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ROCK-2 activation induces malignant conversion in ras<sup>Ha</sup>-mediated transgenic mouse skin carcinogenesis via p53 loss, elevated NFκB and tenascin C-associated rigidity; but p21 inhibits early-stage progression.

To study mechanisms of tumour progression, transgenic mice that expressed a 4-hydroxytamoxifen (4HT)-activated human ROCK 2-estrogen receptor fusion transgene from a keratin 14 promoter [K14.ROCK<sup>er</sup>] were crossed to mice expressing activated ras<sup>Ha</sup> exclusively in epidermal transit amplifying keratinocytes [HK1.ras<sup>1205</sup>]. 4HT-treatments of K14.ROCK<sup>er</sup> mice [3/wk; 26 wks] induced epidermal and follicular hyperplasia but no papillomas; whilst untreated K14.ROCK<sup>er</sup>-HK1.ras<sup>1205</sup> cohorts exhibited papillomas similar to HK1.ras<sup>1205</sup> controls [16wks]. In contrast by 8 weeks, 4HT-treated K14.ROCK<sup>er</sup>-HK1.ras<sup>1205</sup> histotypes comprised a mixed papilloma/well-differentiated squamous cell carcinoma (wdSCC) that exhibited p53 loss, beginning in papilloma basal layers leading to increased proliferation. In addition papilloma histotypes also exhibited novel, ROCK<sup>er</sup>-associated NF-κB expression in basal layer keratinocytes, prior to malignant conversion. By 12 weeks, K14.ROCK<sup>er</sup>-HK1.ras<sup>1205</sup> wdSCCs exhibited further increases in NF-κB expression together with the appearance of tenasin C expression, an extracellular matrix molecule indicative of elevated rigidity; yet despite continued ROCK2 activities, progression to SCC required compensatory p21 expression. K14.ROCK<sup>er</sup>-HK1.ras<sup>1205</sup> papillomatogenesis also required a wound-promotion stimulus, confirmed by breeding K14.ROCK<sup>er</sup> into promotion-insensitive HK1.ras<sup>1276</sup> mice, suggesting a permissive K14.ROCK<sup>er</sup>-mediated malignant conversion [p-Mypt1/actinomyosin-mediated mechano-transduction-tenascin C/rigidity]. Malignancy depended on ROCK expression, as cessation of 4HT-treatment induced a p21-associated differentiation in wdSCC and appearance of novel papilloma outgrowths expressing intense, basal-layer p21 which confined endogenous ROCK2/p-Mypt1/NF-κB to supra-basal layers, and restored basal-layer p53. In later SCCs, 4HT-cessation became irrelevant as endogenous ROCK2 expression increased, driving progression via p21 loss, elevated NF-κB and tenasin C-associated rigidity, with p-Mypt1/actinomyosin-mediated contractility to facilitate invasion. Thus, ROCK2 activation induces malignancy in ras<sup>Ha</sup>-initiated/promoted papillomas in the context of p53 loss, increased proliferation, and novel NF-κB expression; whilst increased rigidity was associated with conversion and progression. However, p21 inhibition of early-stage malignant progression and intense expression in papilloma outgrowths identifies a significant antagonism between p21 and ras<sup>Ha</sup>/ROCK2/NF-κB signalling in skin carcinogenesis.