TUMOUR BIOLOGY

Senescence in premalignant tumours

Oncogene-induced senescence is a cellular response that may be crucial for protection against cancer development[1,2], but its investigation has so far been restricted to cultured cells that have been manipulated to overexpress an oncogene. Here we analyse tumours initiated by an endogenous oncogene, *ras*, and show that senescent cells exist in premalignant tumours but not in malignant ones. Senescence is therefore a defining feature of premalignant tumours that could prove valuable in the diagnosis and prognosis of cancer.

We used a mouse model for cancer initiation in humans: the animals have a conditional oncogenic K-*rasV12* allele that is activated only by the enzyme Cre recombinase[3], causing them to develop multiple lung adenomas (premalignant tumours) and a few lung adenocarcinomas (malignant tumours). Senescence markers previously identified in cultured cells were used to detect oncogene-induced senescence in lung sections from control mice (expressing Cre) and from K-*rasV12*-expressing mice (expressing Cre and activated K-*rasV12*). We analysed $p16^{INK4a}$, an effector of *in vitro* oncogene-induced senescence[1], and *de novo* markers that we identified by using DNA microarray analysis of *in vitro* oncogene-induced senescence (see supplementary information). These *de novo* markers are $p15^{INK4b}$ (also known as CDKN2B), Dec1 (BHLHB2) and DcR2 (TNFRSF10D). In addition, we looked for two features evident in cultured senescent cells, namely the expression of senescence-associated β-galactosidase[4] and the presence of senescence-associated heterochromatin foci[5].

Staining with antibodies against $p16^{INK4a}$, $p15^{INK4b}$, Dec1 and DcR2 revealed abundant positive cells in adenomas, whereas adenocarcinomas were essentially negative (Fig. 1a). By contrast, the proliferation marker Ki-67 revealed a weak proliferative index in adenomas compared with adenocarcinomas (Fig. 1a). Lung cryosections from K-*rasV12* mice stained for senescence-associated β-galactosidase gave an intense signal in the adenomas, whereas adenocarcinomas gave a weak or negative signal (Fig. 1b). Adenomas were also strongly positive for HP1-γ, which indicates the formation of senescence-associated heterochromatin foci[5], whereas adenocarcinomas were negative (Fig. 1c). These results were consistently found when using K-*rasV12* mice carrying Cre transgenic alleles that were expressed either inducibly by tamoxifen (Cre-ER) or constitutively (CMV-Cre)[3]; 5–10 mice were analysed for each marker. The results have also been confirmed by immunoblotting and by quantitative real-time polymerase chain reaction with reverse transcription (see supplementary information; these analyses also included $p19^{ARF}$, an effector of senescence-associated β-galactosidase[6]).

Extending these observations, we combined the K-*rasV12* allele with a transgenic Cre allele that targets the expression of the oncogene to the pancreas. These compound mice develop premalignant lesions (pancreatic intraductal neoplasias) that progress into malignant ductal adenocarcinomas (C.G., A.J.S. and M. Barbacid, unpublished results). As in lung adenomas, these premalignant lesions were positive for our oncogene-induced senescence markers, whereas ductal adenocarcinomas were negative. Similarly, chemically induced skin papillomas, which harbour H-*ras* oncogenic mutations, were also positive for oncogene-induced senescence markers (see supplementary information).

We infer from our findings that oncogene-induced senescence may help to restrict tumour progression. In most cells, oncogenic K-*ras* signalling is attenuated and is therefore not sufficient to trigger tumour formation or senescence[3,7]. Presumably, rare cells that do not fully attenuate oncogenic Ras are at the origin of both premalignant and malignant tumours. We conclude that a substantial number of cells in premalignant tumours undergo oncogene-induced senescence, but that cells in malignant tumours are unable to do this owing to the loss of oncogene-induced senescence effectors such as $p16^{INK4a}$ or p53.

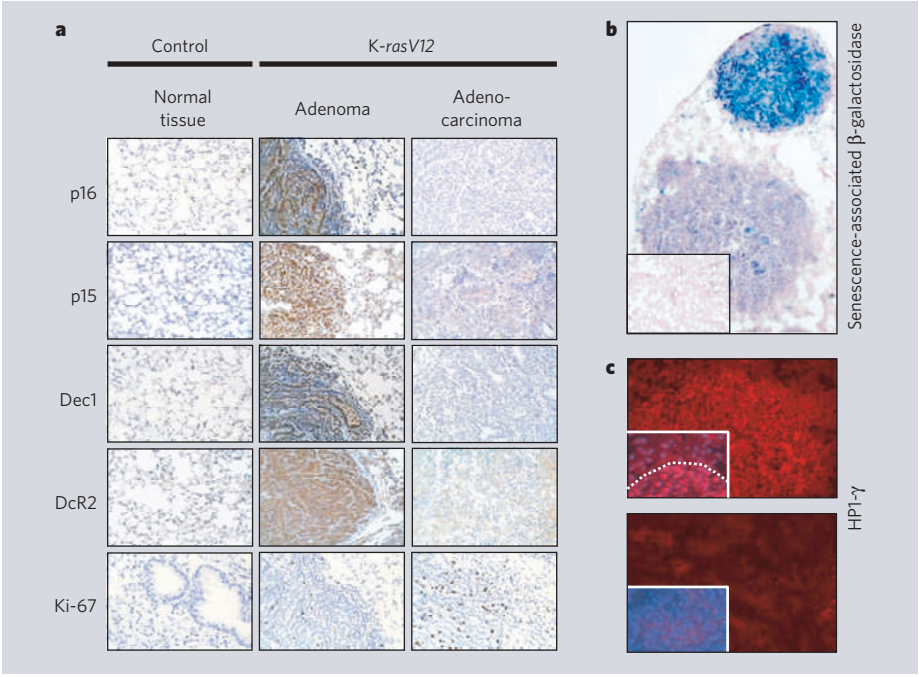

Figure 1 | Premalignant lung adenomas induced by oncogenic K-*ras* are positive for markers of senescence, whereas malignant adenocarcinomas are negative. **a,** Immunohistochemical analysis of the different tissue types for the indicated proteins. **b,** Senescence-associated β-galactosidase expression. A lung section is shown that contains one adenoma (top, blue stained) and one adenocarcinoma (bottom). Inset, negative control. **c,** Immunofluorescence using anti-HP1-γ staining of adenoma (top) and adenocarcinoma (bottom). Insets, double staining with anti-HP1-γ (red) and DAPI (4,6-diamino-2-phenylindole, which stains cell nuclei) (blue). Dotted line, boundary between adenoma and normal tissue. (See supplementary information for further details.)

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