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β-catenin activation is necessary and sufficient to specify the dorsal dermal fate in the mouse

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Abstract

Dorsal dermis and epaxial muscle have been shown to arise from the central dermomyotome in the chick. En1 is a homeobox transcription factor gene expressed in the central dermomyotome. We show by genetic fate mapping in the mouse that En1-expressing cells of the central dermomyotome give rise to dorsal dermis and epaxial muscle and, unexpectedly, to interscapular brown fat. Thus, the En1-expressing central dermomyotome normally gives rise to three distinct fates in mice. Wnt signals are important in early stages of dermomyotome development, but the signal that acts to specify the dermal fate has not been identified. Using a reporter transgene for Wnt signal transduction, we show that the En1-expressing cells directly underneath the surface ectoderm transduce Wnt signals. When the essential Wnt transducer β-catenin is mutated in En1 cells, it results in the loss of Dermo1-expressing dorsal dermal progenitors and dermis. Conversely, when β-catenin was activated in En1 cells, it induces Dermo1 expression in all cells of the En1 domain and disrupts muscle gene expression. Our results indicate that the mouse central dermomyotome gives rise to dermis, muscle, and brown fat, and that Wnt signalling normally instructs cells to select the dorsal dermal fate.

Keywords: Dermomyotome; Skin; Cell fate; Dermis; Wnt; Brown fat

Introduction

The somites give rise to axial skeleton, striated muscle, and dorsal dermis (Christ et al., 2004; Gossler and Hrabe de Angelis, 1998; Hirsinger et al., 2000; Scaal and Christ, 2004). Each somite displays restricted domains of gene expression and these are thought to represent cell populations that will give rise to the various tissue derivatives. The dermal derivative has been the least explored with respect to where the dermal progenitors come from and the identity of signals that instruct the cells of the dermomyotome to adopt a dermal fate (Scaal and Christ, 2004).

Somites segment from the anterior end of the presomitic mesoderm and form epithelial units (Pourquie, 2001). The ventromedial portion of the somites become mesenchymal again to form the sclerotome and the remainder of the somite persists as an epithelial dermomyotome (Christ et al., 2004; Gossler and Hrabe de Angelis, 1998; Hirsinger et al., 2000; Scaal and Christ, 2004). Later, the central portion of the somite undergoes an epithelial to mesenchymal transition (Scaal and Christ, 2004). Historically, the dermis was assumed to derive from this region of the dermomyotome, and it was termed the dermatome (Scaal and Christ, 2004).

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Recently, it has been shown conclusively that the "dermatome" also contributes to fetal and postnatal epaxial skeletal muscle and to adult muscle satellite cells in both chick and mouse (Ben-Yair and Kalcheim, 2005; Gros et al., 2005; Kassar-Duchossoy et al., 2005; Relaix et al., 2005). Thus, it has been proposed that the dermatome instead be called the central dermomyotome (Scaal and Christ, 2004). How the dermal and muscle fates are selected from the broader dermomyotome domain is not known. That the somite gives rise to the dorsal dermis of the trunk was established by orthotopic transplantation of quail presomitic mesoderm into chick hosts (Mauger, 1972; Nowicki et al., 2003). Similar experiments showed that the flank and belly dermis of the trunk comes from lateral plate mesoderm (Mauger, 1972). However, which parts of the presomitic mesoderm, somite, and dermomyotome give rise to the dorsal dermis has not been resolved (Scaal and Christ, 2004).

In one map of somite-derived dermis, the cells which form dermis are present first in the medial presomitic mesoderm and then later in the medial portion of epithelial somites (Cheng et al., 2004; Ordahl and Le Douarin, 1992). The dorsomedial lip (DML) of the dermomyotome generates cells that move further laterally within the plane of the dermomyotome (Cheng et al., 2004; Ordahl et al., 2001), perhaps giving rise to much of the dermomyotome (Ordahl et al., 2001). The DML-derived cells translocate into a domain of cells that express the homeobox transcription factor gene En1 and begin to express En1 (Cheng et al., 2004). Dorsal dermis is thought to derive from cells which expressed En1 (Cheng et al., 2004).

A second map also places somite-derived dermal progenitors in the medial presomitic mesoderm (Olivera-Martinez et al., 2000). Once the dermomyotome has formed, the DML gives rise to mesenchymal cells that migrate early to form the dermis over the neural tube (Houzelstein et al., 2000; Olivera-Martinez et al., 2002). The majority of the dorsal somite-derived dermis arises from dermomyotomal cells that express En1 (Olivera-Martinez et al., 2002).

Finally, one group has found that both medial and lateral halves of the epithelial somites give rise to dermis (Ben-Yair et al., 2003). According to the results from this group, the dermomyotome does not grow from the DML and the dermomyotome exclusive of its medial and lateral lips gives rise to dermis (Ben-Yair et al., 2003). In this map, the dermis over the neural tube arises later in development, from the central dermomyotome (Ben-Yair et al., 2003).

The signals that are required for the formation of dermis from the central dermomyotome are not known. There are several results which suggest that members of the Wnt family are excellent candidates for the signal that promotes the development of dermis from the central dermomyotome. Wnts are expressed in the surface ectoderm (Parr et al., 1993; Rodriguez-Niedenfuhr et al., 2003; Schubert et al., 2002), and blocking Wnts with Dkk in the chick results in a thinner dermis (Chang et al., 2004) and loss of feather follicles (our unpublished studies). Wnts are important in several earlier steps of somite and dermomyotomal development, including segmentation, somite epithelialization, dermomyotome proliferation, and expression of Pax3, Pax7, and En1 in the dermomyotome (Aulehla et al., 2003; Linker et al., 2005; Nakaya et al., 2004; Schmidt et al., 2004; Galli et al., 2004; Capdevila et al., 1998; Fan et al., 1997; Fan and Tessier-Lavigne, 1994; Schmidt et al., 2004). The expression of En1 in the central dermomyotome requires Wnts from the dorsal neural tube in the mouse (Ikeya and Takada, 1998), and from the dorsal neural tube and the surface ectoderm in the chick (Cheng et al., 2004; Olivera-Martinez et al., 2001). After the formation of dermis, signals from the dorsal neural tube are necessary for its survival (Olivera-Martinez et al., 2001). Wnt1 can substitute for the dorsal neural tube signal, but it is not clear if Wnt1 acts directly or indirectly (Olivera-Martinez et al., 2001). Much later in development, Wnt signal transduction in the epidermis is necessary for the development of hair and feathers (Chang et al., 2004; Huelsken et al., 2001; Lo Celso et al., 2004; Silva-Vargas et al., 2005), and it has been suggested that the dermal condensations participating in early stages of hair follicle development might require Wnt signal transduction (DasGupta and Fuchs, 1999; Kishimoto et al., 2000; Ridanpaa et al., 2001). At the intermediate stage when the central dermomyotome generates dermal progenitors, it has been hypothesized, but not tested, that Wnt signaling is necessary for dermal specification (Scaal and Christ, 2004).

Here we determined the fate of the En1-expressing cells of the central dermomyotome in the mouse and found that the central dermomyotome tissue is tripotent in that it gives rise to interscapular brown fat bundles in addition to dermis and muscle. Our results also clarify the fate map of the dermomyotome relative to the dorsal dermis in the mouse. Using a TCF/Lef reporter gene assay, our data indicate that a subset of the En1 population responds to Wnt signalling at the time of dermal specification. Finally, by both loss- and gain-of-function approaches, we show that the decision to form dermis instead of muscle and brown fat is critically controlled by β-catenin through the specification of the dorsal dermal progenitors.

Results

Central dermomyotome contributes to brown fat, dermis, and muscle

The fate of cells that express En1 in the mouse central dermomyotome was determined with an allele of En1 with Cre recombinase coding sequences inserted (En1Cre) (Hanks et al., 1995) and the Rosa-floxed Stop-LacZ Reporter allele (RR) (Soriano, 1999). With these alleles, cells which express En1 and their progeny are marked by β-galactosidase expression (Zinyk et al., 1998). E17.5 fetuses carrying single copies of the En1Cre and RR alleles were sectioned and stained for β-galactosidase expression (Fig. 1A). In the skin, β-galactosidase expression was seen in the dermis but not the epidermis. Unexpectedly, all trunk dermis of the E17.5 fetus at the forelimb level was marked (Fig. 1A, and data not shown). Some back muscles and bundles of interscapular brown fat were labeled as well. The brown fat bundles in the most medial interscapular bundles and in the axillary depots were not labeled (not shown). The marking of

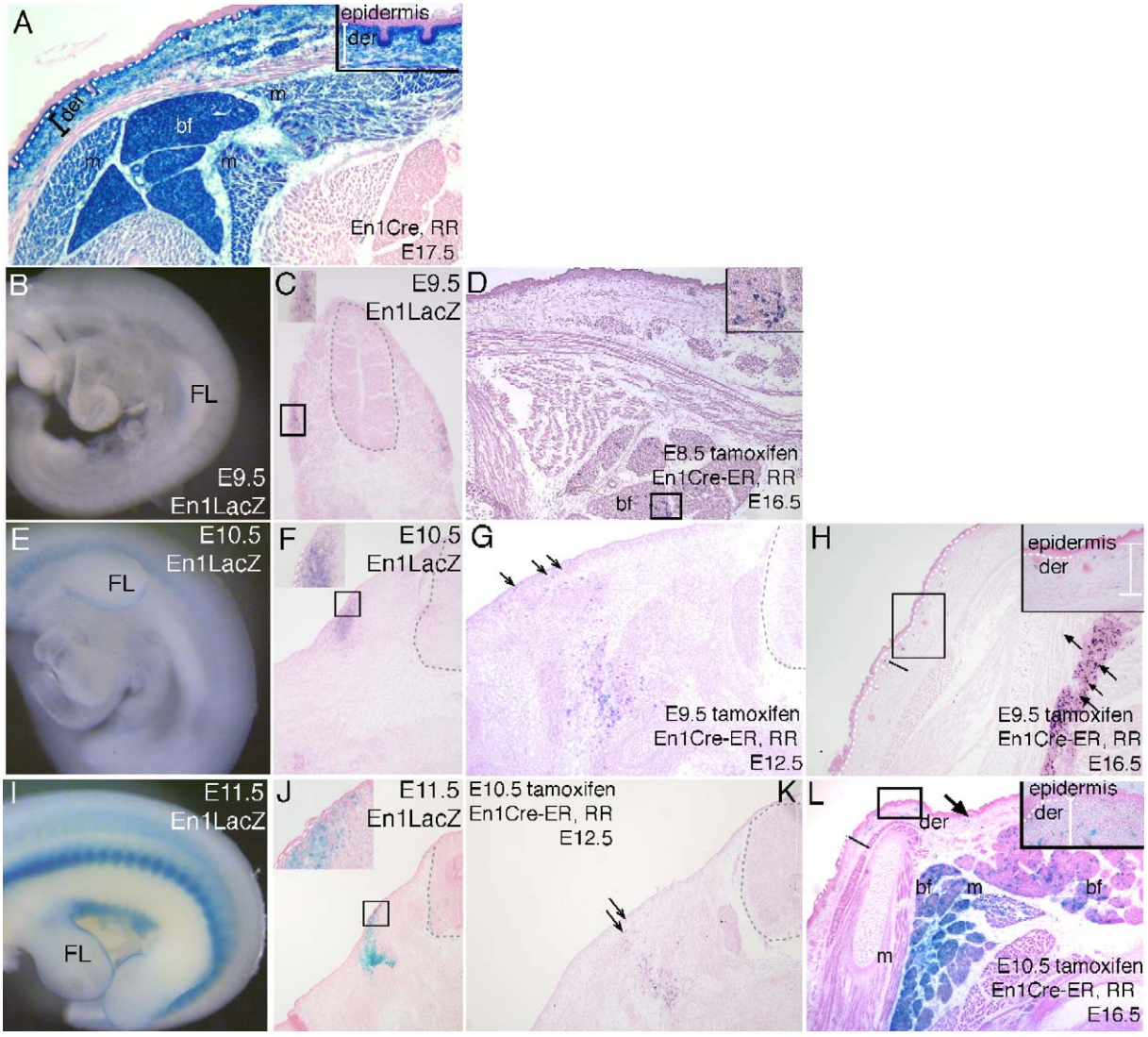

Fig. 1. The En1-expressing cells of the central dermomyotome contribute to epaxial muscle, dermis, and interscapular brown fat. (A) En1 lineage-marked cells (blue) are found in the dermis (der), interscapular brown fat (bf), and epaxial muscle (m) of E17.5 day En1Cre/+, RR/+ embryos at the forelimb level. (B and C) At E9.5, En1 is restricted to a few cells of the central dermomyotome. (B) In E9.5, En1LacZ/+ whole-mount embryos expression is only detectable in the ventral ectoderm of the fore limb bud (FL). (C) In sectioned E9.5 En1LacZ/+ embryos, stained cells are present in the central dermomyotome (inset). (D) En1Cre-ER/+, RR/+ embryos were treated with tamoxifen in utero at E8.5 and harvested at E16.5. The only labeled trunk mesodermal cells were in the interscapular brown fat (bf). (E) In whole-mount E10.5 En1LacZ embryos, expression is visible in cervical and thoracic somites. (F) In E10.5 embryo sections, the expression of En1LacZ (blue stain) is in cells of the somite just underneath the surface ectoderm, as well as in slightly deeper mesenchymal cells (boxed and inset). (G) En1Cre-ER/+, RR/+ embryos were treated on E9.5 with tamoxifen and were examined on E12.5. Stained cells are present in the somite mesenchyme subjacent to the surface ectoderm (arrows) and in deeper cells. (H) En1Cre-ER/+, RR/+ embryos treated on E9.5 with tamoxifen and harvested on E16.5 have labeled cells in the dermis (boxed and inset), epaxial muscle (arrows), and brown fat. (I) On E11.5, whole-mount-stained embryos show that En1LacZ is expressed in somites along most of the anterior–posterior axis, and in the ventral ectoderm and ventral body wall. (J) In the sections, it can be seen that the expression of En1LacZ on E11.5 is present in cells subjacent to the ectoderm (box and inset) and in deeper cells within the somite. (K and L) En1Cre-ER/+, RR/+ embryos treated with tamoxifen on E10.5 results in labeled cells in the dermal precursors and deeper cells at E12.5 and in muscle, brown fat, and dermis at E16.5.

dermis was more widespread and it is different from what has been observed in the fate maps of the chick central dermomyotome (Mauger, 1972; Nowicki et al., 2003; Scaal and Christ, 2004), suggesting that En1 is expressed in dermal precursors or mature dermis. As shown below, En1 is transiently expressed in many dermal progenitors at later stages. Thus, in order to map the fate of the central dermomyotome, labeling of cells was limited to short temporal windows at early stages with an En1 Cre-Estrogen Receptor (ER) fusion knockin (Sgaier et al., 2005). The Cre-ER fusion protein is active only when tamoxifen is experimentally administered, and it is active for about 24 h, starting about 6 h after dosing (Sgaier et al., 2005).

En1 expression in the E9.5 central dermomyotome (Davidson et al., 1988; Davis et al., 1991) is recapitulated by an allele with β-galactosidase coding sequences knocked into the En1 locus (En1LacZ) (Hanks et al., 1995) (Figs. 1B and C). The mouse dermomyotome, and particularly the central dermomyotome, does not show a robust epithelial organization like the chick dermomyotome, so the assignment of labeled cells to the

dermomyotome layer is based on their position and according to the stage of the embryo. To lineage-mark the En1 cells of the E9.5 central dermomyotome, we administered tamoxifen to pregnant females carrying En1Cre-ER/+, RR/+ embryos at 8.5 days of gestation. β-galactosidase-labeled cells were seen in the interscapular brown fat of E16.5 fetuses at the level of the forelimb after E8.5 tamoxifen administration (Fig. 1D). These results demonstrate that En1-expressing cells of the central dermomyotome contribute to interscapular brown fat. However, it was surprising that no dermis or muscle were lineage-marked in these early labeling experiments.

Dermis and epaxial muscle were marked in addition to brown fat when tamoxifen was administered 24 h or more later in development. At E10.5, the En1LacZ allele is expressed in a narrow domain of the mesenchyme of the central dermomyotome directly underneath the surface ectoderm and in adjacent, contiguous mesenchymal cells lying slightly deeper within the somite (Figs. 1E and F). Tamoxifen administered on E9.5-labeled cells deep back muscles and deep mesenchymal cells, and presumed dermal precursors on E12.5 (Fig. 1G). At E16.5, labeled descendents were detected mostly in interscapular brown fat, with labeled cells in nearby back muscles and in the dorsal dermis (Fig. 1H). Staining in the dorsal dermis was restricted to cells in the vicinity of the original En1 expression in the dermomyotome.

At E11.5, En1LacZ is expressed in a narrow domain of cells underneath the surface ectoderm, and in a contiguous strip of mesenchymal cells deeper within the somite (Figs. 1I and J). Labeled progeny from tamoxifen administered on E10.5 were present both superficially and deeper at E12.5 (Fig. 1K), and labeled cells were present in brown fat, muscle, and dermis at E16.5 (Fig. 1L). In this instance, labeled dermal cells were distributed above the En1 domain as well as in the midline over the neural tube. The lateral limit of labeled dermal cells was the medial third of the scapula at E16.5 (Fig. 1L).

Taken together, our results indicate that cells which express En1 in the central dermomyotome on E9.5 give rise to interscapular brown fat. The cells that express En1 on E10.5 give rise to dermis and epaxial muscle, in addition to brown fat, and these tissues are narrowly distributed. Cells which express En1 on E11.5 give rise to the same derivatives but, in addition, also give rise to dermis over the fetal midline. Together, dermal cells derived from the En1-expressing cells from E9.5 to E11.5 contribute to the dorsal dermis from the fetal midline to the medial third of the scapula.

En1 expression precedes Dermo1 in some regions but not others

The timing of expression of En1 and Dermo1, a marker of dermal specification (Houzelstein et al., 2000; Li et al., 1995), was compared. In the mouse, Dermo1 is not expressed in the dermomyotome on E9.5 and E10.5 (Li et al., 1995, and data not shown) when En1 is expressed in the central dermomyotome (Figs. 1B, C, E, and F). On E11.5, cells expressing En1LacZ or lineage-marked by En1Cre are confined to a small medial–lateral domain (Figs. 2A and B), whereas Dermo1 is expressed in subectodermal mesenchyme in mouse dermal progenitors with a gap over the neural tube (Fig. 2C). Thus, in the mouse central dermomyotome, En1 precedes Dermo1 by as much as 48 h.

By E12.5, En1LacZ expression and lineage-labeled cells are present in the dorsal subectodermal mesenchyme over the midline and lateral to the central dermomyotome (Figs. 2D and E). The expression of Dermo1 on E12.5 is similar to that on E11.5, with no detectable expression over the neural tube (Fig. 2F). Thus, lateral to the central dermomyotome, Dermo1 expression precedes En1 expression by as much as 24 h.

By E14.5, En1LacZ expression is reduced from the levels seen earlier (Fig. 2G), but lineage-marked dermis, muscle, and brown fat are evident in En1Cre, RR fetuses (Fig. 2H). On E14.5, Dermo1 RNA is detectable for the first time in the mesenchyme over the neural tube (Fig. 2I). Thus, in the mesenchyme over the midline, En1 expression precedes detectable Dermo1 expression by at least 24 h.

On E16.5, En1LacZ expression was not detected in the dorsal mesodermal tissues of the fetus (Fig. 2J), but lineage-marked cells in En1Cre/+, RR/+ fetuses were present in dermis, muscle, and brown fat bundles (Fig. 2K). Dermo1 is no longer expressed in the dermis on E16.5 (not shown).

Thus, En1 expression precedes Dermo1 expression by 48 h in most of the cells, which give rise to the dermis from the midline to the medial third of the scapula (Fig. 2L). In the flank, Dermo1 expression precedes En1 expression by about 24 h (Fig. 2L).

Canonical Wnt signal transduction is active prior to and coincident with Dermo1 expression

At E10.5, the TCF/Lef-lacZ Wnt reporter transgene is expressed in subectodermal mesenchyme, overlapping En1LacZ expression there (Figs. 3A and C). By E11.5, the TCF/Lef-lacZ transgene is expressed throughout much of the dorsal subectodermal mesenchyme (Fig. 3D), similar to Dermo1. To test if TCF/Lef-LacZ expression is dependent on β-catenin, we removed or stabilized β-catenin using conditional loss-of-function and gain-of-function alleles (Brault et al., 2001; Harada et al., 1999). TCF/Lef-LacZ expression was lost in β-catenin loss-of-function mutant En1 cells (Fig. 3E) and was ectopically expressed in mutant cells carrying the β-catenin gain-of-function allele, including mutant En1 cells at a distance from the surface ectoderm (Fig. 3F). Thus, transcription activated by canonical Wnt signal transduction is active in subectodermal mesenchyme consistent with a role for Wnt signaling in dermal specification.

β-Catenin activity is necessary and sufficient for dermal specification

To determine if dermal specification requires Wnt signal transduction, we mutated β-catenin in the En1-expressing cells of the somite. In E11.5 β-catenin conditional loss-of-function mutant embryos, the domain of Dermo1 expression in the subectodermal mesenchyme was interrupted: Dermo1

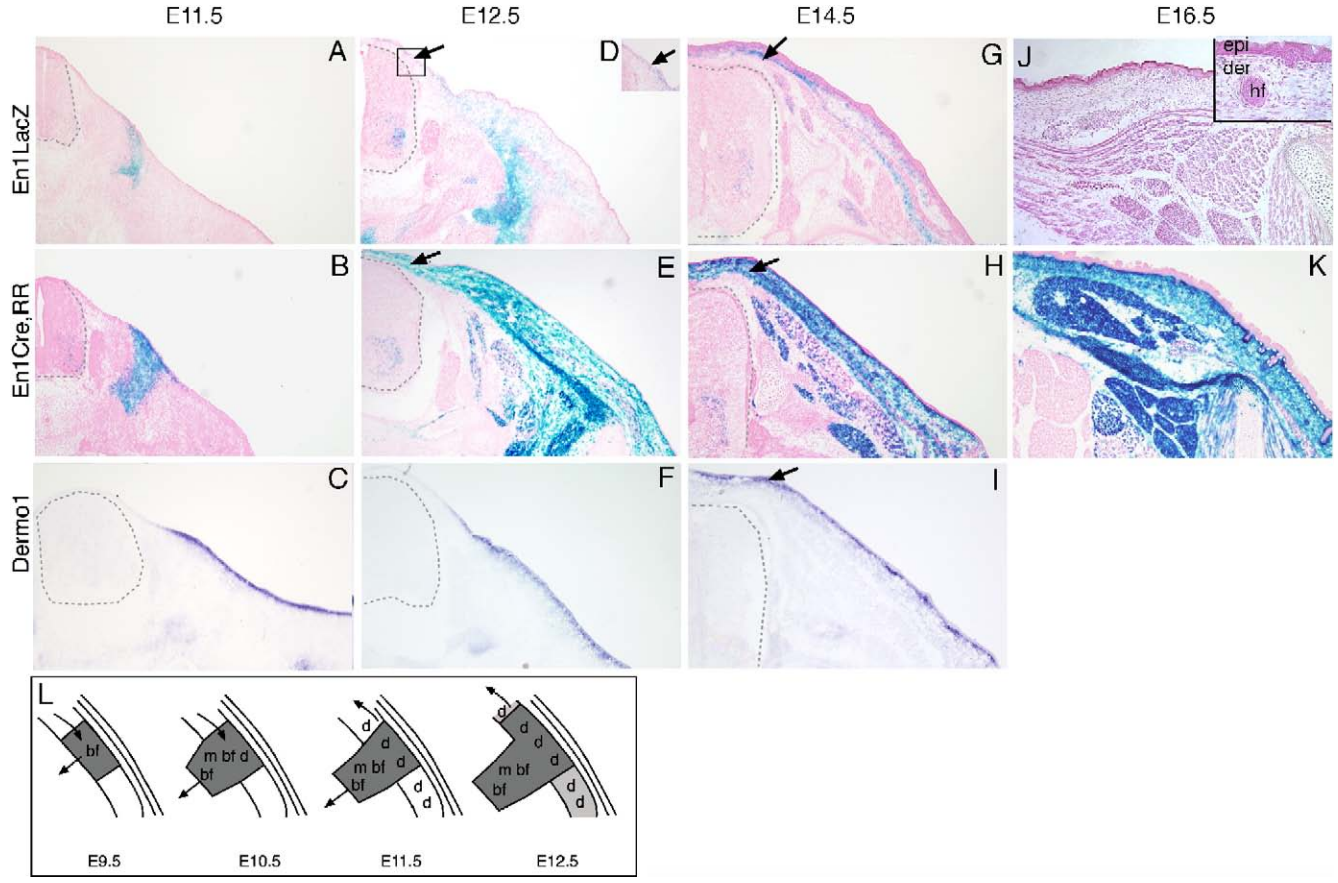

Fig. 2. En1 expression precedes Dermo1 expression medially, but not laterally. (A–C) In E11.5 embryos, Dermo1 is expressed in En1LacZ-expressing and En1Cre-lineage-marked dermal progenitors in the En1 domain, as well as lateral to the En1 domain. (D–F) In E12.5 embryos, En1LacZ-expressing cells and En1Cre-lineage-marked cells (arrows and inset in panel D) are detected over the midline neural tube, but Dermo1 expression is not. Lateral to the original En1 domain, En1LacZ expression and En1Cre lineage-marked cells are detectable in the subectodermal mesenchyme. (G–I) By E14.5, Dermo1 expression can be detected over the fetal midline above the neural tube. (J and K) En1LacZ expression is greatly diminished by E16.5 and is not detectable in the dermis (inset in panel J), whereas En1Cre lineage-marked cells are prevalent in dermis, brown fat bundles, and muscle (K). (L) Schematic of the fate map of the En1-expressing somite cells. En1 is expressed (gray shading) in the central dermomyotome at E9.5 in cells fated to become brown fat (bf). These cells displace to the interior and are replaced by medial cells in the dermomyotome. On E10.5, En1 is expressed in cells directly underneath the surface ectoderm and in deeper cells. Cells that now express En1 are fated to dermis (d) and muscle (m) in addition to the deeper cells which are fated to brown fat. By E11.5, cells adjacent to the surface ectoderm express Dermo1. By E12.5, some cells of the En1 domain move dorsomedially underneath the surface ectoderm, and En1 becomes broadly expressed (lighter gray) in the dermal progenitors.

expression was not detectable in the mutant En1 cells (Figs. 4D and E). Dermo1 expression was present lateral to the mutant cells and was also present at lower levels in a small domain medial to the mutant cells. Thus, β-catenin is necessary for Dermo1 expression in the En1 lineage-marked cells of the somite. To determine if β-catenin is sufficient for Dermo1 expression, we analyzed embryos carrying a gain-of-function conditional allele of β-catenin. Dermo1 was expressed in all cells that expressed stabilized β-catenin, including ectopic expression in mesenchymal cells distant from the surface ectoderm (Figs. 4C and F). Combined, these results indicate that β-catenin is necessary and sufficient for dermal specification as assayed by expression of Dermo1.

To determine if proliferation or programmed cell death was altered when β-catenin was mutated, we determined DNA synthesis and apoptosis on E10.5. Both the β-catenin loss-of-function and gain-of-function mutant cells had lower levels of BrdU incorporation (Figs. 5A–C and Table 1), but the differences in BrdU labeling did not reach statistical significance ($P > 0.05$), nor did the results reach statistical significance when they were partitioned into deep cells and cells within three cell diameters of the surface ectoderm (Table 1). Very few apoptotic cells were detected by TUNEL staining in wild-type embryo sections in the En1 domain, and there were no detectable changes in TUNEL labeling in the mutant cells (Figs. 5D–F). Thus, any changes in proliferation were either too subtle to detect or occurred earlier in development than assayed.

Expression of myogenin is altered in β-catenin mutant cells

To determine whether the specification of muscle progenitors was altered in the β-catenin mutants, we assayed muscle specification by staining for the bHLH myogenic transcription factor myogenin (Sassoon et al., 1989; Smith et al., 1994). At E11.5, cells lineage-marked by the En1Cre allele are contiguous with each other, not mixing with unlabeled cells except at the edges of the domain. When

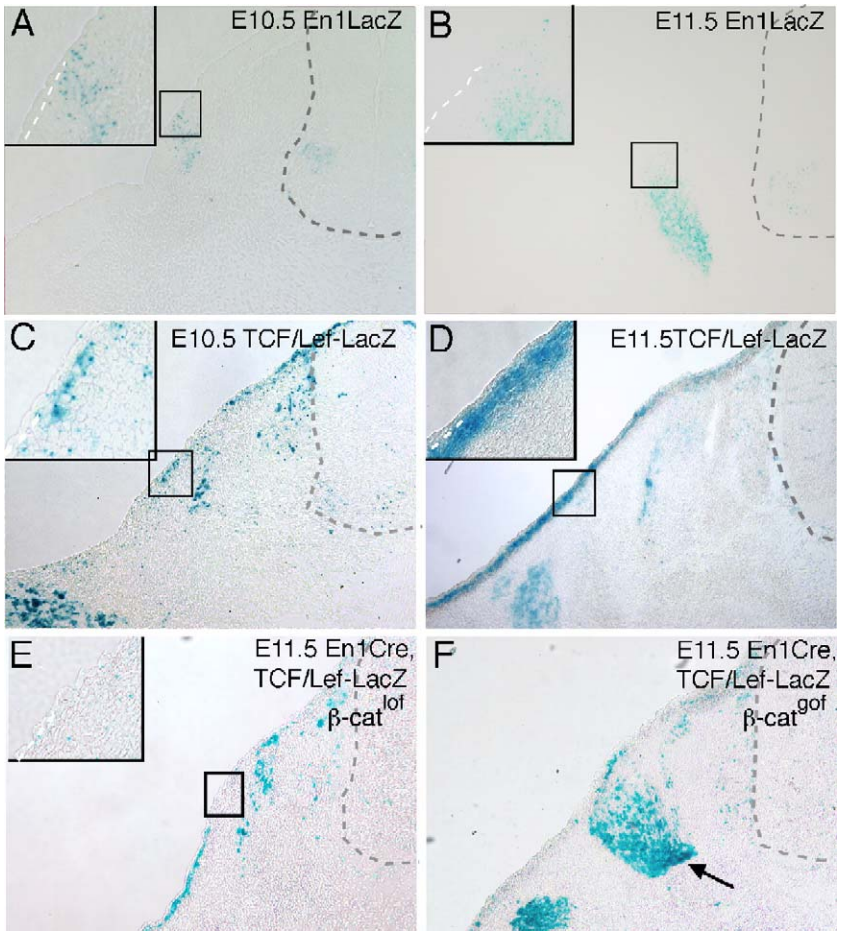

Fig. 3. A Wnt reporter transgene is expressed in the subectodermal mesenchyme. (A and B) En1LacZ expression in the somite at E10.5 and E11.5. (C) At E10.5, the TCF/Lef-LacZ transgene is expressed in subectodermal mesenchyme in the same region as En1 is expressed. (D) On E11.5, the TCF/Lef-LacZ transgene is expressed throughout the subectodermal mesenchyme. (E) In E11.5 embryos with β-catenin tissue-specifically mutated in En1-expressing cells, there is a gap in the TCF/Lef-LacZ transgene expression in the En1 domain. (F) E11.5 embryos with β-catenin stabilized in En1-expressing cells show ectopic expression of the TCF/Lef-LacZ transgene.

E11.5 embryos were sectioned and stained with antibodies for β-galactosidase from the RR allele and for myogenin, it was not always possible to identify positively double-labeled cells because the antibody to β-galactosidase stains puncta in the cytoplasm and the antibody to myogenin stains nuclei. However, because lineage-labeled cells do not mix with unlabeled cells, myogenin-positive nuclei within the En1 domain were considered as En1 lineage-marked cells, which also expressed myogenin. In wild-type lineage-marked embryos, myogenin-positive cells are present in the deeper parts of the En1 domain, but not in the subectodermal mesenchyme (Figs. 6A–C). In β-catenin conditional loss-of-function mutant embryos, there was an increase in the number of lineage-labeled cells, which expressed myogenin, particularly in the subectodermal mesenchyme (Figs. 6D–F). In β-catenin gain-of-function mutant embryos, there was a dramatic reduction in the number of lineage-labeled cells that expressed myogenin (Figs. 6G–I). Taken together, these results demonstrate that there is increased specification of muscle progenitor cells in the β-catenin loss-of-function mutants and decreased specification of muscle progenitors upon stabilization of β-catenin.

Dorsal dermis is absent in β-catenin mutants

Hair follicles develop from the epidermis but their formation depends on interactions with dermis (Schmidt-Ullrich and Paus, 2005). Thus, for an overview of the distribution of functional dermis, 14.5 day fetuses were probed for Ptch1 mRNA by whole-mount in situ hybridization to mark hair placodes (Oro et al., 1997). Ptch1-positive hair placodes were absent on the dorsum of mutant embryos but had a normal density and appearance on the flanks (Figs. 7A–D) despite the fact that all dermis is lineage-marked and mutant for β-catenin (Fig. 8E and data not shown). Embryos mutant for the β-catenin conditional gain-of-function allele died between E12 and E13 so the effect of activation of β-catenin could not be analyzed at these later stages.

Histological analysis of E16.5 mutant fetuses showed that dense, compact mesenchyme characteristic of dermis was absent in the dorsum (Figs. 7F and 8F) and the brown fat abutted the epidermis (Fig. 8F). Laterally, on the flanks of the mutant embryos, dermis and hair placodes were present and appeared normal in section (Figs. 7G and H). β-Catenin is eliminated in En1-expressing cells of the ventral limb

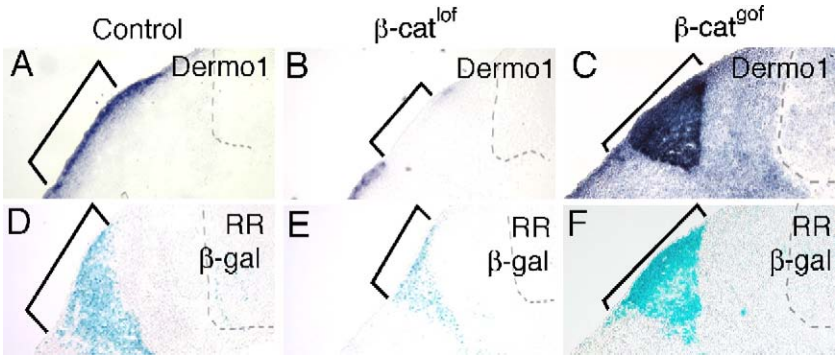

Fig. 4. Dermo1 expression is dependant on β-catenin. Paired adjacent sections of E11.5 embryos were analyzed for Dermo1 expression and En1 lineage-marked cells. (A and D) Dermo1 is expressed in the subectodermal mesenchyme in En1Cre/+, RR/+ embryos. (B and E) Dermo1 expression is not detectable in β-catenin loss-of-function mutant cells. (C and F) Dermo1 is ectopically expressed in the presence of activated β-catenin.

ectoderm (Davis et al., 1991), which also results in loss of hair follicle development in the ventral limb (data not shown).

In E12.5loss-of-function mutants, the surface of the embryo typically showed an indentation at the location of the En1 domain (Fig. 8A), and Dermo1 expression persisted medial and lateral to the domain of mutant cells but was not detected in the dorsal cells (Fig. 8B). By E14.5, the dorsal subectodermal mesenchyme was cell-poor (Fig. 8C). In the flank of E14.5 embryos, Dermo1 was expressed (Fig. 8D) and there was normal appearing compact dermis (Fig. 8E) consistent with the subsequent formation of hair placodes. We interpret the formation of flank dermis in the mutants as due to later mutation of β-catenin in these cells, after dermal specification has occurred.

Taken together, our results demonstrate the importance of Wnt signal transduction in the specification and formation of the dorsal dermis from the En1 domain of the central dermomyotome.

Discussion

In this work, we aimed to identify the signal required for the specification of the dermal lineage from the mouse dermomyotome. We conclusively show with genetic techniques that Wnt signal transduction in the central dermomyotome is required for dorsal dermis specification and development in the mouse embryo. Studies in the chick embryo have demonstrated the role of Wnt1 in survival of dorsal dermal progenitors and later in skin patterning. Using multiple genetic fate mapping techniques and conditional β-catenin mutants, we systematically demonstrate an earlier role of Wnt signaling in selecting dermal fate from a tripotential tissue expressing En1 in the central dermomyotome of the mouse.

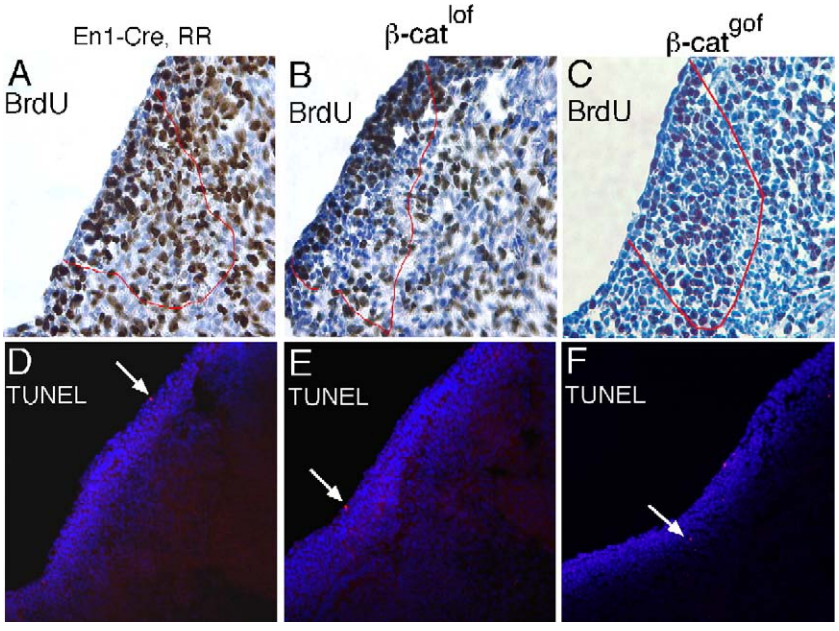

Fig. 5. No change in proliferation or cell death in β-catenin mutant cells. (A–C) There is no change in the number of cells that incorporate BrdU (brown stain) in the β-catenin loss-of-function and gain-of-function cells (see Table 1 for quantitation). The domain of En1 lineage-marked cells is outlined with a red line. (D–F) Apoptosis as measured by TUNEL staining is not changed in the mutant cells.

Table 1
Proliferation in β-catenin mutant cells

β-Catenin genotype	Number of embryos analyzed	En1 domain			En1 domain three cells subjacent to ectoderm		
		BrdU-positive cells (%)	SD	*P*	BrdU-positive cells (%)	SD	*P*
Wild type	5	60.2	8.4		66.3	8.9	
Loss of function	4	46.4	5.1	0.098	54.9	15.9	0.52
Gain of function	4	46.8	4.8	0.082	53.9	10.9	0.78

Triple fate of mouse En1-expressing tissue of the dermomyotome

The genetic fate mapping of En1-expressing cells identifies the En1 central dermomyotome as a major source of interscapular brown fat. At E9.5, En1 is expressed in the central dermomyotome of the somites at cervical and forelimb levels and these cells contributed only to the interscapular brown fat. This restriction was surprising, and at later stages En1 lineage-marked cells generated descendants in the dermis and epaxial muscles as expected, in addition to interscapular brown fat. On E11.5, En1-expressing cells gave rise to dermis over the fetal midline, whereas at earlier stages the dermal progeny were limited to about the extent of the original En1 domain. There are two novel outcomes of these experiments: firstly, that the fate of En1 cells changes in time with an increase in the diversity of cell types made, and secondly, that some interscapular brown fat bundles have their origin in the En1-expressing cells of the central dermomyotome.

The change in fate of En1 cells from brown fat at an early stage to brown fat, dermis, and muscle at later stages in the tamoxifen experiments is unexpected. It is possible but unlikely that the fate restriction to fat at early stages might be apparent rather than real, reflecting the fact that brown fat is the major cell type labeled in all of the tamoxifen induction experiments and other fates were adopted but were missed in the early inductions.

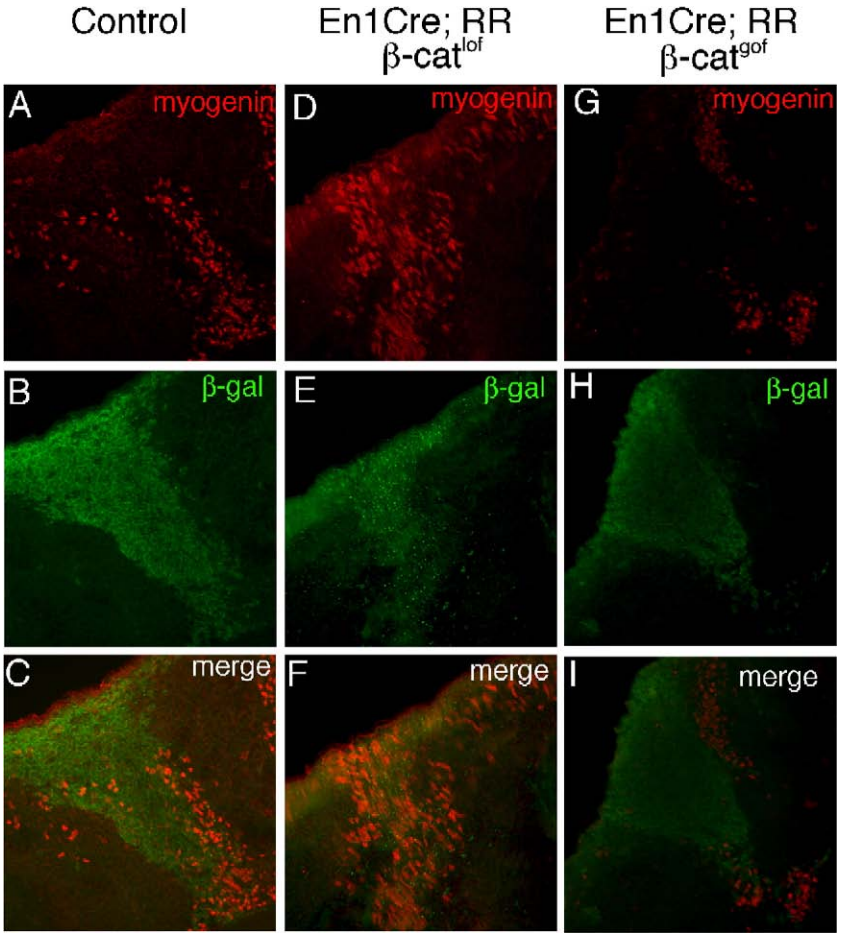

Fig. 6. Myogenin expression is altered in β-catenin mutants. Sections of E11.5 embryos were stained with antibodies to myogenin and to β-galactosidase to identify mutant cells. In wild-type embryos (A–C), myogenin is expressed in deep cells of the lineage-marked domain. In β-catenin loss-of-function mutant cells (D–F), ectopic myogenin-positive cells are found close to the surface ectoderm. In β-catenin gain-of-function mutants (G–I), myogenin-positive cells are absent from the En1 domain.

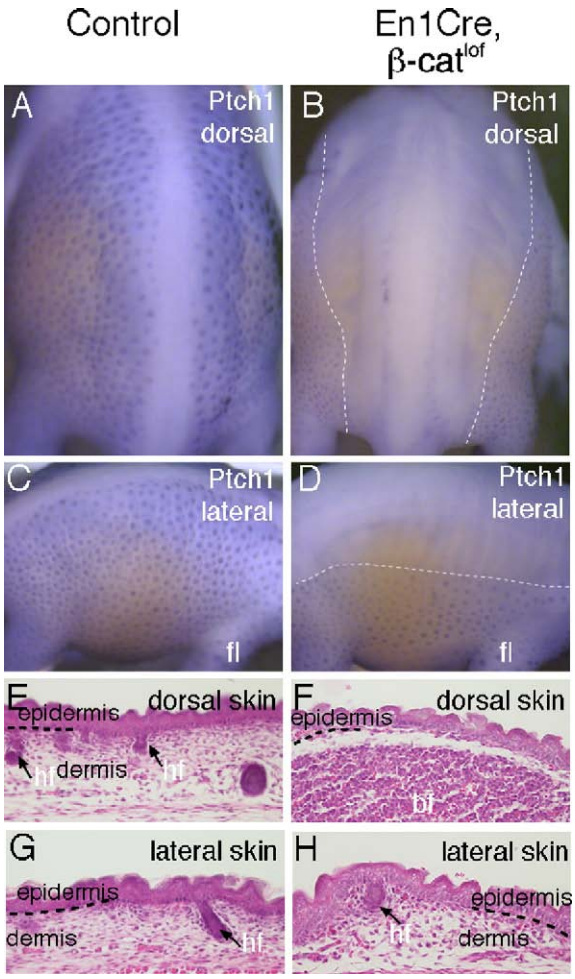

Fig. 7. Dorsal hair placodes and dermis are absent in β-catenin loss-of-function mutants. (A–D) Hair placodes identified with a Ptch1 probe are absent on the dorsum of mutant embryos but are present on the flanks. (E and F) Histological dermis and hair follicles are absent in the dorsal skin of the mutant fetuses. (G and H) Dermis and hair follicles are present in the flank of E16.5 mutant fetuses (fl, forelimb; hf, hair follicle; bf, brown fat; arrows, hair follicles).

Alternatively, our favored interpretation is that the results reflect a real change in fate. An increase in the number of cell types made by En1 cells at later stages would require that early En1 cells be replaced by new cells that turned on En1 and contributed to all three fates. Cells have been shown to move laterally within the dermomyotome from the DML and turn on En1 in the chick embryo (Cheng et al., 2004; Ordahl et al., 2001). It may be that a similar process is at work in the mouse, with early En1-expressing cells of the central dermomyotome moving deep away from the surface, becoming incorporated into brown fat, replaced by medial cells which turn on En1 and have the potential, at least collectively, to form all three cell types. Continued translocation of DML cells into the En1 domain at later stages could also explain why early inductions did not detect progeny in midline dermis, but tamoxifen administration on E10.5 did give rise to labeled cells in midline dermis.

Our fate mapping results identify the origin of some of the interscapular bundles of brown fat in the En1-expressing cells of the central dermomyotome. Brown fat is a thermogenic or heat-producing tissue found only in eutherian mammals (Cannon and Nedergaard, 2004; Nicol et al., 1997; Rothwell and Stock, 1985). The interscapular bundles are the major depots of brown fat in the mouse, and their embryological origin was not known previously. Not all interscapular brown fat bundles were labeled by En1 lineage marking—the more medial interscapular brown fat bundles were not lineage-labeled by En1Cre nor were axillary brown fat bundles (data not shown). It seems reasonable that the more medial bundles of brown fat derive from dermomyotome medial to the En1 domain, possibly the Wnt11-expressing cells of the dermomyotome, although this possibility was not examined directly. Other small contributions to interscapular brown fat are not excluded by these experiments – for example, some undetected population which also expresses En1 – but the fact that the marked brown fat bundles are surrounded by marked muscle and connective tissue and the extensive labeling of brown fat in the tamoxifen induction experiments make it unlikely.

Although there is little known about the early development of brown fat, there is one mutant which supports our finding that brown fat shares a developmental origin with muscle. Mice mutant for the myogenic transcription factor myogenin have a deficit of epaxial muscle and an excess of brown fat bundles in the cervical and interscapular region (Hasty et al., 1993). Similarly, fetuses mutant for both Myf5 and MyoD have an excess of bundles of fat cells in the place of some epaxial muscles (Kablar et al., 2003), although it was not said if this ectopic fat is brown or white. An alternative explanation for the excess of fat in mutants lacking muscles is that the adipocytes are released from inhibitions on its growth rather than arising from a change in specification. Nonetheless, considered together with our current experiments, we conclude that in the mouse, the central dermomyotome is a tripotent tissue.

Fate maps of dorsal dermis in the mouse

In the chick, the fate of different parts of the somite and dermomyotome with respect to the dorsal dermis has not been resolved with agreement on all details, in particular, the origin of the dermis over the midline (Scaal and Christ, 2004). Our work in mouse may be different from chick but it conclusively shows the origin of the midline dermis in the mouse. The results obtained by temporally restricted activation of Cre recombinase to 24-h windows show that the central dermomyotome generates dermis and that it contributes to dermis over the fetal midline. The extent of contribution cannot be determined quantitatively from the data due to the low activity of the Cre-ER fusion protein, but the results from the mutant phenotype suggest that the contribution is substantial. In the β-catenin conditional loss-of-function mutants, Dermo1 expression is lost in the En1 domain, but toward the midline from the En1 domain there is a small group of cells that continue to express Dermo1. This group of cells is likely composed of Wnt11-expressing cells medial to the En1 domain in the dermomyotome. In the β-catenin conditional mutants, this small group of cells is not sufficient to rescue later midline Dermo1 expression, the

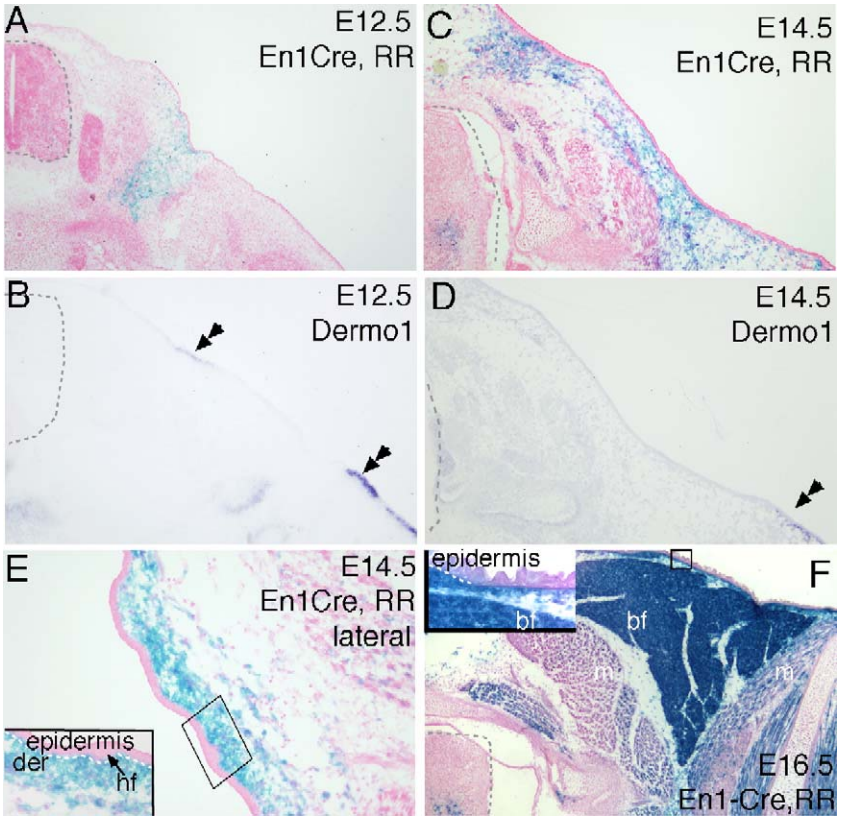

Fig. 8. Development of dermis is disrupted in En1Cre β-catenin loss-of-function mutant cells dorsally, but not in the flank. (A) In E12.5 embryos, En1 lineage-marked cells are absent from the subectodermal mesenchyme and there is an indentation in the surface of the embryo over the En1 domain. (B) At E12.5, the expression of Dermo1 is not detectable in mutant cells. (C) At E14.5, β-catenin loss-of-function cells are present in a loose, cell-poor mesenchyme under the dorsal surface ectoderm. (E) Laterally on E14.5, lineage-labeled β-catenin loss-of-function cells organize into a typical appearing dense dermis (der). (F) By E16.5, dorsal dermis is absent and brown fat (bf) bundles are immediately adjacent to the surface ectoderm.

formation of midline dermis, or the induction of dorsal hair placodes. These results suggest that some cells medial to the En1 domain do contribute to the midline dermis, but that their contribution is minor.

Our fate mapping results and the analysis of the mutants taken together suggest that there is little lateral dispersion of En1 cells from the central dermomyotome in the dermal layer, with the lateral limit probably corresponding to the lateral boundary of the early expression of En1 in the dermomyotome and the medial third of the scapula at later stages. Our results in the mouse and the results of others in the chick (Ben-Yair et al., 2003) do not appear to be consistent with a substantial early and direct contribution of DML cells to the midline dermis as suggested for the mouse (Houzelstein et al., 2000) and chick (Olivera-Martinez et al., 2002). However, as argued above, they are consistent with translocation of DML cells into the central dermomyotome where they turn on En1 (Cheng et al., 2004) and then give rise to dermis, including the midline dermis later in development.

The formation of dermis and induction of hair follicle in the flank of the mutant mice occurs despite the fact that the dermis in this region of the fetus is lineage-marked and mutant for β-catenin. We suggest that the difference between flank and dorsal dermal phenotypes is due to the different timing of β-catenin loss in these two tissues. En1 is expressed in the flank dermis after Dermo1 expression, and thus presumably after specification of dermal progenitors. Thus, in the β-catenin conditional loss-of-function mutants where excision is driven by Cre expressed from the En1 locus, loss of β-catenin should occur after dermal specification in the flank. β-catenin is required within the other layer of the skin, the epidermis, for placodes to form (Huelsken et al., 2001). Within the dermis, transient β-catenin expression and β-catenin-directed transcriptional activity have been observed in the dermal condensations preceding hair follicle formation, and it has been suggested that dermal β-catenin might be necessary for hair development (DasGupta and Fuchs, 1999). However, the present results show that β-catenin is not required in the dermis after dermal progenitor specification, at least for development of hair placodes in the flank up to stage E17.5.

When β-catenin is activated in the somite, all mutant cells express Dermo1, suggesting that the cells are respecified to the dermal progenitor fate. A comparable marker for brown fat progenitors is not available, but all or most cells with activated β-catenin fail to express myogenin. Unless the Dermo1-positive cells in the β-catenin gain-of-function experiment also are simultaneously specified to brown fat, it seems likely that activation of Wnt signal transduction has inhibited brown fat specification. With loss of β-catenin, Dermo1 expression is lost in the somite, and there is an apparent increase in myogenin-

positive cells and some of these cells are located ectopically where dermal progenitors are normally found. It is not clear if the myogenin-positive cells were respecified to the myogenic fate, or whether they are normally specified cells that are abnormally displaced to the subectodermal mesenchyme due to the absence of dermis. However, the fact that there are no significant differences in the proliferation of the mutant cells and no detectable cell death, it seems likely that these cells have been respecified. Respecification of somite cells has been observed previously in mutant embryos, where cells that were in the myogenic lineage instead adopt dermal and skeletal fates in the absence of Myf5 (Tajbakhsh et al., 1996). Contradictory to our report, Wnt signaling has been shown to promote myogenesis. Our report on myogenin expression in β-catenin mutants suggests an inhibitory role of Wnt signaling in adopting myogenic fate. Taken together, our results are consistent because Dermo1 expression complement myogenin expression in the gain- and loss-of-function for β-catenin mutants. The inhibitory role of Wnt signaling in myogenic fate may be due to a specific population of cells from the En1 lineage-marked cells in the central dermomyotome.

Instructive role for β-catenin in dorsal dermis specification

Together, the lineage mapping results and the outcomes with loss-of-function and gain-of-function roles point to an instructive role for β-catenin and canonical Wnt signaling in dermal specification. En1-expressing cells of the somite were fate mapped to dermis, muscle, and interscapular brown fat, demonstrating that the central dermomyotome tissue is tripotent in the mouse. The changes in expression of the dermal and muscle progenitor markers with manipulation of β-catenin suggest that Wnt signaling is necessary and sufficient for cells to express Dermo1 and adopt the dermal progenitor fate. The absence of changes in cell death or DNA synthesis suggests that if Wnt signal transduction alters survival or growth, the effect is minor. The complementary changes in myogenic fates support the idea that the role of Wnt signalling is instructive. The temporal requirement for β-catenin at the time of dermal specification, and not for subsequent differentiation and survival, shows that the role of Wnt signaling is limited to the window around specification. The pattern of expression of the reporter gene for β-catenin transcriptional activity in normal embryos suggests that cells underneath the surface ectoderm convert Wnt signals into transcriptional activity. This transcriptional activation is present only in En1 lineage cells close to the surface ectoderm, those which express Dermo1 and presumably later form dermis and not the deeper cells that become muscle and brown fat. This pattern of Wnt signal transduction and specification is most consistent with a Wnt signal produced locally from the surface ectoderm. The surface ectoderm is the site of expression of multiple Wnt genes in the mouse and at least one Wnt in the chick (Parr et al., 1993; Rodriguez-Niedenfuhr et al., 2003; Schubert et al., 2002; Tajbakhsh et al., 1998). Deeper cells derived from the En1 domain might adopt brown fat and muscle fates due to attenuated Wnt signals, or due to the predominance of other signals. Our results do not suggest what mechanism selects between the muscle and brown fat fates but do indicate that Wnt signalling is inhibitory for the adoption of these fates. From these results, we propose that Wnt signals from the surface ectoderm are transduced by central dermomyotome cells close the surface ectoderm, and that this instructs cells to select the dermal progenitor fate from the three fates available to them.

How transduction of Wnt signals causes central dermomyotome cells to select the dermal fate is not clear. Although Wnt signals induce and maintain En1 expression in the somite (Cheng et al., 2004; Ikeya and Takada, 1998; Olivera-Martinez et al., 2002) (our unpublished results), En1 is not necessary for the development of dermis, muscle, or brown fat (data not shown). The identification of the genes that respond to β-catenin activation and select the dermal fate is the next step in understanding dermal specification.

Methods

Mice

En1Cre (Kimmel et al., 2000), En1LacZ (Loomis et al., 1998), En1Cre-ER (Sgaier et al., 2005), Rosa-floxed Stop-LacZ Reporter (Soriano, 1999), TCF/Lef-LacZ reporter (Liu et al., 2003), β-catenin conditional loss-of-function (Brault et al., 2001), and β-catenin conditional gain-of-function (Harada et al., 1999) mice and embryos were genotyped as described. Tamoxifen was administered by gavage to pregnant females as described (Sgaier et al., 2005). Three to seven embryos from 2 to 3 different litters were analyzed. Pregnant mice were injected intraperitoneally with 100 mg/kg BrdU (Sigma).

In situ hybridization, immunocytochemistry, histochemistry, and histology

Embryos were fixed for 30 min to 1 h in 4% paraformaldehyde in PBS, washed in PBS, and equilibrated in a graded series of solutions up to 25% sucrose and embedded in OCT (Tissue-Tek). Frozen sections at the forelimb level were processed for in situ hybridization (Holmes and Niswander, 2001). For whole-mount in situ hybridization, E14.5 embryos were fixed in 4% paraformaldehyde in PBS overnight and then processed as described (Henrique et al., 1995), except that the protease treatment was 7.5 μg/ml proteinase K for 15 min at room temperature and the color reaction was developed with BM Purple (Roche). The mouse Dermo1 probe was a gift from Eric Olson and the Ptch1 plasmid was a gift from Matt Scott. For immunocytochemistry, frozen sections were treated as described (Yamada et al., 1993). Antibodies used were against rat myogenin (monoclonal, 1:1.5; Developmental Studies Hybridoma Bank, IA), β-galactosidase (goat, 1:500,; Biogenesis), BrdU (monoclonal, 1:8; Roche), as well as appropriate secondary antibodies conjugated to FITC or Cy3 (1:250; Jackson Immunoresearch). Histochemical staining for β-galactosidase expression from reporter transgenes was performed on frozen sections of embryos processed as described (Rivera-Perez et al., 1999). For histology, E16.5 embryos were fixed for 8 h in 4% paraformaldehyde in PBS, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Cell proliferation and apoptosis

Two hours after BrdU injection, embryos were harvested, fixed for 1 h in 4% paraformaldehyde in PBS, and processed for frozen sections as described above. Sections were treated with 1 M HCl at 50°C for 2 min, washed, and stained with mouse anti-BrdU (2 μg/ml, Roche) followed by biotinylated secondary antibody (1:250 Vector), amplified using the reagents of an ABC kit (Vectastain), and visualized by 3,3′-diaminobenzidine staining and counterstaining with hematoxylin. Apoptotic cell death was assayed by TUNEL using TMR Red (Roche) according to the kit's instructions.

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