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CORRESPONDENCE

Altered nucleocytoplasmic staining patterns of p62/SQSTM1 in cutaneous squamous cell carcinoma precursors

Autophagy is an evolutionarily conserved adaptive process that mediates a myriad of stress responses.[1] The autophagy cargo receptor, p62/sequestosome 1, shuttles between the cytoplasmic and nuclear components of the cell and promotes autophagic degradation of ubiquitinated proteins.[1,2] Similar to other genes such as tumor protein p53 (TP53) or nuclear factor erythroid 2-related factor 2 (NRF2) that play a role in stress response,[3] p62 is involved in malignant progression.[2,3] Decreased p62 expression levels can indicate the progression of oral squamous cell carcinoma (oSCC)[4] or cutaneous squamous cell carcinoma (cSCC).[5] The prognostic value of decreased nuclear p62 expression levels in oSCC was stressed,[4] suggesting that SCC malignant progression involves aberrant p62-mediated DNA damage responses.[1,2] Actinic keratosis (AK) or Bowen's disease (BD) is distinctive etiologies that may be precursors of cSCCs.[6–8] AK patients obviously exhibit aberrant TP53 nuclear accumulation, whereas BD patients do not,[9] indicating the presence of altered p62 nucleocytoplasmic shuttling states in these conditions. To address this, we analyzed the p62 expression profile in the cytoplasmic/nuclear compartments of cSCC and its precursors (Supporting information).[9]

A total of 18 AK, 25 BD, 26 cSCC (originated from 6 AK and 20 non-AK),[9] and 19 normal skin samples were included in this study. Most specimens from the normal skin group exhibited moderate nuclear and cytoplasmic p62 expression. The AK and cSCC groups exhibited the highest and lowest staining levels in the nucleus, respectively (Figure 1), suggesting that the malignant progression of cSCC is accompanied by decreased nuclear p62 expression levels.[4] In terms of cytoplasmic staining, the BD group showed a greater degree of staining, exhibiting substantially higher expression levels than the normal skin, whereas the AK group did not

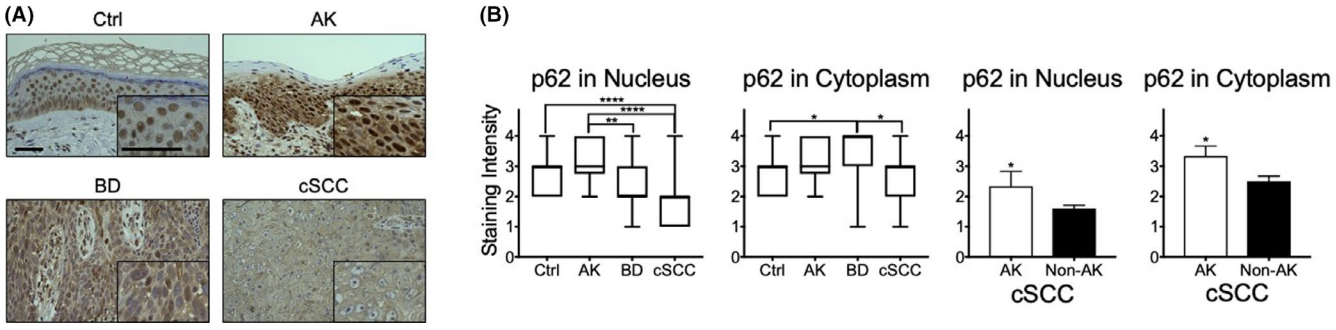

FIGURE 1 p62 nucleocytoplasmic expressions profiles in cSCC and precursors. A, Representative immunohistochemical images of p62 in healthy control (Ctrl), actinic keratosis (AK), Bowen's disease (BD), or cutaneous squamous cell carcinoma (cSCC) samples. Bar = 50 μm. B, Staining intensity analysis of p62 in the nucleus and the cytoplasm in four groups. AK exhibits the highest p62 expression levels in the nucleus, whereas cSCC exhibits the lowest. Both AK and BD show stronger p62 expression in cytoplasm compared to Ctrl and cSCC. AK-associated cSCC has stronger p62 expression in both the nucleus and cytoplasm compared to non-AK counterpart. The staining intensity of the nucleus and cytoplasm is as follows: Grade 1, none; Grade 2, weak; Grade 3, strong; and Grade 4, very strong. Ctrl, $n = 19$; AK, $n = 18$; BD, $n = 25$; cSCC, $n = 26$ (originated from AK, $n = 6$; from non-AK, $n = 20$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.001$, one-way ANOVA or unpaired *t*-test.

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(Figure 1). Intriguingly, a cSCC subgroup analysis revealed that the AK-associated cSCC group[9] had stronger p62 expression levels in both cellular compartments than the non-AK-associated counterpart (Figure 1).

In line with previous reports, we found that reduced p62 expression levels were associated with SCC progression.[4,5] This agrees with the results of an experiment suggesting that autophagy protects against carcinogenesis with concomitant emergence of p62-positive cytoplasmic inclusion bodies and DNA damage.[2] Nonetheless, it is worth noting that cSCC precursors exhibited distinctive staining patterns. The BD group had lower nuclear p62 expression levels than the AK group, whereas cytoplasmic expression levels were comparable. Given that cumulative ultraviolet radiation causes DNA damage in AK,[6] the strong nuclear p62 signal may indicate a high tumor mutation burden that also activates TP53/NRF2-mediated stress responses.[3] In contrast, infection with oncogenic human papilloma viruses (HPVs) hampers the anti-tumor/pro-apoptotic activity of the tumor suppressor TP53.[8] Because HPV infection underlies BD pathogenesis,[10] the low nuclear and high cytoplasmic p62 signal in the BD group may reflect distinctive etiopathologies. Given that p62 nucleocytoplasmic shuttling constitutes an inherent DNA damage response,[3] the distinctive nucleocytoplasmic expression patterns among the cSCC precursors could reflect alterations in p62-mediated biological responses.[3] In addition to the prognostic value of p62,[4,5] our findings may provide insight into the cutaneous carcinogenesis of distinctive etiologies.[6]

DECLARATION SECTION

Approval of the research protocol: All procedures were approved by the Tsukuba University Hospital Ethics Committee.

Informed Consent: Patients diagnosed at Tsukuba University Hospital were included in this study with written informed consent.

Registry and the Registration No: N/A

Animal Studies: N/A

CONFLICT OF INTEREST

The author declared no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.