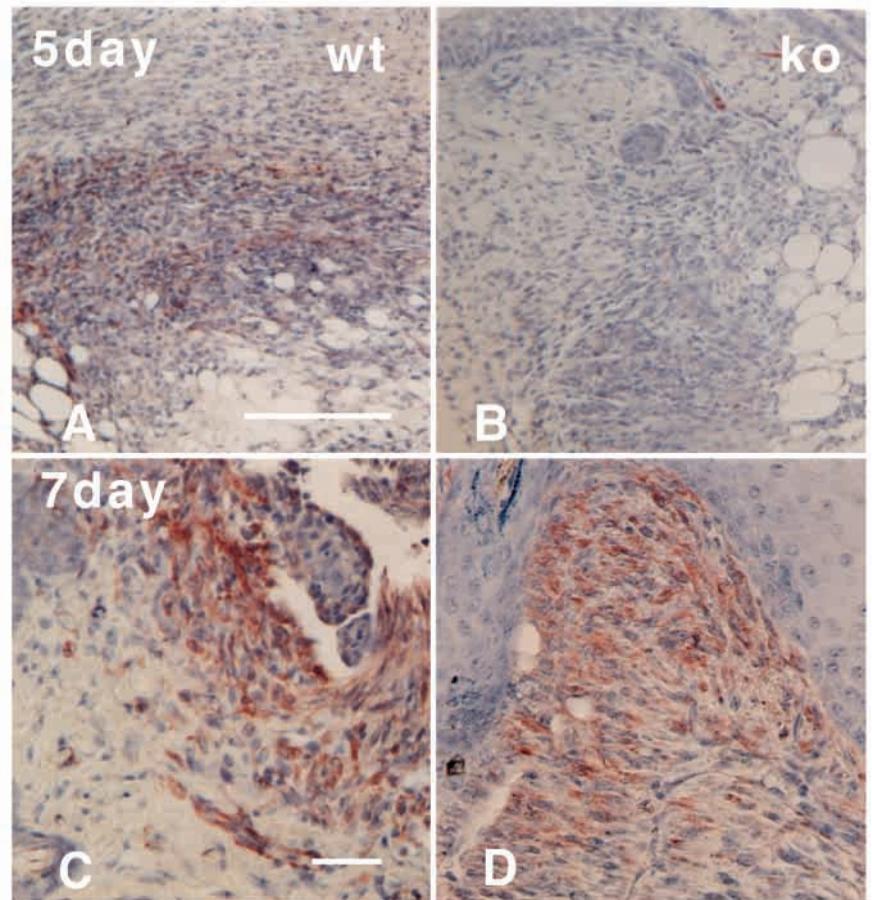
Fig. 3. Myofibroblasts in adult wild-type and vimentin-deficient wounds. (A) α-Smooth muscle immunostaining (brown) in the 5 day wild-type wound reveals large numbers of myofibroblasts in the wound granulation tissue. (B) By contrast in vimentin deficient wounds at the same time post-wounding there are no myofibroblasts in the wound connective tissue. (C) At 7 days post-wounding, myofibroblasts are still present in the granulation tissue of wild-type wounds, but are restricted to a sub-epidermal domain. (D) At 7 days, myofibroblasts are now also present in vimentin deficient wounds, but are not localised to the sub-epidermal domain. Bars: 50 μm (A,B); 10 μm (C,D).

with wild-type wounds (Fig. 3). In wild-type mice, myofibroblasts were first seen at 5 days after wounding (Fig. 3A), but in vimentin deficient mice there was no α-smooth muscle staining in the wound at this timepoint, except for that associated with small blood vessels (Fig. 3B). Staining in vimentin deficient wounds did not appear until 7 days (Fig. 3D). At this time, the localisation of myofibroblasts also appeared different in the two wound genotypes; in wild-type mice the majority of these cells were restricted to the subepidermal layers (Fig. 3C), whilst in vimentin deficient wounds, myofibroblasts appeared evenly distributed throughout the granulation tissue (Fig. 3D). For both wild-type and vimentin deficient mice, myofibroblast staining was back to zero at 14 days (data not shown), indicating that regression, presumably by apoptosis (Desmouliere and Gabbiani, 1996), proceeds as normally. Clearly vimentin deficient fibroblasts can transform into myofibroblasts but their delay in doing this, and possibly their mis-localisation within the wound granulation tissue, may, at least partially, account for the hindered wound healing in these mice.

Mesenchymal contraction is almost entirely blocked in wounded vimentin null embryos, whilst re-epithelialisation proceeds normally

In order to test whether vimentin is required by fibroblasts during embryonic tissue repair, where transformation of fibroblasts into myofibroblasts does not occurr (McCluskey and Martin, 1995), we have wounded vimentin deficient limb bud stage (E11.5) mouse embryos and compared their rate of wound closure with that of wild-type equivalents. We amputate the left hind limb bud leaving an exposed oval-shaped patch of mesenchyme with dimensions approximately 500×750 μm (Fig. 4A and B), and measure the extent of healing after 12 hours and 24 hours of culture. As previously reported these wounds heal over leaving a smooth flat surface where the limb bud had been – they repair by a combination of re-epithelialisation and contraction of the wound mesenchyme, and never show any sign of limb regeneration (McCluskey and Martin, 1995). Our scanning electron microscope studies (Fig. 4C-E) reveal wound area remaining open in wild-type embryos after 12 and 24 hours to be 50 and 0% (fully closed), respectively (ns=5 and 4 – Fig. 4C and E), whilst vimentin deficient embryos are significantly hindered with 82% and 51% of initial wound area still exposed at 12 and 24 hours of culture (ns=2 and 3 – Fig. 4D and E).

Since our SEM data give an overall impression of the total wound closure effort, including both that due to re-epithelialisation and that due to connective-tissue contraction, we performed a second study where only the connective-tissue contraction component of the repair process was measured (Fig. 4F-H). In wild-type embryos a DiI marked patch of wound exposed mesenchyme contracted to 61% and 41% of its initial area (ns=8 and 9) after 12 and 24 hours of culture respectively, whilst in vimentin deficient embryos mesenchymal contraction was severely reduced with only 88% and 79% (ns=4 and 6) shrinkage from initial wound area, after 12 and 24 hours (Fig. 4F-H). These data are suggestive that the major effect of vimentin absence on embryonic wound closure
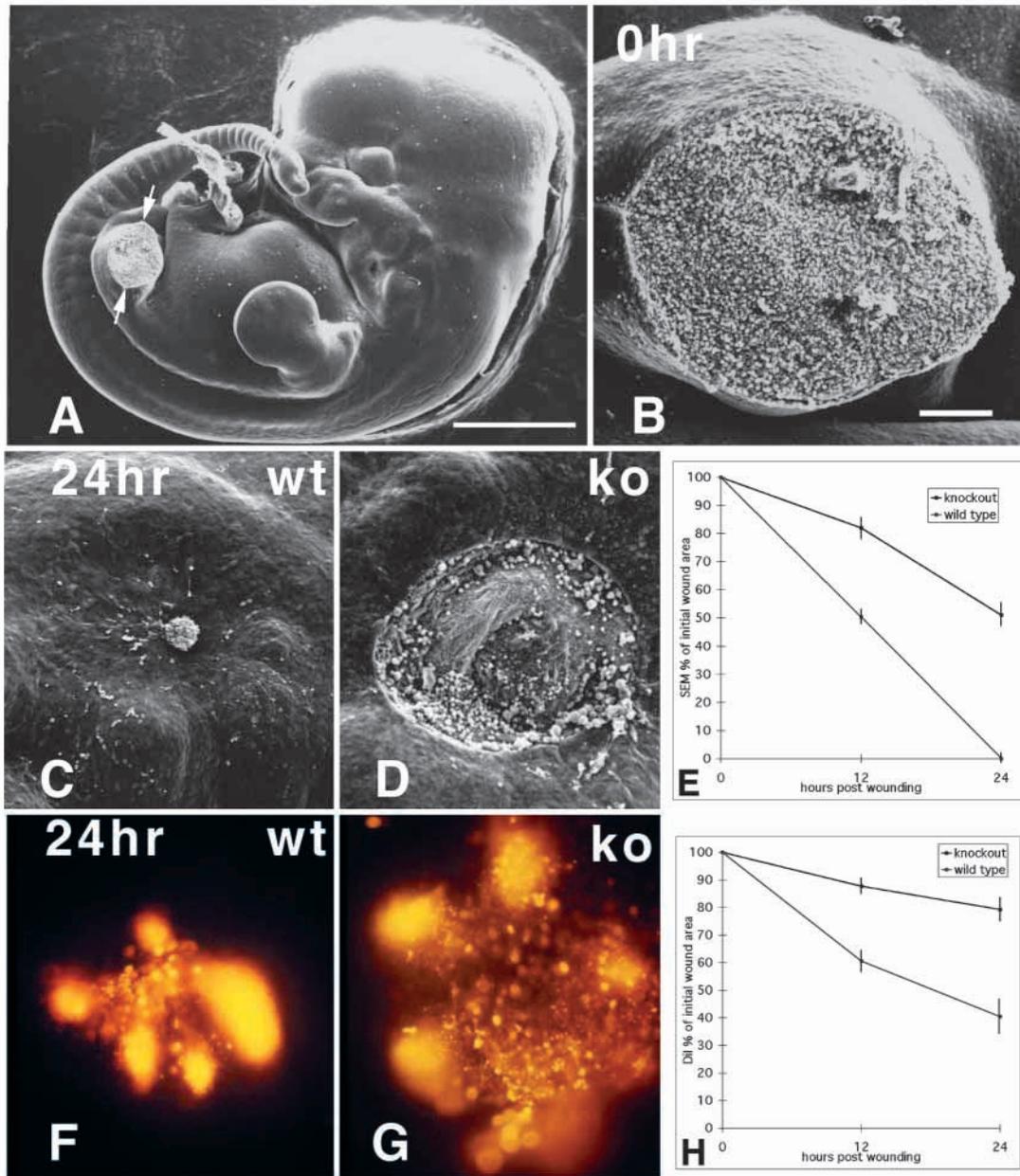
Fig. 4. Wound closure in wild-type and vimentin deficient embryos. (A) Scanning electron micrograph of an E11.5 mouse embryo with left hind limb bud amputated to leave an oval-shaped excisional wound (arrows). (B) Higher magnification detail of this '0'hr wound. (C) After 24 hours the wild-type wound is closed. (D) By contrast, at 24 hours post wounding the vimentin deficient wound is still open. (E) Graphical representation of wound closure (re-epithelialisation + connective tissue contraction) as measured from scanning electron micrographs. Grey and black points indicate area measurements in wild-type and vimentin deficient embryos during the 24 hour culture period. Error bars are s.e.m.s. (F) 24 hours post wounding, the wild-type wounds have significantly contracted their DiI-marked wound mesenchyme. (G) By contrast, at the same timepoint, the vimentin deficient wound has barely contracted. (H) Graphical representation of the connective-tissue contraction component of wound closure as measured from DiI marked specimens. Grey and black points indicate area measurements in wild-type and vimentin deficient embryos during the 24 hour culture period. Error bars are s.e.m.s. Bars: 1 mm (A); 100 µm (B,C,D,F,G).

is to significantly hinder mesenchymal contractility whilst, as one might expect, the epithelial component of wound closure is largely unaffected.

DISCUSSION

In this paper we report a clear phenotype for the vimentin deficient mouse, which otherwise appears to develop into adulthood entirely normally. We show that wound healing in the adult is dramatically impaired at the level of fibroblast invasion into, and contraction of, connective tissue. By contrast to our studies in the keratin 8 deficient embryo, where wound healing was normal (Brock et al., 1996), we find that repair in the vimentin deficient embryo is dramatically impaired by a failure of mesenchymal contraction. While the absence of

vimentin intermediate filaments is apparently compensated for by the embryo as it undergoes the various natural morphogenetic tissue movements critical for shaping itself, vimentin is clearly essential when mesenchymal cells are required to exert the powerful contractile forces that tug and draw wound edges together. In the embryo, over the timecourse we have examined, this block appears almost total, whilst in adult repair, we see a delay in the transformation of fibroblasts into contractile myofibroblasts, and consequently an extended lag phase before contraction begins.

In our original description of mice deficient in vimentin we reported no obvious wound healing phenotype since a superficial study of tail snip and ear punch wounds (as required for genotyping) suggested a normal timecourse of healing (Colucci-Guyon et al., 1994), but these were clearly not ideal 'in vivo' wound models. We also saw no difference in histological sections taken from vimentin deficient versus wild-type wound sites after the repair process was complete. However, recently we showed that vimentin deficient fibroblasts isolated in tissue culture display a reduced capacity for several functions that would presumably be important for tissue repair in vivo, and for this reason we decided to re-visit the question of tissue repair in these mice. In particular, we had observed slower migration into a monolayer scratch wound and an impaired capacity to contract collagen matrices (Eckes et al., 1998). Our current data, using an adult back skin wound model, do indeed show that vimentin deficient mice are impaired in tissue repair, and suggest that the same fibroblast failings observed in vitro may underly this in vivo wound healing phenotype. We observe that both fibroblast invasion and subsequent contraction of wounds is retarded in these mice.

The observed macroscopic delay in repair of these wounds, by comparison with wild-type mice, peaks at 5 days, when α-smooth muscle actin positive myofibroblasts are present in wild-type wounds but absent from vimentin deficient wounds. We presume that the delay in wound contraction is due to absence of these specialist contractile cells. This delay in myofibroblastic transformation may be directly related to the reduced contractility of vimentin deficient fibroblasts – certainly, it has been postulated that generation of significant mechanical strain may be a key trigger for switching of fibroblasts into myofibroblasts (Grinnell, 1994, 1999). Alternatively, transformation might be a density dependent process which is retarded in vimentin deficient individuals because of the slow invasion of fibroblasts. Indeed, in embryonic wound closure, which is not dependent on myofibroblast contractility, but entirely driven by fibroblast tractional/migrational forces (McCluskey and Martin, 1995), we show that vimentin appears to remain necessary for mesenchymal wound contraction. Our proliferation studies in adult wounds suggest that the reduced numbers of cells in the connective tissue of vimentin deficient wounds is a direct consequence of impaired fibroblast migration, rather than reduced proliferative capacity.

How might intermediate filaments be required for these processes and how are adult mice able to compensate after a delay period and heal their wounds, whilst embryos are not? The simplest interpretation of our data is that fibroblasts lacking intermediate filament support are unable to exert sufficient tractional force to efficiently migrate forward through their 3-D matrix surround into the wound defect, or to tug on that matrix in order to contract the wound. This incapacity is compensated for as the fibroblast transforms into a myofibroblast. When this happens in the adult wound, contraction then proceeds, but in the embryo, where this cell transformation never occurs, wound contraction almost completely fails. Our in vitro studies suggest that this in vivo failure of fibroblasts might not simply be a matter of cell fragility due to the missing structural support provided by vimentin, but might result from poor organisation of the other, non-intermediate filament cytoskeletal elements (Eckes et al., 1998). In tissue culture, vimentin deficient fibroblasts appear to have unusually bundled actin filaments and smaller numbers of actin anchoring adhesion sites. As Ingber and coworkers have speculated, it is likely that the various cytoskeletal networks are intimately dependent on one another (Wang et al., 1993; Ingber et al., 1994). Indeed, Bershadsky and colleagues (1987) have shown that vimentin filaments often terminate at, and may direct the assembly of, focal adhesions. Clearly, by disrupting the intermediate filaments in these cells we may also have disabled their capacity to adhere to and tug on the matrix surround using their actinomyosin contractile machinery and related adhesion complexes. How the newly upregulated actin and myosin isoforms expressed by myofibroblasts might override this vimentin dependency deserves further study.

Given the incapacity of mesenchymal cells to contract their wound bed in vimentin deficient embryos, it seems surprising that these mice have an otherwise normal phenotype and are seemingly able to undertake all natural morphogenetic movements just as wild-type embryos. Of course, much of embryonic morphogenesis involves bending and folding of epithelial sheets, which one would not expect to be dependent on mesenchymal cell strength, but a significant number of key morphogenetic events are dependent on mesenchymal aggregations, for example, the condensation of cells that precedes formation of cartilaginous longbones in the developing limbs, or beneath the hair placodes of the developing skin (reviewed by Bard, 1992). There is good in vitro evidence that embryonic fibroblasts are capable of generating quite substantial tractional forces, sufficient to contort silicone and collagen substrata, and it has been postulated that similar is true in vivo, and that this is what drives mesenchymal aggregation (Stopak and Harris, 1982). Our wounding studies in vimentin deficient embryos suggest that this is unlikely. We show that embryonic fibroblasts in which vimentin is absent are unable to exert powerful tractional forces in vitro or in vivo, and yet vimentin deficient embryos do not suffer impaired skeletal development or unusual hair pattern. We therefore postulate that mesenchymal morphogenetic events may not involve the generation of hefty tractional forces during development, because if they did, then these events would fail in the vimentin deficient embryo.

In summary, we have shown a new phenotype for the vimentin null mouse. As an embryo or an adult, these mice show impaired wound healing, with the defect residing in the connective-tissue component of this process. The fact that vimentin deficient fibroblasts seem unable to generate significant tractional forces in vitro or in vivo and yet the vimentin null mouse is essentially normal, leads us to speculate that such forces may not be necessary to drive the major morphogenetic events that occur during development.

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