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ROCK-2 activation induces malignant conversion in *ras^{Ha}*-mediated transgenic mouse skin carcinogenesis via p53 loss, elevated NFκβ 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^{er}*] were crossed to mice expressing activated ras^{Ha} exclusively in epidermal transit amplifying keratinocytes [*HK1.ras^{1205}*]. 4HT-treatments of *K14.ROCK^{er}* mice [3/wk; 26 wks] induced epidermal and follicular hyperplasia but no papillomas; whilst untreated *K14.ROCK^{er}-HK1.ras^{1205}* cohorts exhibited papillomas similar to *HK1.ras^{1205}* controls [16wks]. In contrast by 8 weeks, 4HT-treated *K14.ROCK^{er}-HK1.ras^{1205}* 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^{er}-associated NF-κβ expression in basal layer keratinocytes, prior to malignant conversion. By 12 weeks, *K14.ROCK^{er}-HK1.ras^{1205}* wdSCCs exhibited further increases in NF-κβ expression together with the appearance of tenascin C expression, an extracellular matrix molecule indicative of elevated rigidity; yet despite continued ROCK2 activities, progression to SCC required loss of compensatory p21 expression. *K14.ROCK^{er}-HK1.ras^{1205}* papillomatogenesis also required a wound-promotion stimulus, confirmed by breeding *K14.ROCK^{er}* into promotion-insensitive *HK1.ras^{1276}* mice, suggesting a permissive *K14.ROCK^{er}-HK1.ras^{1205}* papilloma context [wound-promoted/NF-κβ^{+ve}/p53^{-ve}/p21^{+ve}] preceded *K14.ROCK^{er}*-mediated] malignant conversion [p-Mypt1/ actinomyosin-mediated mechano-transduction-tenascin C/rigidity]. Malignancy depended on ROCK^{er} 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-κβ 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-κβ and tenascin C-associated rigidity, with p-Mypt1/actinomyosin-mediated contractility to facilitate invasion. Thus, ROCK2 activation induces malignancy in ras^{Ha}-initiated/promoted papillomas in the context of p53 loss, increased proliferation, and novel NF-κβ 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^{Ha}/ROCK2/NF-κβ* signalling in skin carcinogenesis.