ASPP2 suppresses squamous cell carcinoma via RelA/p65–mediated repression of p63

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Squamous cell carcinoma (SCC) is highly malignant and refractory to therapy. The majority of existing mouse SCC models involve multiple gene mutations. Very few mouse models of spontaneous SCC have been generated by a single gene deletion. Here we report a haploinsufficient SCC mouse model in which exon 3 of the Tp53BP2 gene (a p53 binding protein) was deleted in one allele in a BALB/c genetic background. Tp53BP2 encodes ASPP2 (ankyrin repeats, SH3 domain and protein rich region containing protein 2). Keratinocyte differentiation induces ASPP2 and its expression is inversely correlated with p63 protein in vitro and in vivo. Up-regulation of p63 expression is required for ASPP2Δ exon3+/– BALB/c mice to develop SCC, as heterozygosity of p63 but not p53 prevents them from developing it. Mechanistically, ASPP2 inhibits Np63 expression through its ability to bind Idx8 and enhance nuclear RelA p65, a component of the NF-κB transcription complex, which mediates the repression of p63. Reduced ASPP2 expression associates with tumor metastasis and increased p63 expression in human head and neck SCs. This study identifies ASPP2 as a tumor suppressor that suppresses SCC via inflammatory signaling through NF-κB–mediated repression of p63.

Significance

Squamous cell carcinoma (SCC) is one of the most common human tumor types with high malignancy. Most SCC mouse models involve multiple genetic mutations. Here we present the ASPP2Δ exon3+/- Balb/c mouse as a haploinsufficient SCC mouse model and identify ASPP2 as a suppressor of SCC and a potent repressor of p63 expression. The finding that ASPP2 represses p63 expression via NF-κB provides a molecular insight into how inflammatory signaling may affect SCC development. All these demonstrate that ASPP2 may serve as a molecular signature of SCC and the characterized ASPP2Δ exon3+/- Balb/c mouse may provide a much-needed experimental model to extend our understanding of SCC as well as enable us to develop better strategies to treat SCC.

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of three members: ASPP1, ASPP2, and iASPP. ASPP1 and ASPP2 stimulate, whereas iASPP inhibits, the activities of p53 and its family members p63 and p73 (21–23). Studies with genetically modified mice have shown that ASPP2 is a haploinsufficient tumor suppressor, and that ASPP2 and p53 co-operate to suppress the onset of lymphomas and sarcomas (24, 25). Recent studies have also shown that ASPP2 is a key mediator of RAS-induced senescence, a property independent of p53 (26, 27), suggesting that ASPP2 can suppress tumor growth through p53-dependent and -independent pathways. In contrast to ASPP2, iASPP (the inhibitory ASPP) is mainly expressed in basal epithelial cells. In vitro, iASPP is down-regulated together with p63 upon keratinocyte differentiation. Nuclear iASPP colocalizes with p63 to maintain stratified epithelial homeostasis (28, 29), and is a potent inhibitor of p53, apoptosis, and cellular senescence (23, 28, 30). These findings suggest that the ASPPs may play a key role in epithelial tumor development. We show here that ASPP2Δexon3/+ BALB/c mice represent a haploinsufficient spontaneous SCC mouse model. Mechanistically, ASPP2 suppresses SCC by inducing nuclear p65–mediated repression of p63.

Results

Keratinocyte Differentiation Induces ASPP2 Expression, Which Inversely Associates with p63 Expression in Vivo and in Vitro. To clarify ASPP2’s role in regulating the homeostasis of squamous epithelia, we first examined its expression in the epidermis. ASPP2 was mainly detected in the differentiated layers of human and mouse skin. Double immunofluorescence (IF) staining showed that ASPP2 expression was almost mutually exclusive from that of p63. This was more pronounced in human skin (Fig. L4), which has more extensive stratification than murine skin (Fig. SL4). This expression pattern was also observed in human cervical squamous epithelium (Fig. S1B), in which ASPP2 and iASPP expression was inversely correlated (Fig. 1B). In murine esophageal squamous epithelium ASPP2-expressing cells coexpressed keratin-4, a marker of differentiation, and were almost absent from the proliferative keratin-14 (K14)-expressing basal layer (Fig. S1C). In human esophagus ASPP2 was also mainly detected in differentiated suprabasal epithelial cells (Fig. S1D).

The impact of keratinocyte differentiation on ASPP2 expression was examined in mouse primary keratinocytes (PKs) in vitro. High calcium-induced mouse PK differentiation induced ASPP2 expression, the timing of which coincided with p63 down-regulation, and up-regulation of the differentiation marker envoplakin (Fig. 1C and Fig. S1E). In contrast, iASPP was down-regulated upon PK differentiation (28). These data suggest that ASPP2 may promote epithelial differentiation.

ASPP2Δexon3/+ BALB/c Mice Develop Spontaneous SCC with High Frequency and Early Onset. p53 deficiency caused more epithelial tumors in mice with a BALB/c background than a C57BL6 background (31). We thus examined the tumor suppressive properties of ASPP2 in ASPP2Δexon3/+ and ASPP2Δexon3/+ BALB/c mice (32). The genotype birth ratio followed the expected Mendelian segregation (Fig. S2A). The overall survival rate of ASPP2Δexon3/+ mice was significantly reduced compared with heterozygous and WT littermates, with only approximately 50% surviving to 20 wk of age (Fig. S2B). Two of 13 ASPP2Δexon3/+ mice developed spontaneous SCC at 19 to 20 wk. As a result of early mortality of the ASPP2Δexon3/+ mice, tumor studies were carried out between WT and ASPP2Δexon3/+ BALB/c mice. ASPP2Δexon3/+ BALB/c mice started to develop spontaneous SCC as early as 20 wk of age. Over 80 wk, almost 50% of ASPP2Δexon3/+ mice developed tumors, compared with fewer than 10% of WT mice. The difference in tumor-bearing frequency was statistically significant (P = 0.0002; Fig. 24), whereas tumor frequency in male vs. female ASPP2Δexon3/+ mice was not, suggesting that ASPP2’s tumor-suppressive function has no sex bias (Fig. S2C). All tumors found in ASPP2Δexon3/+ BALB/c mice were well-differentiated squamous cell carcinomas, contrasting with those observed in ASPP2Δexon3/+ WT mice: retinoblastoma (one of 37), lymphomas (two of 37) and carcinoma (one of 37; Fig. 2B). Most of the ASPP2Δexon3/+ BALB/c tumors were located in the neck, abdomen, flanks, and chest (Fig. S2D). Mice were killed when tumors reached approximately 1 cm diameter in size (Fig. S2E). Histological analysis of the ASPP2Δexon3/+ tumors revealed that they all belonged to poorly differentiated SCCs, the main histological characteristics being barrel-shaped masses of tumoral cells (Fig. S2 F, i) and desmoplastic stroma (Fig. S2G). Necrosis was frequently observed in groups of proliferating cells, as seen in Fig. S2 F, i. Poor differentiation was underscored by a high number of pleomorphic cells with vesicular nuclei (Fig. S2 F, i) and keratinous pearls. Invasion of the blood vessels was also found (Fig. S2H). Large numbers of mitotic nuclei were observed, indicating a high proliferative index (Fig. S2I). Immunohistological analysis showed that the majority of tumor cells expressed high K14 and keratin-1 (K1) levels, two well-known markers for SCC, but were negative for vimentin, a mesenchymal cell marker. High levels of nuclear p53 were detected in all SCCs derived from ASPP2Δexon3/+ BALB/c mice (Fig. 2C). Double IF analysis also confirmed that K14, K1, and p63 proteins were coexpressed in the tumor cell population (Fig. S2J), whereas vimentin and keratin-18 were mainly found in the surrounding stromal compartment and normal sweat ducts within the tumor mass, respectively (Fig. S2K). By using antibodies and primers able to distinguish between the two N-terminal p63 isoforms, it was found that tumors analyzed mainly over-expressed ΔNp63 rather than TAp63 (Fig. S2 L and N). These results demonstrate that ASPP2 is a haploinsufficient tumor suppressor, and reduced ASPP2 expression associates with increased ΔNp63 expression.

Heterozygosity of p63, but Not p53, Prevents ASPP2Δexon3/+ BALB/c Mice from Developing SCC. Mutational inactivation of p53 is frequently observed in human SCC (33). ASPP2 was originally identified as an activator of p53 (25) and cooperates with it to suppress tumor growth in vivo (24). Thus, reduced ASPP2 expression could dampen p53’s activity and predispose to SCC. The onset of SCC formation in ASPP2Δexon3/+ BALB/c mice should be induced in a p53+/− background. On the contrary, ASPP2 and p63 expression are mutually exclusive in normal epithelia, and all tumors derived from ASPP2Δexon3/+ BALB/c mice express high levels of nuclear p63. ASPP2 may suppress SCC by repressing p63’s expression in stratified epithelia. Reduced p63 expression might prevent SCC development in ASPP2Δexon3/+ BALB/c mice. To test these hypotheses, ASPP2Δexon3/+ BALB/c mice were backcrossed onto p53−/− mice first and then onto p63−/− mice. The results presented indicate that p63, but not p53, is a major factor in SCC development.
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BALB/c mice develop spontaneous SCC with high frequency and early onset. (A) Tumor-free survival and (B) tumor incidence and spectrum spontaneously developed in ASPP2+/− (WT) and ASPP2Δexon3/+ (Het) BALB/c mice over 88 wk (***p = 0.0002, log-rank (Mantel–Cox) test). (C) Immunohistochemical staining of vimentin, K14, K1, and p63 in tumor sections. (Scale bars: 50 μm.)

mice were crossed with p53+/− or p63+/− BALB/c mice to generate a compound mouse. ASPP2Δexon3+/p53+/+ or ASPP2Δexon3+/-; p63−/+ mice were then intercrossed. As reported by Vives et al., ASPP2Δexon3+/-; p53−/+ mice mainly developed carcinomas (66% carcinomas, 34% sarcomas), and tumor onset was earlier than in ASPP2Δexon3+/p53−/+ mice, but the ASPP2Δexon3Δexon3+Δp53Δ/+ cohort was too small to reach statistical significance as a result of premature lethality. ASPP2Δexon3+/p53+/+ mice had significantly higher carcinoma incidence (90% carcinomas, 10% lymphomas) than ASPP2Δ+/p53−/+ mice and poorer tumor-free survival (P = 0.03). In a p53 heterozygous background, ASPP2Δexon3Δexon3+Δp53Δ/+ mice mainly developed carcinomas (66% carcinomas, 34% sarcomas), and tumor-free survival rate was significantly worse than ASPP2Δ+/Δp53Δ/+ mice (P < 0.00001). ASPP2Δexon3Δexon3+Δp53Δ−/+ mice developed tumors (45% carcinomas, 55% of lymphomas and sarcomas) faster than ASPP2Δ+/+Δp53Δ−/+ mice (P = 0.03). This confirms our earlier finding that ASPP2 and p53 cooperate in tumor suppression. In a p53-null background, ASPP2Δexon3Δexon3+/Δp53Δ−/+ and ASPP2Δ+/Δp53Δ−/+ mice developed lymphomas and sarcomas exclusively with similar onset regardless of ASPP2 status, supporting the notion that p53 is the dominant tumor suppressor and is downstream of ASPP2 (Fig S3 B−G). This study shows that p53 heterozygosity failed to enhance SCC in ASPP2Δexon3Δexon3−/+ BALB/c mice, as the percentage of carcinomas observed in ASPP2Δexon3Δexon3−/+; p53−/+ and ASPP2Δexon3+.Δp53Δ−/+ mice was 26%, 19%, and zero, respectively (Fig. S3C, bars marked by triangles).

The ability of ASPP2 to interact with p63 to suppress SCC was next tested in compound mice obtained from an intercross of ASPP2Δexon3Δexon3−/+; p63−/+ BALB/c mice. Because of the early lethality of p63−/+ and ASPP2Δexon3Δexon3−/+ mice (12, 13, 24), the study was performed in the remaining four genotypes. Remarkably, none of the ASPP2Δexon3Δexon3−/+; p63−/+ mice developed any spontaneous tumor by 80 wk (Fig. 3A), demonstrating that p63 heterozygosity is sufficient to prevent ASPP2Δexon3Δexon3−/+ BALB/c mice from developing spontaneous SCC. Approximately 24% of the ASPP2Δexon3Δexon3−/+; p63Δ−/+ mice developed spontaneous tumors, all of which were SCCs (Fig. 3B and Fig. S3H). Hence, ASPP2 is likely to suppress SCC development by repressing p63 expression.

ASPP2 Represses p63 Expression. Double IF staining of p63 and ASPP2 in SCC tumors from ASPP2Δexon3Δexon3−/+ mice showed very few ASPP2-positive cells (5%) scattered within the tumor mass, whereas most tumor cells expressed p63 (78%; Fig. S4A). These tumors retained the WT ASPP2 allele (Fig. S4B), supporting the notion that ASPP2 is a haploinsufficient suppressor of SCC. Importantly, in ASPP2-enriched tumor regions, ASPP2 and p63 expression were mutually exclusive, and no cells coexpressed ASPP2 and p63 (Fig. 4A). Double IF staining of human cutaneous SCC confirmed that ASPP2 and p63 expression were again mutually exclusive. ASPP2 was expressed in only a few cells within the tumor mass or in residual normal stratified epithelium, whereas most tumor cells expressed p63 (Fig. S4C). The molecular mechanisms controlling ASPP2 and p63’s mutually exclusive expression were analyzed in ASPP2Δ+/+ and ASPP2ΔΔexon3Δexon3−/+ mouse PEs and mouse embryonic fibroblasts (MEFs). In both cell types, the amount of Δ6p63 transcript was much higher than that of TAp63, measured by real-time quantitative PCR (RT-qPCR) using isoform-specific primers (Fig. S4D). An approximately three- or sixfold increase in ΔNp63 mRNA was observed in ASPP2Δexon3Δexon3−/+ compared with ASPP2Δ+/+ PKs and MEFs, respectively, using RT-qPCR. Very little change was detected in TAp63 expression levels under the same conditions (Fig. 4B and Fig. S4 E and F). A small increase in ΔNp63 protein was also observed (Fig. S4G). These findings suggest that ASPP2 may repress p63 expression. To investigate how the expression levels of ASPP2, iASPP, and p63 were analyzed in a panel of 34 human SCC cell lines by immunoblotting. Interestingly, iASPP was expressed at similar levels in nearly all lines tested, most of which also expressed p63, although expression levels varied. Most of the lines expressed low levels of ASPP2, and the mean of p63 expression was significantly different and higher than that of ASPP2 (P = 0.0009; Fig. S4 H and I). An inverse correlation between p63 and ASPP2 expression was also observed in some cell lines (Fig. S4H, red and green frames). The signal intensity of ASPP2 and p63 (normalized by using iASPP levels) was quantified in each cell line and is shown in Fig. S4I. The values obtained from the ratios between p63 and ASPP2 expression levels for each line were used to produce a scatter-plot graph (Fig. S4K). Approximately 22% of the cell lines had similar p63:ASPP2 expression ratios (~1; Fig. S4K, yellow dots), whereas the remaining 78% had imbalanced expression. Most of the SCC cell lines (59%) had a high p63:ASPP2 ratio (Fig. S4K, red dots). In approximately 19% of the lines, values of the p63:ASPP2 ratios were less than 1 (Fig. S4K, blue dots). The cell lines with profoundly imbalanced ASPP2 and p63 expression patterns provided us with an experimental system with which to investigate how ASPP2 may negatively regulate p63 expression in vitro. UPCL-SCC-040 cells (hereafter “040 cells”; SI Materials and Methods) express high levels of p63 in almost all cells, whereas ASPP2 expression is barely detectable (Fig. 4D and Fig. S4H). The 040 cells were used to test whether increased ASPP2 level could repress p63 expression. V5-tagged ASPP2 was transiently transfected into 040 cells, and anti-V5 and anti-p63 antibodies were used for double IF staining to detect the exogenously introduced V5-ASPP2 and endogenous Fig. 2. ASPP2Δexon3Δexon3−/+ BALB/c mice develop spontaneous SCC with high frequency and early onset. (A) Tumor-free survival and (B) tumor incidence and spectrum spontaneously developed in ASPP2+/− (WT) and ASPP2Δexon3/+ (Het) BALB/c mice over 88 wk (***p = 0.0002, log-rank (Mantel–Cox) test). (C) Immunohistochemical staining of vimentin, K14, K1, and p63 in tumor sections. (Scale bars: 50 μm.)

Fig. 3. Heterozygosity of p63 prevents ASPP2Δexon3Δexon3−/+ BALB/c mice from developing SCC. (A) Tumor-free survival and (B) tumor incidence and spectrum in ASPP2+/p63 compound mice with indicated genotypes over 80 wk (**p = 0.0086 by log-rank (Mantel–Cox) test).
p63. In empty vector-transfected V5− cells, fewer than 1% of cells lacked p63 expression. Remarkably, in approximately 70% of transfected cells expressing detectable ASPP2-V5, endogenous p63 expression was undetectable. Exogenously expressed iASPP-V5 failed to affect endogenous p63 expression, with only approximately 2% of iASPP-V5–expressing cells losing endogenous p63 expression (Fig. 4 C and D). Similar results were obtained in another human SCC cell line, HSC3 (Fig. S4L). A reduction of ΔNp63 mRNA of approximately 40% was detected in 040 cells upon transient expression of ASPP2-V5 (Fig. S4M). The ability of increased iASPP or reduced ASPP2 expression to influence p63 expression was further tested in the immortalized human keratinocyte cell line HaCat. As earlier, iASPP overexpression did not affect p63 expression, whereas ASPP2 RNAi induced endogenous p63 expression in HaCat cells (Fig. S4 N and O). These data illustrate that ASPP2 but not iASPP is able to specifically repress ΔNp63 expression.

**ASPP2 Represses p63 Expression by Binding IκB and Inducing Nuclear p65/NF-κB.** We showed previously that ASPP2 partially overlaps with β-catenin at cell–cell junctions and can be detected in the same protein complex (32). A more recent study showed that nuclear β-catenin can directly transactivate ΔNp63 expression (34). We tested whether ASPP2 could repress p63’s expression by inhibiting β-catenin–mediated induction of ΔNp63. The expression of endogenous p63 was assessed by IF staining of 040 cells transfected with plasmids expressing ASPP2 or β-catenin constitutively active mutant (ΔN89 β-catenin), separately or together. As expected, exogenous ASPP2 expression repressed endogenous p63 expression. Expression of ΔN89 β-catenin alone failed to affect p63 expression and when coexpressed with ASPP2, it failed to prevent ASPP2 from repressing p63’s expression, even though it was detected as a nuclear protein in the same cells in which ASPP2-V5 was expressed. Endogenous nuclear β-catenin expression was almost undetectable in 040 cells (Fig. S5A). These data suggest that the ability of ASPP2 to repress p63 expression is likely to be independent of nuclear β-catenin.

The Notch signaling pathway is one of the main pathways that negatively regulate p63’s expression in stratified epithelial cells (11). Notch1 is under positive transcriptional regulation by p53 (35) and is mainly expressed in the differentiated layers of the epithelium. Whether Notch1 could mediate ASPP2-induced p63 repression was tested by using Notch1 RNAi with an ASPP2-V5 expression plasmid. The presence of Notch1 RNAi almost abolished Notch1 expression, but failed to influence ASPP2’s ability to repress p63 expression in 040 cells (Fig. S5 B and C), suggesting that ASPP2 inhibits p63 expression independently of Notch1.

RelA/p65 has been shown to be nuclear in differentiated, but cytoplasmic in basal p63+, epidermal layers (6). RelA/p65 can also repress ΔNp63 expression in a transcription-dependent and -independent manner in epithelial cells (10, 11). As ASPP2 is mainly expressed in the differentiated layers of the epithelium, and a known binding protein of RelA/p65 (36), we tested its ability to repress p63 expression by binding and activating RelA/p65. Endogenous RelA/p65 was mainly cytoplasmic in 040 cells. Double IF staining showed that exogenously expressed cytoplasmic ASPP2-V5 induced nuclear accumulation of RelA/p65 in approximately 66% of ASPP2-V5–expressing cells, compared with fewer than 5% of empty vector-transfected V5− cells (Fig. 5A). RelA/p65 was observed in approximately 64% of cells (Fig. 5B). Knockdown of RelA/p65 by RNAi largely prevented ASPP2-V5-induced nuclear accumulation of p63 in 040 cells (Fig. 5C). The percentage of ASPP2-V5–expressing cells was more than 60% in control RNAi-transfected cells, but reduced to approximately 20% in RelA/p65 RNAI-transfected cells (Fig. 5B). ASPP2-V5 was also transfected into 040 cells alone, or in the presence or absence of IκBα, an inhibitor that binds and retards RelA/p65 in the cytoplasm to inhibit its transcriptional activity. When expressed alone, only 2% of transfected cells had reduced p63 expression. As before, ASPP2 reduced p63 expression in approximately 70% of transfected cells when IκBα was expressed with ASPP2-V5, down-regulating p63 was observed in only 25% of cotransfected cells (Fig. 5C and Fig. S5D), an effect only observed in cells expressing high IκBα levels (Fig. S5D, yellow arrows). In low IκBα-expressing cells, ASPP2 still repressed p63 expression (Fig. S5D, white arrows). These results suggest that ASPP2 may counteract IκBα to induce nuclear RelA/p65 to repress p63’s expression. It was confirmed that ASPP2 status has minimal impact on RelA/p65 and IκBα expression in ASPP2+/− and ASPP2Δ exon3/Δ exon3 MEFs (Fig. S5E). Interestingly, an anti-ASPP2 antibody immunoprecipitated IκBα and p65/NF-κB in ASPP2+/− MEFs (Fig. S5F and G). We next examined whether ASPP2 expression was able to complex with ASPP2 in a reciprocal immunoprecipitation assay (Fig. S5 F and G). IκBα immunoprecipitated with RelA/p65 and p105/p50, but not with β-catenin (Fig. S5F) or IκBα (Fig. S5H) under the same conditions. The amount of the IκBα–RelA/p65 complex detected in ASPP2Δ exon3/Δ exon3 MEFs was visibly higher than that in ASPP2+/− MEFs (Fig. S5E and Fig. S5H). These results suggest that ASPP2 may repress p63’s expression by competing with RelA/p65 to bind IκBα, thus inducing RelA/p65’s nuclear accumulation.

**Down-Regulation of ASPP2 Associates with Increased p63 Expression and Tumor Metastasis in Human Head and Neck SCC.** In human samples, ASPP2’s expression was mainly confined to adjacent normal squamous epithelium, and was strongly decreased in the neighboring tumor mass (Fig. 6A). ASPP2 expression was subsequently examined in a cohort of 318 human head and neck SCCs...
Fig. 5. ASPP2 inhibits p63 by inducing nuclear p65/NFκB. (A) Double IF staining of 040 cells using anti-V5 (red) and anti-RelA/p65 (green) antibodies. Arrows label cells expressing ASPP2-V5 and nuclear RelA/p65. The graph shows the percentage of nuclear RelA/p65 expressing cells in indicated transfected samples (**P < 0.001). (B) Triple IF staining of 040 cells to detect transfected ASPP2-V5 (red), endogenous RelA/p65 (green), and endogenous p63 (magenta) in cells treated with control (CTR) or RelA/p65 (NFκB) RNAi. Arrows label ASPP2-V5-expressing cells. The bar graph shows the percentage of V5/p63+ cells in transfected samples as indicated. (**P = 0.0083). (C) Graph shows the percentage of cells with low or undetectable p63 in transfected samples as indicated. (**P = 0.001). (D and E) Lysates from ASPP2 WT and Δexon3 MEFs were immunoprecipitated with a control IgG (IP CTR) or indicated antibodies (IP ASPP2, IP IκB) and immunoblotted with antibodies as labeled. (Scale bars: 10 μm). Bar graph values are the mean ± SD from three different experiments.

Fig. 6. ASPP2 expression is decreased in human SCC samples. (A) ASPP2 immunostain of human HNSCC. Areas of normal epithelium and tumor mass are shown at higher magnification. (Scale bars: 50 μm and 10 μm.) (B) Diagram to summarize the interplay between ASPP2, IκB, and RelA/p65 in regulating ΔNp63 expression.
SCC cells, whereas p63 and RelA/p65 are predominantly nuclear, the previously identified protein/protein interactions are unlikely to mediate ASPP2-induced repression of p63 expression and nuclear RelA/p65 induction. Cytoplasmic ASPP2 is more likely to function through its ability to bind IkB in the cytoplasm, and regulate the nuclear localization of RelA/p65 and p63’s expression (Fig. 6B). Overexpressing IkB can bypass ASPP2’s ability to repress p63 expression in the SCC cell line 040, consistent with the notion that constitutive IkB expression can block NF-κB signaling and induce SCC in vivo (6). Indeed, in suprabasal epithelial cells, ASPP2 cytoplasmic expression corresponds with nuclear expression of RelA/p65. The finding that ASPP2 uses the IkB-RelA/p65 pathway to repress p63’s expression is of particular interest in light of the observed difference in ASPP2’s tumor suppressive function according to mouse strain used. Emerging evidence indicates differences between BALB/c, 129SvJ, and C57BL6 mice in their inflammatory response, such as their ability to heal corneal epithelia wounds (38). Thus, future investigations may elucidate whether differences in NF-κB signaling in the aforementioned mouse strains may influence ASPP2’s tumor-suppressive function.

Finally, the observation that reduced ASPP2 expression is strongly associated with metastatic human HNSCCs agrees with a previous report of reduced ASPP2 mRNA expression in human breast cancer metastases (39). This finding also agrees with the ASPP2 expression in BALB/c mouse model, in which reduced levels of ASPP2 are sufficient to cause undifferentiated and often invasive spontaneous SCC. Together, these studies show that ASPP2 is a key haplosufficient tumor suppressor of SCC.

Materials and Methods

ASPP2 Δexon3 C57BL6/Jx129SvJ mice (26) were backcrossed in a BALB/c background for nine generations. pS3Δκ− and p63Δκ− BALB/c mice were generated by G.L. and F.D.M., respectively. All animal procedures were approved by the University of Oxford’s ethical review committee and licensed by the UK Home Office (license number PPL 302862). Human tumor samples were obtained with full consent. Cell-culture experiments were performed by using MEF's, PKs, or SCC cell lines. More details are provided in SI Materials and Methods.

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