Relationship between Keloid Formation and YAP/TAZ Signaling

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Summary: YAP (yes-associated protein) and TAZ (transcriptional coactivator with PDZ-binding motif) are part of a classical pathway that controls contact inhibition in the Hippo pathway. YAP and TAZ were recently reported to act as nuclear relays of mechanical signals that communicate extracellular matrix rigidity and cell shape. However, the role of YAP/TAZ signaling in keloid formation is unclear. Here, we used immunohistochemistry to investigate YAP/TAZ expression in keloid and unaffected lesions. YAP/TAZ expression in keloid fibroblasts had a greater tendency to localize to the nucleus relative to that seen in fibroblasts from unaffected tissues. Meanwhile, keratinocytes or endothelial cells from either keloid or unaffected lesions showed no significant differences in YAP/TAZ expression patterns. These results suggest that YAP/TAZ nuclear localization in keloid fibroblasts might activate Hippo signaling and may play an important role in gene expression that affects keloid formation and stiffness. (Plast Reconstr Surg Glob Open 2017;5:e1357; doi: 10.1097/GOX.0000000000001357; Published online 13 June 2017.)

INTRODUCTION

Keloids are raised, red fibrotic tissues that form after inflammation or injury and are recognized as an abnormal wound healing response. Keloids often stiffen and extend beyond the borders of the original wound to invade into normal skin. However, the exact mechanisms that promote keloid growth or invasion into normal skin are unclear. Clinical observations indicate that the growth of keloids is strongly related to their stiffness, as evidenced by the softening of keloids after treatment with drugs such as steroids, and that keloids rarely grow where the skin is soft (e.g., eyelids or scrotum). In the clinic, relief of scar tissue tension can suppress the growth of keloids. However, the way in which keloid tissue responds to skin stiffness remains unclear.

The Hippo signaling pathway, which was initially identified in Drosophila, plays an important role in both organ size and tumorigenesis through the regulation of cell proliferation and apoptosis. The Yorkie-homologues yes-associated protein (YAP) and transcriptional coactivator with PDZ-binding motif (TAZ) are transcription coactivators that bind to transcription factors and following activation are localized to the nucleus. Further analysis showed that YAP function is required for cancer-associated fibroblasts to promote matrix stiffening, cancer cell invasion, and angiogenesis. In addition, nuclear YAP and TAZ were reported to convey mechanical signals exerted by extracellular matrix (ECM) rigidity, whereas cell shape and ECM stiffness induce corresponding regulation of YAP/TAZ nuclear activity. From these reports, we hypothesized that rigidity in the keloid lesions may activate YAP/TAZ signaling. However, the relationship between YAP/TAZ signaling and keloid formation has not been explored. To assess whether YAP/TAZ signaling participates in keloid invasion and growth, we investigated YAP and TAZ expression in keloid and unaffected lesions.

MATERIALS AND METHODS

Keloids and unaffected normal skin tissue around the keloids were obtained surgically with the patient’s informed consent. Ten tissue samples were taken from keloids located on the shoulder, chest, or abdomen of 8 individuals (Table 1: K1–K10, male:female ratio, 2:3; mean age, 36.2; Fig. 1). This protocol was approved by the Keio University School of Medicine institutional review board.

Excised skin specimens were fixed with 4% paraformaldehyde, and paraffin sections (4 μm) were taken. Slides were stained with anti-YAP/TAZ signaling antibody (1:100 dilution, Cell Signaling Technology) and anti-smooth muscle actin (SMA) antibody (1:100 dilution; Dako, Santa Clara, Calif.). Fluorescent images were obtained using a confocal laser scanning microscope (FV1000; Olympus, Tokyo, Japan).

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To analyze YAP/TAZ localization in fibroblasts, keratinocytes, and endothelial cells from keloids and unaffected regions, the YAP/TAZ nucleus–positive cell index (percentage of cells with YAP/TAZ localized to the nucleus) was calculated by counting the number of immunoreactive cells and total cells in the keloid and unaffected areas (n = 4 per group). Cell counting was performed by an independent observer blinded to the sample identity. Statistical differences were determined using a nonparametric Mann-Whitney U test. P values less than 0.05 were considered significant.

RESULTS

In keloids, YAP/TAZ expression was detected in keratinocytes, endothelial cells, and fibroblasts. Immunofluorescence and 3,3’-diaminobenzidine tetrahydrochloride staining showed that YAP/TAZ (red) was expressed in both the nucleus and cytoplasm of keratinocytes, endothelial cells, and fibroblasts (Fig. 2). The percentage of fibroblasts from keloid areas that showed YAP/TAZ nuclear localization (77.4% ± 9.1%) was significantly increased compared with that in unaffected areas (34.1% ± 13.4%; P = 0.00001468; Fig. 3A). Meanwhile, the YAP/TAZ distribution inside the keloid lesion was not significantly different (data not shown). Although YAP/TAZ signaling is reported to occur in keratinocytes, YAP/TAZ localization in most keratinocytes from these samples was cytoplasmic rather than nuclear, and there were no significant differences between keloid areas and unaffected areas (7.2% ± 7.9% versus 11.0% ± 10.3%; Fig. 3B). YAP/TAZ localization in vascular endothelial cells stained with αSMA was mainly cytoplasmic, and again no significant differences were seen between keloid areas and unaffected areas (17.2% ± 9.7% versus 17.9% ± 6.0%; Fig. 3C).

DISCUSSION

Canonical biochemical components of the Hippo and Wnt signaling pathway YAP and β-catenin were found to exhibit undefined mechanical sensitivity. Phosphorylated YAP/TAZ localized to the cytoplasm decreases tumor growth, whereas unphosphorylated YAP/TAZ is mainly localized in the nucleus and promotes cell and tumor growth. Several previous studies showed that elevated YAP/TAZ expression levels and activity correlate with various human cancers. In addition, Yap1 is a determinant of the proliferative capacity of epidermal stem cells and YAP/TAZ reportedly plays a role in wound healing in skin by modulating the expression of transforming growth factor (TGF)-β signaling pathway proteins. ECM stiffness induces corresponding regulation of YAP/TAZ nuclear activity. In the presence of high stiffness or elasticity, YAP/TAZ is active. Nuclear YAP/TAZ acts as a cofactor for smad signaling and enhances smad nuclear localization that in turn promotes TGF-β signaling. Based on these findings, we checked the simultaneous levels of smad 2/3 in keloids and found no significant differences between keloid areas and unaffected areas (data not shown), which suggests that another pathway might be involved in regulation of YAP/TAZ activity in keloids.

Although keloids are a benign disorder, they can proliferate and infiltrate into normal skin. When keloids progress, the tissue becomes hard compared with normal skin. In terms of these characteristics, keloids closely resemble cancer tissues. In our study, we found that keloid fibroblasts had increased nuclear expression of...
YAP/TAZ relative to unaffected areas. In contrast, there were no significant differences in YAP/TAZ nuclear localization in keratinocytes and endothelial cells from either keloid or unaffected tissues. These results suggest that fibroblasts in keloid tissue could receive "stiffness signals" that activate Hippo signaling and play an important role in keloid formation and stiffness by affecting gene expression.

In our study, YAP/TAZ expression in keloid fibroblasts had a greater tendency to localize to the nucleus relative to fibroblasts from unaffected tissues. Meanwhile, keratinocytes or endothelial cells from either keloid or unaffected tissues showed no significant differences in YAP/TAZ expression patterns. This participation of YAP/TAZ signaling in keloid invasion and growth suggests that agents that affect YAP/TAZ signaling pathways could be developed to provide novel approaches to treat keloids.

Fig. 3. YAP/TAZ nuclear localization in fibroblasts in the keloid and unaffected areas. The YAP/TAZ nucleus–positive cell index (percentage of localized nucleus to whole YAP/TAZ–positive cells) was calculated by counting the number of immunoreactive cells and total cells in the keloid or unaffected areas (n = 4 per group). YAP/TAZ nuclear localization of fibroblasts in keloids was significantly increased compared with that of the unaffected area (A). There were no significant differences between keratinocytes (B) and endothelial cells (C). Statistical differences were determined using a nonparametric Mann-Whitney U test. *P values less than 0.05 were considered significant; **P < 0.001.

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