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PDGF signalling in the dermis and in dermal condensates is dispensable for hair follicle induction and formation

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Abstract: Embryonic hair follicle (HF) induction and formation is dependent on signalling crosstalk between the dermis and specialized dermal condensates on the mesenchymal side and epidermal cells and incipient placodes on the epithelial side, but the precise nature and succession of signals remain unclear. Platelet-derived growth factor (PDGF) signalling is involved in the development of several organs and the maintenance of adult tissues, including HF regeneration in the hair cycle. As both PDGF receptors, PDGFRα and PDGFRβ, are expressed in embryonic dermis and dermal condensates, we explored in this study the role of PDGF signalling in HF induction and formation in the developing skin mesenchyme. We conditionally ablated both PDGF receptors with Tbx18^{Cre} in early dermal condensates before follicle formation, and with Prx1-Cre broadly in the ventral dermis prior to HF induction. In both PDGFR double mutants, HF induction and formation ensued normally, and the pattern of HF formation and HF numbers were unaffected. These data demonstrate that mesenchymal PDGF signalling, either in the specialized niche or broadly in the dermis, is dispensable for HF induction and formation.

Key words: dermal papilla cells – hair follicle morphogenesis – hair follicle stem cells – PDGF signalling – stem cell niche

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Background

Hair follicle (HF) induction and formation is a highly complex process controlled by successive signals between epidermal cells and incipient placodes on the epithelial side and the dermis and specialized dermal condensates (DCs) as the mesenchymal counterpart (1). Several studies have identified key signalling pathways that are involved in the regulation of HF induction and formation, including Wnt, Eda/Edar/NFkB, Fgf and Shh signalling [reviewed in (1)]. Platelet-derived growth factor (PDGF) signalling is instrumental in embryonic development and adult tissue function of several tissues, including gonads, lung, kidney, intestine, brain and skin (2). Global deletion of the PDGF receptors, PDGFRα and PDGFRβ, in knockout mice results in early embryonic lethality with specific defects suggesting unique physiological functions (2). However, both receptors mostly share overlapping expression patterns suggesting functional compensation in several tissues. In the skin, mice lacking PDGFRα exhibit strong skin defects including widespread dermal hypoplasia (3), while PDGF-A null mice show increasing loss of dermal mesenchyme and reduced hair development after birth (4). PDGF signalling was also suggested to be instrumental for HF regeneration during the hair cycle (S1). Finally, neonatal pups or embryonic skins treated with blocking antibodies against PDGFRα failed to form HFs (5, S2). In this study, we determined the role of PDGF signalling in HF induction and formation with definitive genetic methods by conditionally ablating both PDGF receptors in the developing dermis and DCs.

Questions addressed

Does dermal PDGF signalling play a role during HF induction and/or formation?

Experimental design

To assess the potential involvement of dermal PDGF signalling in HF formation, we ablated PDGFRα and PDGFRβ specifically in the DC at E14.5 using previously described Tbx18^{Cre} mice (6). Prx1-Cre mice were used to ablate PDGFRs in the entire ventral dermis before DC formation (7) to test a potential role of PDGF signalling in HF induction. More detailed information is available in Data S1.

Results

In the skin, previous reports have linked PDGF signalling to dermis development and the control of adult hair regeneration in the hair cycle (4, S1). To identify a potential role of this pathway in dermal condensates (DCs) during embryonic HF morphogenesis, we first confirmed the expression of PDGFRα and PDGFRβ at E14.5, the beginning of HF formation after induction. Expression of both PDGF receptors was detected by qRT-PCR in the dermal compartment of E14.5 back skin (Fig. 1a). Immunofluorescence staining for both PDGFRs confirmed broad expression in the dermis and in DC cells in both dorsal and ventral skin (Fig. 1b, red). DCs were identified as GFP-positive cell clusters (green) in Sox2^{GFP} embryos (8) and staining for EDAR marked HF placodes (white) (9).

Next, to explore the functional role of PDGF signalling during HF induction and formation, we ablated both PDGF receptors by crossing PDGFRα$^{fl/fl}$;PDGFRβ$^{fl/fl}$ double-floxed mice (S3, S4) with two different Cre lines in a Sox2^{GFP} background: Tbx18^{Cre} for ablation specifically in DCs at E14.5 in the back skin (6) and Prx1-Cre for knockout in the entire ventral dermis at E11.5 prior to HF induction (7). Efficient double knockout (dKO) gene ablation of both PDGFRα/β with Tbx18^{Cre} (dKO^{Tbx18}) and Prx1-Cre (dKO^{Prx1})

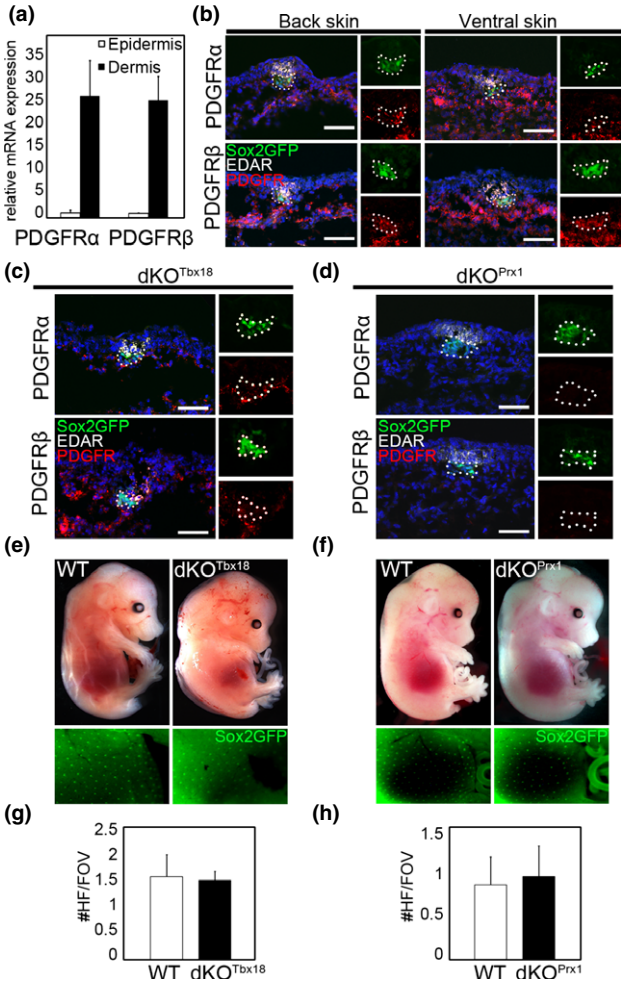

Figure 1. PDGF receptors α and β are expressed in the dermis and dermal condensates of E14.5 skin and are dispensable for HF induction. (a) qRT-PCR of FACS-sorted cells from E14.5 back skin shows high PDGFRα and PDGFRβ expression in the dermis. (b) Immunofluorescence staining for PDGFRα and PDGFRβ demonstrating widespread expression in back and ventral skin at E14.5. Note that both PDGFRs are also expressed in GFP^{+} dermal condensates (DCs) of Sox2^{GFP+} mice. (c) Immunofluorescence staining of E14.5 dKO^{Tbx18} back skin shows efficient PDGFRα and PDGFRβ ablation in Sox2^{GFP+} DCs. (d) Immunofluorescence staining of E14.5 dKO^{Prx1} ventral skin shows efficient ablation of PDGFRα and PDGFRβ in the entire dermis including Sox2^{GFP+} DCs. (e) E14.5 WT and dKO^{Tbx18} show a comparable Sox2^{GFP+} DC pattern. (f) E14.5 WT and dKO^{Prx1} show a similar Sox2^{GFP+} DC pattern. (g–h) Quantification of HFs per field of view (FOV), assessed by staining for placode marker EDAR. HFs form in similar numbers in E14.5 dKO^{Tbx18} (g) and dKO^{Prx1} (h) skin compared to controls ($n \geq 3$, ≥20 FOVs for each). Dapi (blue) highlighted nuclei. Scale bar = 50 μm.

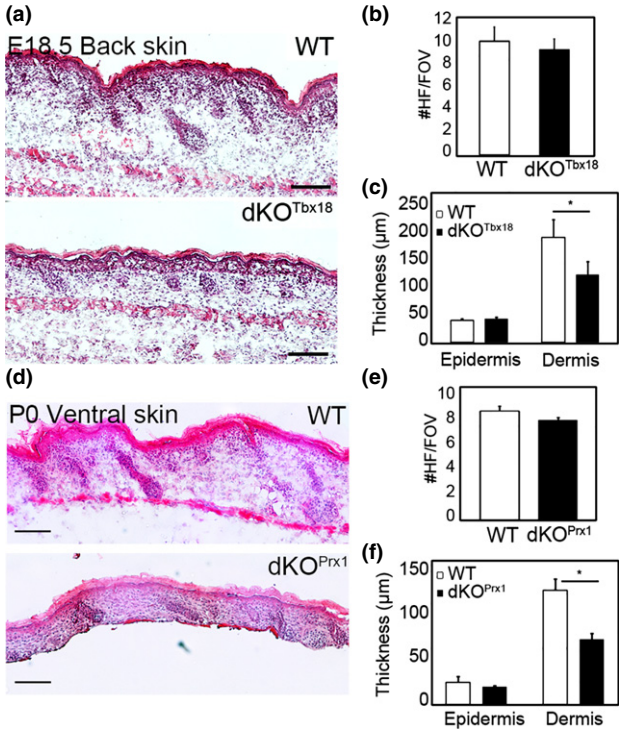

Figure 2. PDGF signalling in the dermis and DCs is not required for HF formation. (a) Haematoxylin/eosin staining of E18.5 WT and dKO^{Tbx18} back skin sections. (b) Quantification of total HFs per field of view (FOV; $n = 3$, ≥50 FOVs for each). Comparable HF numbers in E18.5 WT and dKO^{Tbx18} back skin. (c) Thickness measurement of E18.5 back skin ($n = 3$, ≥30 FOVs for each). dKO^{Tbx18} dermis is significantly thinner than WT. (d) Haematoxylin/eosin staining of E18.5 WT and dKO^{Prx1} ventral skin sections. (e) Quantification of total HFs per field of view ($n = 2$, ≥20 FOVs for each). WT and dKO^{Prx1} show comparable HF numbers. (f) Thickness measurement of E18.5 ventral skin of WT and dKO^{Prx1} ($n = 2$, ≥30 FOVs for each). Mutant dermis is significantly thinner than WT. *$P < 0.05$ using Student's *t*-test. Scale bar = 100 μm.

was confirmed by immunofluorescence at E14.5 (Fig. 1c, d). Some dKO^{Tbx18} embryos presented a haemorrhage and oedema phenotype (Fig. 1e, Fig. S1) as previously described for PDGFRα or PDGFRβ single null mutants (3, S5). Sox2^{GFP}-positive DCs were detectable in a similar pattern in dKO and WT controls after both broad dermal and DC-specific ablation (Fig. 1e, f). Likewise, staining for placode marker EDAR (Fig. 1c, d) and subsequent quantification of formed placodes showed similar numbers in dKO^{Tbx18} back skin (Fig. 1g) and dKO^{Prx1} ventral skin (Fig. 1 h) compared to WT (Fig. 1b). Taken together, these data demonstrate that PDGF signalling is dispensable for HF induction, that is specification of placodes and DCs.

To assess a potential role of PDGF signalling in the DC and DP in HF downgrowth and formation, we examined later stages of HF morphogenesis in dKO^{Tbx18} and dKO^{Prx1} mutant skin. Analysis of E18.5 haematoxylin/eosin-stained sections of dKO^{Tbx18} back skin revealed normal HF formation without apparent morphological changes (Fig. 2a). Immunofluorescence staining for PDGFRα and PDGFRβ confirmed efficient ablation of both receptors in the entire dermis (Fig. S2a) as Tbx18^{Cre} displays widespread dermal Cre activity after E16.5 (6). ITGA8^{+} DCs and DPs in developing HFs were identified (S6), confirming formation of 1st, 2nd and 3rd wave HFs in both WT and dKO^{Tbx18} E18.5 back skins (Fig. S2a). Quantification of total HF numbers revealed no significant difference between WT and dKO^{Tbx18} embryos (Fig. 2b), although dKO^{Tbx18} skin had slightly fewer 3rd wave HFs than WT control (Fig. S2b). This minor difference might be due to broad ablation of PDGF signalling, but is likely caused by the onset of embryonic lethality, as E18.5 was the latest point we obtained dKO^{Tbx18} mice (Fig. S2c). Thickness measurements of dKO^{Tbx18} skin revealed significantly thinner dermis in double mutants compared to WT controls (Fig. 2c), which is consistent with similar observations in PDGF-A null mutants (4). dKO^{Prx1} mutants on the other hand were viable and developed normally. Analysis of ventral skin sections at P0 confirmed that both PDGFRs were absent (Fig. S2d).

In this knockout model of broad dermal PDGFR ablation, haematoxylin/eosin staining demonstrated normal HF formation in dKO^{Prx1} skin (Fig. 2d), and quantification of HF numbers showed no significant difference between dKO^{Prx1} and controls (Fig. 2e). As with dKO^{Tbx18} embryos, the thickness of the dermis was strongly decreased in dKO^{Prx1} compared to WT (Fig. 2f). Taken together, both PDGFR double-mutant models demonstrate that dermal PDGF signalling is not required for HF formation and maturation.

Conclusions

Platelet-derived growth factor signalling has been involved in many developmental processes and was shown to be crucial for maintenance of different adult tissues. Previous reports suggested that activation of this pathway should be important for HF morphogenesis and regeneration. To specifically address its role in HF morphogenesis, we ablated both PDGFRs broadly in the dermis prior to HF induction and in DCs during 1st wave HF formation. We found that dermal PDGF signalling is not required for HF induction nor subsequent HF downgrowth and formation. Lastly, we confirmed that dermal ablation of this pathway leads to a thinner dermis. Taken together, these results highlight the involvement of PDGF signalling in dermal development but show that it is dispensable for HF morphogenesis. The importance of PDGF signalling during HF regeneration remains to be assessed.

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Author contributions

A.R. and M.R. conceived and designed experiments; A.R., R.S. and M.T. performed experiments; C.C. contributed essential reagents and tools; A.R. and M.R. analysed and interpreted data; A.R. prepared figures; and A.R. and M.R. wrote the manuscript.

Conflict of interests

The authors declare no conflict of interest.

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Supporting Information

Additional supporting data may be found in the supplementary information of this article.

Data S1. Supplementary materials and methods.

Figure S1. E14.5 dKO^{Tbx18} show a range of hemorrhage and edema phenotypes.

Figure S2. Efficient ablation of both PDGFRs in E18.5 dKO^{Tbx18} embryos and P0 dKO^{Prx1} mice.