TO THE EDITOR

The wound healing process relies on the proper execution and regulation of signaling pathways and transcriptional regulators to properly coordinate the wound healing response (Eming et al., 2014). Although epithelial lining tissues have a remarkable ability for tissue repair, the efficiency of such repair varies depending on the tissue considered (Iglesias-Bartolome et al., 2018). For example, wounds of the oral mucosa heal more effectively than wounds of the skin (Iglesias-Bartolome et al., 2018). Recently, our laboratory performed a comparative analysis on human oral and cutaneous skin wounds and determined that a wound-activated transcriptional network regulated by the transcription factor SOX2 establishes a network that primes the oral epithelium for rapid wound repair (Iglesias-Bartolome et al., 2018). Reprogramming of cutaneous skin by SOX2 corresponded to increased expression of several wound-activated keratin genes, including keratin (K)6 gene K6, gene K16, and gene K17 at baseline before wounding, further showing priming of cutaneous skin for rapid wound healing (Iglesias-Bartolome et al., 2018; Uchiyama et al., 2019). In addition to the wound-activated keratin genes, we identified three potential SOX2-binding sites (sites 1, 2, and 3) in the promoter of K75 (Supplementary Figure S2). Among them, we identified SUN2, an inner nuclear membrane protein and component of the linker of nucleoskeleton and cytoskeleton complex (LINC) (Ostlund et al., 2009). Keratins have been shown to interact with nuclear components and can be present in the nuclear interior (Hobbs et al., 2016). One of the main functions of LINC is to transduce mechanical signals from the plasma membrane to the nucleus where it can influence gene expression (Carley et al., 2021; Ostlund et al., 2009; Stewart et al., 2015; Ueda et al., 2022), suggesting that K75 interaction with LINC may regulate the wound healing response by regulating gene expression. To confirm mass spectrometry data, we performed a proximity ligation assay to confirm K75–SUN2 interaction in primary human KCs overexpressing SOX2. Transduction efficiency of SOX2 was confirmed by immunofluorescence staining (Supplementary Figure S3). Proximity ligation assay confirmed K75–SUN2 interaction, which was found to occur primarily at the cytoplasmic face of the nuclear periphery (Figure 2a). This was further confirmed by immunofluorescence staining in primary human KCs overexpressing anti-SOX2 antibody compared with those of IgG control (Figure 1d). These findings show K75 to be a direct target of SOX2.

To further investigate the role of K75 in regulating the wound healing response, we identified potential protein binding partners by mass spectrometry. Protein interactions with keratins have been previously described and have shown to play an essential role in regulating KC migration (Rotty and Coulombe, 2012; Wang et al., 2018). We transfected K75 construct in murine ameloblast-like cells, a type of oral epithelial cells, and found several binding partners for K75 (Supplementary Figure S2). Among them, we identified SUN2, an inner nuclear membrane protein and component of the linker of nucleoskeleton and cytoskeleton complex (LINC) (Ostlund et al., 2009). Keratins have been shown to interact with nuclear components and can be present in the nuclear interior (Hobbs et al., 2016). One of the main functions of LINC is to transduce mechanical signals from the plasma membrane to the nucleus where it can influence gene expression (Carley et al., 2021; Ostlund et al., 2009; Stewart et al., 2015; Ueda et al., 2022), suggesting that K75 interaction with LINC may regulate the wound healing response by regulating gene expression. To confirm mass spectrometry data, we performed a proximity ligation assay to confirm K75–SUN2 interaction in primary human KCs overexpressing SOX2. Transduction efficiency of SOX2 was confirmed by immunofluorescence staining (Supplementary Figure S3). Proximity ligation assay confirmed K75–SUN2 interaction, which was found to occur primarily at the cytoplasmic face of the nuclear periphery (Figure 2a). This was further confirmed by immunofluorescence staining in primary human KCs overexpressing
Figure 1. *K75 is a direct transcriptional target of SOX2*. (a) Immunofluorescence staining of unwounded skin tissue from SOX2-transgenic mice treated with vehicle or tamoxifen to detect K75 (red) and DAPI (blue). Bar = 50 μm. (b) qPCR of K75 from SOX2-transgenic mice treated with vehicle or tamoxifen. n = 2. Data expressed as mean ± SD. *P < 0.05. (c) Genome track of K75 gene showing three potential SOX2-binding sites (sites 1, 2, and 3). (d) qPCR analyses on chromatin samples from mouse primary keratinocytes isolated from SOX2-transgenic mice treated with tamoxifen or vehicle after immunoprecipitation with anti-SOX2 or IgG control antibodies. n = 2. Data expressed as mean ± SD. *P < 0.05, **P < 0.01, and ***P < 0.001. chr, chromosome; K, keratin; Tam, tamoxifen; Veh, vehicle.
Figure 2. K75 is a component of the LINC complex and is essential for promoting the SOX2-mediated rapid healing effects. (a) Proximity ligation assay of primary human keratinocytes overexpressing SOX2 to detect K75 and SUN2 (red) interaction and DAPI (blue). Bar = 5 μm. The magnification of the dashed box is shown at the bottom of the image. (b) Super-resolution imaging of primary human keratinocytes overexpressing SOX2 to detect the localization of K75 (green), SUN2 (red), and SOX2 (magenta). The magnification of the dashed box is shown at the bottom of the image. (c) Western blot and quantification showing the expression of K75, SUN2, SOX2, and RPS14. (d) Western blot and quantification showing the % Keratinocyte migration.
SOX2 (Figure 2b and Supplementary Figure S4). Super-resolution imaging further showed cytoplasmic K75, and SUN2 detection was observed primarily at the nuclear periphery (Figure 2b and Supplementary Movie S1). Interestingly, we found K75 to be expressed in control KCs (Supplementary Figure S4). KCs grown in culture are known to have a wound-activated phenotype that consists of induced expression of the wound-activated keratins K6, K16, and K17. Our data support that cytoplasmic K75 interacts with SUN2 at the periphery of the nuclear envelope. These findings show K75 to be an additional marker of the wound-activated phenotype of KCs and identify a previously unreported role for K75 as a component of the LINC complex.

We next tested whether the K75–SUN2 interaction is essential for the rapid wound healing effects mediated by SOX2. We utilized a KC scratch assay in which primary human KCs were transduced with SOX2 and knockdown of K75 and SUN2 was performed with small interfering RNA. Knockdown efficiency of K75 and SUN2 was confirmed by western blot (Figure 2c). We show that overexpression of SOX2 resulted in increased K75 expression and KC migration (Figure 2c and d). Knockdown of either K75 or SUN2 resulted in inhibition of KC migration and reversed SOX2 effects. Our findings describe K75 as a component of the LINC complex and that it has an essential role in mediating the SOX2 rapid healing response during wound healing.

Data availability statement


Ethics statement

All procedures were approved by the National Institute of Arthritis and Musculoskeletal and Skin Diseases Animal Care and Use Committee.

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AUTHOR CONTRIBUTIONS

Conceptualization: APS, MIM; Data Curation: APS, AU, EH, DB, SW, MC, EB, LMJ, OD, MIM; Formal Analysis: APS, AU, EH, DB, SW, MC, EB, LMJ, OD, MIM; Funding Acquisition: MIM; Investigation: APS, AU, EH, DB, SW, MC, EB, LMJ, OD, MIM; Methodology: APS, MIM; Project Administration: MIM; Resources: EB, LMJ, OD, MIM; Supervision: MIM; Validation: APS, AU, EH, DB, SW, MC, EB, LMJ, OD, MIM; Writing - Original Draft Preparation: APS, DB, MIM; Writing - Review and Editing: APS, DB, MIM.

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expression of K75, SUN2, and SOX2 in primary human keratinocytes after siRNA knockdown. RPS14 served as a loading control. (d) Scratch assay of primary human keratinocytes after siRNA of K75 and SUN2. The rate of keratinocyte migration was quantified 24 hours after the scratch. n = 3 per group. Data are expressed as mean ± SEM. *P < 0.05. K, keratin; LINC, linker of nucleoskeleton and cytoskeleton complex; siK75, small interfering RNA targeting K75; siNeg, small interfering RNA targeting negative control; siRNA, small interfering RNA; siSun2, small interfering RNA targeting SUN2.


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