The role of Runx family in the murine epidermis

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SUMMARY

Keratinocytes contribute to the epidermal homeostasis mainly. Keratinocytes regularly undergo differentiation and proliferation so that the homeostasis is maintained normally. Many factors are involved in these regulation. However, the regulatory mechanism of keratinocytes is complicated and its full picture has not been found out. Runx-related transcription factor (RUNX) proteins belong to a family of metazoan transcription factors that serve as master regulators. The Runx gene family has three members, Runx1(R1), Runx2 and Runx3(R3). RUNX functions have been unclear in keratinocytes in detail. In this study, we focused on the roles of RUNX in keratinocyte. First, we confirmed whether R1 and R3 (R1/3) are expressed in keratinocytes. We used primary culture of mouse keratinocytes. The transcripts of R1/3 were detected by RT-PCR. Keratinocyte expressed R1/3. Next, we overexpressed R1/3 in primary mouse keratinocytes. R1/3 overexpression decreased the amounts of keratin1 (K1) and keratin10 (K10). Conversely, R1/3 knockdown increased the amounts of K1 and K10. R1/3 inhibited the induction of K1/10, which were early differentiation markers. We analyzed the mechanism to modulate K1/10 by R1/3. We tried to immunostain R1/3. Immunohistological analysis showed that R1/3 were detected in the nuclear of immature keratinocytes but moved out of the nuclear of differentiated keratinocytes. ChIP assay showed that R1/3 bound to Runx consensus sequences of *K1/10* genome in undifferentiated state but not in differentiated state. R1/3 directly regulated the expressions of K1/10. We assessed the role of R1/3 to proliferation. R1/3 overexpression induced keratinocytes growth arrest. R1/3 knockdown did not advance proliferation. We generated transgenic mice lacking R1(or R3) in basal keratinocytes. In these mice, K1/10 were detected in basal keratinocytes, but the proliferation did not changed. Last we applied Imiquimod to these mice. However there was no difference between transgenic mice and wild type. Taken together, we propose that R1/3 regulate partially differentiation and proliferation of keratinocytes.

INTRODUCTION

Runx-related transcription factor (RUNX) family is implicated in stem cell regulation, tissue development, and oncogenesis. The Runx gene family has three members, Runx1, Runx2 and Runx3. Runx1 and Runx3 (Runx1/3) play important roles in several organs, including blood, muscle, gastric mucosa, nervous system and hair follicles by affecting cell survival, proliferation, and differentiation. However, it is unknown whether and how Runx1/3 regulate in the epidermis. The purpose of this study is to investigate the role of Runx1 and Runx3 in the differentiation and proliferation of keratinocytes.

HYPOTHESIS

Runx1/3 regulate differentiation and proliferation of keratinocytes

MATERIALS & METHODS

Figure 1. Runx1/3 were expressed in keratinocytes.

Figure 2. Runx1/3 suppressed the proliferation of keratinocytes.

Figure 2. Next, we examined whether Runx1/3 regulate keratinocyte proliferation by monitoring bromodeoxyuridine(BrdU) incorporation in cultured keratinocytes which were overexpressed Runx1/3. Under low-calcium conditions, overexpressed Runx1/3 induced growth arrest (a). However regardless of reduction in the amount of Runx1/3 by their siRNAs, the proliferation of cultured keratinocytes did not alter significantly (b). Taken together, Runx1/3 are negative regulators of cell proliferation in cultured keratinocytes.

Figure 3. Runx1/3 suppressed keratin1 and 10 expression in keratinocytes.

Figure 3. To clarify the role of Runx1/3 in keratinocytes, we examined whether both of them regulated the production of the markers of keratinocyte differentiation. We overexpressed Runx1/3 in primary mouse keratinocytes using an adenovirus transduction system (a). We observed that Runx1/3 overexpression decreased levels of K1 and K10 (b,c). Conversely, reduction in the amount of the Runx1/3 by each two independent siRNAs specific to their sequence increased the expression of K1 and K10 (data not shown). These results of Fig. 3. suggest that both Runx1/3 inhibited K1 and K10 expressions in keratinocytes *in vitro*.

Figure 4. Runx1/3 bound promoter lesion of K1 and K10 directly and controlled these expression.

Figure 5. To assess the translocation of Runx1/3, we tried immunostaining Runx with the primary keratinocytes, which were in undifferentiated condition and differentiated condition. Immunohistological analysis showed that Runx1/3 were detected in nuclear of undifferentiated keratinocytes but were removed from nuclear of differentiated keratinocytes (a,b). These results suggest that Runx1/3 translocated from nuclear to cytoplasm after differentiation of keratinocytes. To clarify the mechanism how Runx1/3 regulate K1 and K10 expression, we examined whether Runx1/3 bind to K1 and K10 promoter lesion by chromatin immunoprecipitation assay. We designed the original primers each other, which amplicon consists of 'CACAG', Runx1/3 binding consensus sequences. Under low-calcium conditions, Runx1/3 bound to K1 and K10 promoter lesion (c). However they uncoupled to K1 and K10 promoter (d). Taken together, Runx1/3 directly regulated the expressions of K1 and K10.

Figure 5. In the skin specific Runx1 or Runx3 knock-out mice, the expressions of keratin1 and 10 decreased but the proliferation of keratinocyte did not altered.

Figure 5. Finally, we examined that Runx1/3 regulate epidermal differentiation and proliferation in transgenic mice that did not express Runx1 or Runx3 in epidermis specifically. We crossed K5Cre mice and Runx1 f/f mice (or Runx3 f/f mice) to generate K5Cre-Runx1 (or Runx3) f/f mice lacking Runx1 (or Runx3) in K5-expressing cells, including keratinocytes (a,b). Phenotype and Histologic analyses showed that there were no significant difference in skin between wild-type mice and Runx-transgenic mice (c,d). Immunohistochemical analysis of K1 and K10 showed that K1 and K10 were induced in basal keratinocytes significantly (c,d). As it was expected *in vitro*, the loss of Runx1/3 expression inhibited early differentiation *in vivo*. Immunohistochemical analysis of the cellular proliferation marker K67 revealed that there was no difference in the ratio of K67-positive cells to total basal cells between wild-type mice and Runx1 (or Runx3) conditional knock-out mice (data not shown). Thus, the loss of Runx1/3 expression does not alter keratinocyte proliferation *in vivo*.

CONCLUSION

Runx1/3 inhibits both early differentiation and proliferation of keratinocyte.

Runx1/3 binds promoter lesion of K1 and K10 directly and controls these expression.

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Conflict of Interest (COI) of the Principal Presenter :
No potential COI to disclose