YAP Expression and Activity Are Suppressed by S100A7 via p65/NFκB-mediated Repression of ΔNp63

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Abstract

In several squamous cell carcinoma (SCC) cells, it has been previously observed that induction of the S100 calcium-binding protein A7 (S100A7) is repressed by YAP via the Hippo pathway. This report now demonstrates that S100A7 also represses YAP expression and activity by ΔNp63 in cancer cells. Stable overexpression of S100A7 activates the NFκB pathway and inhibits the expression of ΔNp63. Caffeic acid phenethyl ester (CAPE), as a specific inhibitor of NFκB, counteracts the inhibitory effect of S100A7 on the expression of ΔNp63 and its target genes. Depletion of S100A7 significantly promotes ΔNp63 expression. These data indicate that S100A7 acts as a suppressor of ΔNp63. Mechanistic examination finds that ΔNp63 not only directly binds to the region of YAP promoter and induces its expression, but also inhibits the Hippo pathway and enhances YAP activity. Importantly, either the positive correlation between S100A7 and YAP phosphorylation at S127 or the negative correlation between S100A7 and ΔNp63 is also observed in skin SCC tissues. Chemosensitivity analysis reveals that S100A7 enhances cancer cells’ resistance by inhibition of YAP expression and activity. These results demonstrate that S100A7 is an upstream modulator of the Hippo pathway and extend our understanding of S100A7 functions in cancer.

Implications: S100A7 is a new upstream regulator of the Hippo signaling pathway and reduces chemosensitivity of SCC cells through inhibitions of YAP expression and activity. Mol Cancer Res; 1–12. ©2017 AACR.

Introduction

S100A7 (psoriasin) is present within the epidermal differentiation complex on 1q21 chromosome and was initially identified in the skin of patients suffering from psoriasis, a hyperproliferative skin disease characterized by abnormal differentiation (1, 2). In addition, increased S100A7 expression has been reported in nearly all types of SCC tissues as well as adenocarcinomas of the breast (3–7). Our recent works uncovered that the S100A7 acted as a dual regulator in promoting proliferation and suppressing differentiation in several SCC cells (8–11). S100A7 has also been shown to be attributed to resistance of anoikis resulting in tumor growth by activating several signaling pathways (12, 13). Interestingly, S100A7 has been induced by cytokines, cell dense and suspension culture, and by activation of the Hippo pathway (8, 9).

The Hippo pathway, initially discovered in Drosophila, is an evolutionarily conserved and potent regulator of cell growth and apoptosis (14). Core components of the Hippo pathway include a kinase cascade of MST1/2 and LATS1/2. YAP (Yes-associated protein), as a transcription coactivator, is the ultimate effector of the Hippo pathway (15–17). In response to stimulation, LATS1/2 phosphorylate YAP at serine 127 and confine it to the cytoplasm, where it can no longer bind with TEAD (TEA domain) to promote or repress YAP-dependent target genes as YAP in nucleus (14). Recent advances have rapidly expanded the understanding that YAP acts as an oncogene in various types of cancers (18). For example, YAP has been identified as a candidate oncogene in human chromosome 11q22 amplicon (19). Furthermore, YAP cooperates with the myc oncogene to stimulate tumor growth in vivo (19), and active YAP mutants induce oncogenic cell transformation in vitro (20). On the other hand, YAP was shown to serve as a cofactor for p73-dependent apoptosis in response to DNA damage (21).

p63 is composed of multiple isoforms with overlapping and unique activities. The p63 isoforms can be placed into two groups: the transactivation domain isoforms (TAp63), which structurally resemble p53 and act as tumor suppressors; and the ΔN isoforms (ΔNp63), which bind to p53, TAp63, and TAp73 and inhibit their function, thus acting as oncogenes (22–24). Unlike ΔNp63, TAp63 is not expressed or is present at low levels in a variety of cancers (25, 26). Recent studies reported that NFκB can suppress p63 expression through the direct...
interaction of p65 with the proximal region of the p63 promoter (26). In contrast, expression of IkB-α results in strong induction of p63 expression, indicating that p63 is, indeed, negatively regulated by NFκB (27, 28). In addition, some crosstalk between p63 and the Hippo pathway has been discovered recently (25, 29), for example, ΔNp63 directly interacts with the Hippo effector YAP and is a mediator of YAP function in the epithelium of lung airways (30). p63 coamplified with ACTL6A in SCC to drive YAP activity, which resulted in regeneration, proliferation, and poor prognosis (31).

Our recent studies uncover that S100A7 induction is repressed by YAP via the Hippo pathway in several SCC cells (10–12). Although S100A7 expression is negatively regulated by nuclear YAP, both S100A7 and YAP perform the similar functions in promoting cells’ proliferation and inhibiting squamous differentiation (8–10). In this report, we provide evidence that S100A7 also inhibits YAP expression and activity through p65/NFκB-mediated repression of ΔNp63, and S100A7 represses drug-induced apoptosis via inhibition of YAP.

Materials and Methods

Cell lines

Human carcinoma cell lines HCC94, A431, and FaDu were purchased from the Chinese Academy of Sciences Committee Type Culture Collection Cell Bank and were authenticated by short tandem repeat analysis at HK Gene Science Technology Co. The cells were cultured in RPMI1640 containing 10% FCS, penicillin (100 U/mL), streptomycin (100 U/mL), and 2 mmol/L glutamine, and grown at 37°C in a humidified atmosphere with 5% CO2.

Plasmids

For pCMV14-Flag-ΔNp63, the ΔNp63 cDNA fragment was amplified using 5’-AGATATCATGTTAGCCATCTGAAAAACATGCGCC-3’ and 5’-ATCTAAGATGCTCCCCTCTTGTATGCAGCGC-3’, and then was cloned into the mammalian expression vector pCMV14 (Invitrogen) using EcoR5 and XbaI restriction enzymes (Takara).

siRNA and transfection

To silence the expression of S100A7, MST1, LATS1, YAP, and ΔNp63, all siRNAs as well as the non-targeting control siRNAs were purchased from Gene Pharma and transfected using the Transfection Reagent (Polyplus) according to the manufacturer’s protocol. The sequences of siRNAs are in Supplementary Information (Supplementary Table S1).

Drug treatment

DDP (Qilu Pharmaceutical Co., Ltd., 5050221DB) and VP-16 (Jiangsu Hengrui Medicine Co., Ltd. by Share Ltd., 15101538) were used as indicated. The specific inhibitor Caffeic acid phenethyl ester (CAPE; Sigma, C8221-1G) was used to inhibit NFκB signaling pathway in cells.

Reverse transcription and quantitative RT-PCR

Total RNA was extracted from cells for the generation of single-stranded cDNA. Quantitative RT-PCR (qPCR) was performed using an ABI 7300 Real-Time PCR System (Applied Biosystems) with the PowerSYBR Green PCR Master Mix (Applied Biosystems) in a final volume of 20 μL. GAPDH was used as an endogenous control for each sample. The primers used for each of the genes are listed (Supplementary Table S2).

Western blot analysis

Western blotting analysis was performed as described previously (11). The following antibodies were used: S100A7 (1/1,000; Abcam, ab13680); YAP (1/500, Santa Cruz Biotechnology, sc-101199); pYAP (S127; 1/1,000; Cell Signaling Technology, 3003S); MST (1/1,000; Cell Signaling Technology, 3682S); pLATS1 (1/1,000; Cell Signaling Technology, 8654S); ΔNp63 (1/1,000; Biologen. 619002); PARP (1/1,000; Cell Signaling Technology, 9542S); anti-Flag tag (CWBio, CW0287A); anti-His tag (MBL, D291-3); GAPDH (1/4,000; ZSGB-BIO, TA-08), and β-actin (1/4,000; ZSGB-BIO, TA-09) were used as loading controls.

Immunofluorescence staining

Immunofluorescence staining analysis was performed as described previously (11). To examine the expression pattern of S100A7, ΔNp63, and YAP in cells, cells were plated on coverslips and then cultured for 24 hours before handled. Cells were fixed with 3.4% paraformaldehyde for 20 minutes and then permeabilized with 0.5% PBS-Triton X-100. After blocking in 3% PBS-BSA for 30 minutes, slides were incubated with anti-S100A7 (Abcam), anti-ΔNp63 (Biologend), or anti-YAP (Cell Signaling Technology) for 1 hour at 37°C, and antibodies were diluted in 1% BSA. After washing with PBS, slides were incubated with goat anti-mouse TRITC (tetramethyl rhodamine isothiocyanate) 555- (ZSGB-BIO, ZF-0316) or goat anti-rabbit FITC (fluorescein isothiocyanate) 488- (ZSGB-BIO, ZF-0312) conjugated secondary antibodies for 1 hour at 37°C. The nuclei were stained using DAPI (4’, 6-diamidino-2-phenylindole). The targeted proteins were detected using confocal microscopy (ZEISS LSM700) and a ZEISS LSM700 laser-scanning confocal microscope imaging system.

IHC

IHC was performed as described in our previous study (11). Anti-S100A7 (1/200), anti-ΔNp63 (1/100), and anti-pYAP-S127 (1/200) were separately incubated with the specimens. The goat anti-rabbit/mouse secondary antibody was purchased from MAIXINBIO (KIT-5010). S100A7, ΔNp63, and pYAP-S127 expression was detected using a fluorescence microscope (ZEISS ImagerA1).

High content live-cell imaging systems

S100A7 promote p65 nuclear translocation was detected by high content live-cell imaging system. Cells were seeded in the cover glass, fixed after 24 hours, and permeabilized as for the cell immunostaining. The cells were then blocked with 3% BSA-PBS solution. P65 antibody was applied for 1 hour at 37°C followed by washing and subsequent incubation with FITC-labeled goat anti-rabbit IgG for 1 hour at 37°C. Finally, the nuclei were stained using Hoechst 33342 for 10 minutes at room temperature. High content live-cell imaging systems was applied to detect the p65 nuclear translocation, and “translocation enhanced” analytic model was used to given translocation rate.

Cell apoptosis analysis

Apoptosis was measured by flow cytometry. Staining was carried out by Annexin V-FITC/PI Apoptosis Detection Kit.
SuperMix (TransGen Biotech). The primers for p63-binding site

Chromatin immunoprecipitation

Precleared chromatin of HCC94 was immunoprecipitated with a rabbit polyclonal antibody against ΔNp63 (619002, BioLegend) and normal rabbit IgG (7074PZ, Cell Signaling Technology). Precipitated DNA was amplified with 2× EasyTaq SuperMix (TransGen Biotech). The primers for p63-binding site of the YAP1 promoter predicted by Genomatix software were: site1 (−2,325 to −2,295 bp): 5'-CTGGAGTGCACTGGTGCTGAT-3' (forward), and 5'-GTGTTGGCATGTCCCTGTAGT-3' (reverse); site2 (−1,522 to −1,492 bp): 5'-GCCCTACCAACCAAAGTGCATA-3' (forward), and 5'-AGCAAAGAAAAATCGCTG-3' (reverse; ref. 29).

Statistical analysis

All of the experiments were repeated at least twice. Statistical analysis was performed using GraphPad Prism software. The statistical significance was evaluated using Student t test (two-tailed) to compare two groups of data. The asterisks indicate significant differences between the experimental groups and corresponding control condition. Differences were considered statistically significant at a P value of less than 0.05. P values <0.05, <0.01, <0.001, <0.0001 are indicated with one, two, three, and four asterisks, respectively.

Results

S100A7 represses YAP expression and its activity

Our previous research revealed that YAP acted as a suppressor on S100A7 induction in several SCC cells. Considering S100A7 and YAP perform some similar functions in promoting cells' proliferation and inhibiting squamous differentiation, we wonder whether S100A7 also in return regulates YAP expression or activity. To test our hypothesis, we performed stable overexpression of S100A7 in A431 cells (S100A7-overexpressed A431 cells) which expressed low level of S100A7. As expected, S100A7 overexpression significantly inhibited the expression of YAP and also resulted in activation of the Hippo pathway that was indicated by increase in LATS1-T1079 (LATS1-HM) and YAP-S127 phosphorylation (Fig. 1A). On the contrary, depletion of S100A7 in HCC94 cells, which expressed high level of S100A7, strikingly improved YAP expression and inhibited the Hippo pathway (Fig. 1B). Using quantitative RT-PCR (qPCR), we also analyzed the expression of CTGF (connective tissue growth factor) and/or CYR61 (cysteine-rich angiogenic inducer 61), two direct endogenous markers of YAP activity. Indeed, CYR61 transcription had a significant reduction in S100A7-overexpressed A431 cells (Fig. 1C), whereas CYR61 and CTGF expressions were extremely increased after knockdown of S100A7 in HCC94 cells (Fig. 1D). Importantly, the similar results were also obtained in S100A7-silenced FaDu cells (Supplementary Fig. S1A and S1B). The negative correlation of S100A7 and nuclear YAP was further validated in A431 cells by double immunofluorescence (IF) staining. Representative images are shown in Fig. 1E. We found that overexpressed S100A7 markedly attenuated the fluorescence intensity of YAP and decreased its nuclear accumulation. Collectively, our data uncover that S100A7 not only represses YAP expression, but also activates the Hippo pathway in cancer cells.

S100A7 suppresses YAP expression and activity via repression of ΔNp63

As S100A7 is neither a transcription factor nor a phosphokine, we speculate that S100A7 is unable to regulate YAP expression and its phosphorylation directly. A more recent study showed that ΔNp63 could directly regulate YAP expression through binding to the YAP promoter (29). Therefore, we speculated that S100A7 repressed YAP expression and its activity through inhibition of ΔNp63. To test our hypothesis, we examined the expression of ΔNp63 in S100A7-overexpressed A431 cells and S100A7-silenced HCC94 cells by Western blot analysis. As expected, S100A7 overexpression obviously inhibited ΔNp63 expression in A431 cells (Fig. 2A). The opposite result was also observed in S100A7-silenced HCC94 cells (Fig. 2B). Interestingly, TAp63 was nearly undetectable in all tested cells (Supplementary Fig. S2A), and TAp63 had no regulatory effect on YAP expression and activity (data not shown). Next, to confirm the regulatory effect of ΔNp63 on YAP expression and its activity, we silenced ΔNp63 using p63 siRNA in A431 and HCC94 cells. Indeed, knockdown of ΔNp63 markedly repressed YAP expression and activity, which was reflected by a decrease in YAP protein levels and an increase of YAP phosphorylation in A431 (Fig. 2C) and HCC94 cells (Fig. 2D). We also noted that CYR61 and CTGF decreased after ΔNp63 knockdown (Fig. 2E and F). Similar results were also observed in FaDu cells (Supplementary Fig. S2B and S2C). Double IF analysis also confirmed that knockdown of ΔNp63 significantly decreased YAP expression and its nuclear accumulation in both A431 and HCC94 cells (Fig. 2G and H). To confirm whether S100A7 could influence the promoter effect of ΔNp63 on YAP expression and activity, we transfected ΔNp63 plasmid into S100A7-overexpressed A431 and the corresponding control cells. As anticipated, overexpression of ΔNp63 almost entirely recovered the expression of total YAP and the level of pYAP-S127 in S100A7-overexpressed A431 cells compared with the control cells (Fig. 3A). Similarly, in HCC94 cells, the total YAP was decreased and pYAP-S127 was increased in double deletion of S100A7 and ΔNp63 compared with single silencing of S100A7 (Fig. 3B). In fact, both the total YAP expression and its activity nearly reached the levels of the control cells. Finally, to validate the correlation of these three proteins in vivo, three consecutive sections of skin SCC tissues microarrays were stained by specific S100A7, ΔNp63, and pYAP-S127 antibodies, respectively. Consistent with our observation in vitro, S100A7 presented the similar expression pattern with pYAP-S127, whereas both proteins were negatively correlated with ΔNp63 staining (Fig. 3C). In skin SCC tissues expressing high S100A7, ΔNp63 was not expressed (Fig. 3C, H, black arrow), or low expressed in S100A7 relatively weak areas (Fig. 3C, H, white arrow). ΔNp63 was generally highly expressed in skin SCC tissues with S100A7 low expression or nonexpression (Fig. 3C, L). These data in vitro and in vivo directly or indirectly support the ability of S100A7 to repress YAP expression, and this activity is likely to be dependent on ΔNp63.

S100A7 inhibits ΔNp63 expression by activation of the NFκB signaling pathway

As p63 is negatively regulated by NFκB pathway in both skin cancers and stratified epithelial cells, and Rel/p65 can also repress ΔNp63 expression in a transcription-dependent in
epithelial cells (26–28), we examined the activity of NFκB and its target gene expressions (cox-2 and survivine) in S100A7-overexpressed A431 cells. Efficient S100A7 overexpression greatly enhanced the phosphorylation of IKKα/β, IκB, p65 (Fig. 4A), and the transcription of cox-2 and survivine (Fig. 4B). To further confirm the subcellular location of p65, we extracted the whole-cell protein and nucleoprotein from the S100A7-overexpressed and the control cells and examined the protein level of p65 by Western blot analysis. The data showed that overexpression of S100A7 really improved p65 nuclear translocation (Fig. 4C). The quantitative observation of nuclear p65 was performed in A431 cells by high-throughput microscopy (Fig. 4D). The percentage of nuclear p65 staining was 23.80±15% and 10.19±44% in S100A7-overexpressed A431 cells and the control cells, respectively (Fig. 4E). CAPE is a specific inhibitor of NFκB (32). To further confirm these results, we treated S100A7-overexpressed cells and the control cells with or without CAPE. The results show that CAPE treatment obviously counteracts the inhibitory effect of S100A7 on the expression of ΔNp63 and keratin5/14 (two target genes of ΔNp63; Fig. 4F).
Collectively, our results fully illustrated that S100A7 inhibits the expression of ΔNp63 via activation of the NFκB signaling pathway.

ΔNp63 inhibits the Hippo pathway and directly regulates YAP expression

To investigate how ΔNp63 regulated YAP expression and activity, we silenced ΔNp63 in A431 and HCC94 cells using p63 siRNA and detected the Hippo pathway. We found that knockdown of ΔNp63 moderately increased MST1 expression and LATS1-HM phosphorylation in both A431 and HCC94 cells (Fig. 5A), whereas ΔNp63 overexpression in the same cells appeared as the opposite phenomenon (Fig. 5B). Strikingly, the finding that ΔNp63 decreased MST1 expression led us to hypothesize that ΔNp63, as a nuclear transcription factor, likely repressed MST1 expression that resulted in inhibition of the Hippo pathway and YAP phosphorylation. To assess this hypothesis, we examined the transcription level of LKB1 (a kinase of MST1), MST1, and LATS1 in the cells after silencing of ΔNp63. The result showed that MST1 mRNA level was

Figure 2. S100A7 acts as an inhibitory factor of ΔNp63, which is positively regulated with YAP. A and B, Stable overexpression or depletion of S100A7 in A431 cells (A) or HCC94 cells (B), respectively. The expression of ΔNp63 and S100A7 was determined by Western blotting. β-Actin was used as a loading control. C and D, Depletion of ΔNp63 in A431 cells (C) or HCC94 cells (D), respectively. The expression of ΔNp63, YAP, pYAP-S127 was determined by Western blotting. β-Actin was used as a loading control. E and F, qRT-PCR analyses of S100A7, CYR61, or/and CTGF in A431 (E) and in HCC94 cells (F) after silencing of p63. Error bars, SDs of three different experiments. †, P < 0.05; ††, P < 0.01; †††, P < 0.001; ††††, P < 0.0001; t test. E and F, Representative immunofluorescence images of YAP and ΔNp63 subcellular location were detected in siNC and sip63 A431 (G) and HCC94 (H) cells. DAPI was a nuclear counter stain. Scale bar, 20 μm.
significantly upregulated in the absence of ΔNp63, whereas LKB1 and LATS1 had no change when compared with the control cells (Fig. 5C). Besides, transient silencing of MST1 impaired the increase in YAP phosphorylation by S100A7 overexpression in A431 cells (Fig. 5D). In addition, the finding that ΔNp63 could directly bind to the region of YAP promoter by chromatin immunoprecipitation (ChIP) provided the support for direct transcriptional induction of YAP expression by ΔNp63 (Fig. 5E). Together, these results strongly support that ΔNp63 increases YAP activity through inhibition of the Hippo pathway, which is mediated by the repression of MST1 expression, and promotes YAP expression through its direct binding with the YAP promoter.

S100A7 represses chemotherapy-induced apoptosis via inhibition of YAP

Except for its controlling of cell growth in physiologic condition, YAP also plays an important role in the regulation of chemosensitivity of cancer cells (21, 33). Considering the relationship of S100A7 and YAP, we hypothesize that both
YAP and S100A7 may be involved in the regulation of chemosensitivity in our tested cells. To substantiate this possibility, A431 and HCC94 cells were treated with DDP and VP16 to induce apoptosis. Interestingly, stably expressed S100A7 in A431 cells had a significant inhibiting effect on drug-induced apoptosis. Conversely, overexpressing YAP promoted apoptosis, but this effect could be rescued by overexpression of S100A7 in A431 cells, suggesting that S100A7 effectively overcame apoptotic induction by YAP (Fig. 6A–D). Consistently, the DDP/VP16–induced apoptosis in HCC94 cells was significantly promoted by single removal of S100A7 or overexpression of YAP, and an obvious improvement of apoptosis was observed by both combinations (Fig. 6E–H). Furthermore, corresponding PARP cleavage was also examined by Western

**Figure 4.**
S100A7 inhibits ΔNp63 expression by activation of the NFκB signaling pathway. A, The expression of IKKα, IKKβ, p-IKKα/β, IκBα, p-IκBα, p65, IκBα, p65, and S100A7 in S100A7–overexpressed A431 cells was determined by Western blotting. β-Actin was used as a loading control. B, The transcription level of ΔNp63, Cox-2, and survivin in S100A7–overexpressed A431 cells was examined by qRT-PCR. Error bars, SDs of three different experiments. *, P < 0.05; **, P < 0.01; t-test. C, The holoprotein and nucleoprotein were extracted from the control and S100A7–overexpressed A431 cells. p65, p50, and S100A7 expression was examined by Western blotting. β-Actin was used as a loading control. Histone H3 was used as a loading control of nucleus proteins. D, p65 nuclear translocation was visualized by high-throughput microscopy. p65 staining was indicated by green color and the nuclei staining was indicated by red color. E, The histograms demonstrate the percentage of nuclear p65 staining in control or S100A7–overexpressed A431 cells. F, qRT-PCR analyses of ΔNp63, Keratin-5, and Keratin-14 in control or S100A7–overexpressed A431 cells with or without the CAPE. Error bars, SDs of three different experiments. *, P < 0.05; **, P < 0.01; t-test.
blotting. The results demonstrated that the detectable PARP cleavage only occurred in A431 and HCC94 cells treated with both drugs. Consistent with the percentage of apoptosis, overexpression of S100A7 resisted DDP- and VP16-induced PARP cleavage in A431 cells, whereas overexpression of YAP significantly promoted PARP cleavage. S100A7 also had a significant inhibiting effect on YAP-induced PARP cleavage (Fig. 6I–L).

**Discussion**

In this study, we demonstrated that S100A7 activates the Hippo pathway through NFκB signaling pathway, and resists chemotherapy-induced apoptosis in SCC cells. On the basis of our results, we have proposed a working model for S100A7 function, which is summarized in Fig. 7. In this model, first, S100A7 attenuated YAP activity through NFκB/p65-mediated activation of the Hippo pathway. Second, S100A7 suppressed YAP expression through NFκB/p65-mediated repression of ΔNp63. The supporting data showing that overexpression of S100A7 in A431 cells could activate NFκB signaling pathway,
which resulted in the translocation of p65 into the nucleus, and then the nuclear p65 suppressed ΔNp63 expression. After that, downregulation of ΔNp63 induced MST1 expression. Finally, YAP activity was suppressed by activation of the Hippo pathway via upregulation of MST1 expression. Conversely, silencing of S100A7 in HCC94 cells could increase ΔNp63 expression that resulted in an increase of YAP expression and its activity. After that, p63 positively regulated YAP expression through its ability to directly bind the promoter of YAP. Therefore, we conclude that S100A7 represses both YAP expression and its activity. Although accumulating evidence suggests that MST1/2 are not absolutely required for the regulation of YAP under various conditions (14), our results demonstrate that MST is critical for S100A7 activating the Hippo pathway in the tested cancer cells.

Although NFκB is antiapoptotic and positively contributes to tumorigenesis in hematopoietic tissue, reduced p65/NFκB has been observed in human SCC and acts as a tumor suppressor in the epidermis (26). For example, mice overexpressing the NFκB inhibitory protein IκBζ develop spontaneous SCCs (26). ASSP2 as a tumor suppressor represses SCC development via p65/NFκB-mediated repression of ΔNp63 expression (26). These results provided a molecular link between the NFκB pathway and ΔNp63. ΔNp63 is restricted to transit-amplifying cells and stem cells residing in the basal layer. ΔNp63 expression is downregulated during skin differentiation and it regulates genes that result in basal cell proliferation (34, 35). Forced expression of ΔNp63 is sufficient to drive stem-like proliferation and tumorigenesis in primary keratinocyte (36). Hence, ΔNp63 is considered as an oncogene and overexpression of ΔNp63 is a hallmark of SCCs (37). In this study, we found that overexpression of S100A7 could activate IκKα/β, the key regulatory step in NFκB pathway and increased in p65 phosphorylation at Ser536 and its nuclear localization. We further confirmed that S100A7 overexpression significantly decreased the expression of ΔNp63, but increased the
expressions of Cox-2 and survivin in A431 cells. Conversely, silencing of S100A7 promoted ΔNp63 expression in HCC94 cells. As NIK (NFκB-inducing kinase) can phosphorylate and activate Iκκα and Iκκβ and is also regulated by MAPK/Erk cascade (38, 39), we speculate that the effect of S100A7 on NFκB pathway is likely through the regulation of NIK or MAPK/Erk activity in the cells. In addition, we also treat S100A7-overexpressed A431 cells and the control cells with or without CAPE (an inhibitor of NFκB). The results revealed that overexpression of S100A7 significantly inhibited the expressions of ΔNp63 and keratin 5/14, whereas CAPE treatment obviously counteracted the inhibitory effect of S100A7 on the abovementioned gene expressions. These findings further illustrated that S100A7 represses ΔNp63 expression via NFκB pathway. Because TAp63 was undetectable in all the tested cells, it is reasonable to ignore the functions of TAp63 in this study. A variety of signals have been reported to either activate or inhibit the Hippo kinase cascade. In epithelial cells, apical–basal polarity regulates activities of the Hippo pathway through interactions between the upstream components and intercellular function–associated proteins in response to cell–cell contact (40). Recent reports found that serum deprivation or energy stress also activates the Hippo pathway (41, 42). Extracellular hormones can regulate the Hippo pathway via G-protein–coupled receptors (43). Cytoskeletal dynamics appear to be the major mediators for YAP/TAZ regulation by cell detachment and mechanical forces (44, 45). Therefore, upstream regulators of the Hippo pathway are being unveiled. In this study, we demonstrate that S100A7 not only promotes LATS1 kinase activity, but also suppresses YAP expression, strongly supporting that S100A7 is a new upstream regulator of the Hippo pathway. In addition, based on the new definition of the Hippo pathway, proteins that specifically influence LATS1 kinase activity and the functional output of YAP/TAZ should be considered as “Hippo pathway” components (46), our results also suggest that S100A7 is also considered as a new member of the Hippo pathway components.

Interestingly, our previous study uncovered that S100A7 induction was also repressed by YAP through activation of the Hippo pathway in A431, HCC94, and FaDu cells under suspend and dense culture or F-actin disruption (8, 9). Combined with the findings in this study, we conclude that there is a negative correlation of S100A7 and YAP, and reciprocal negative regulation between the two proteins in SCC cells, at least in A431, HCC94, and FaDu cells. To investigate the functions of S100A7 and YAP in SCC cells, we found that S100A7 and YAP have the similar or opposite functions in the abovementioned cells. First, overexpression of S100A7 in A431 cells promoted cell proliferation and inhibited differentiation, whereas the opposite results were obtained in silencing of S100A7 in HCC94 cells (11). Similarly, knockdown of YAP in all these three cells resulted in a decrease in the growth of cells (8, 9). Second, either inhibition or activation of chemotherapy-induced apoptosis was observed in S100A7-overexpressed cells or S100A7-silenced cells, suggesting that S100A7 expression reduced the chemosensitivity in these cells. Conversely, overexpression of YAP really promoted chemotherapeutic-induced apoptosis in both A431 and HCC94 cells. Why did S100A7 and YAP perform the similar effects in SCC cell proliferation and differentiation but the converse in chemotherapeutic-induced apoptosis? A possible explanation is the opposite function of YAP in cancer cells with or without chemotherapeutic treatments. Some data support our hypothesis. For example, the nuclear translocation of YAP is increased in cells exposed to cisplatin and then YAP is recruited by promyelocytic leukemia gene (PML) into the nuclear bodies (NB) to promote p73 transcriptional activity. YAP also contributes to p73 stabilization in response to DNA damage and promotes p73-dependent apoptosis (21, 47, 48). Therefore, transcriptional coactivator YAP determines p73 gene targeting in response to DNA damage (21). Collectively, YAP can interact with a variety of transcriptional factors to play similar or even opposite biological activities according to microenvironmental treatment.

Taken together, this study uncovers for the first time that S100A7 functions as a new upstream regulator of the Hippo pathway. Our findings extend the understanding of S100A7 functions and contribute to elucidating the roles of S100A7 and YAP during the development and chemotherapy of SCCs.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Concept and design: Y. Li, F. Kong, R. Wang, D. He, X. Xiao
Development of methodology: Y. Li, F. Kong, Q. Shao, E. Hu, C. Jin, X. Xiao
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Y. Li, F. Kong
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Y. Li, F. Kong, E. Hu, J. Liu, C. Jin, X. Xiao
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