Senescence surveillance maintains genomic and functional differentiation in pre-malignant tumors

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Introduction Different animal models as well as histological studies using samples from cancer patients clearly showed that cellular senescence, in particular cytokine-induced senescence (CIS), is a barrier against tumor progression (1-3). Yet, recent data question this concept suggesting that senescent cells may also promote tumor progression (4, 5). By adopting a senescence-associated secretory phenotype (SASP), senescence signaling may even induce dedifferentiation and stemness (5). To shine more light on this controversial question, we investigated the effects of CIS on tumor progression and differentiation. For this, we used pre-malignant β -cancers from RIP-Tag2 mice that express three differentiation antigens with physiological functions: synaptophysin, insulin and glucose transporter-2 (Glut-2), and treated the mice with Tag-specific T helper 1 (T_H1) cells to induce CIS *in vivo* (Fig. 1).

Results First, we analyzed the genomic stability of the RIP-Tag2 cancers by comparative genomic hybridization (CGH). We show that T_H1 -cell-mediated immunity prevented the transition of pre-cancerous adenomas into β -cancers by inhibition of tumor growth and stabilization their genome. Thus, CIS stopped proliferation (Fig. 2, left) and simultaneously prevented major genomic aberrations, i.e. chromosomal amplifications and losses that repeatedly occurred in RIP-Tag2 cancers of sham-treated mice (Fig. 2, right). To study the differentiation status of RIP-Tag2 cancers, we set up an immunofluorescence method to analyze the expression of the differentiation antigens. Tumor progression caused a gradual loss of the functionally relevant differentiation markers insulin and Glut-2 whereas synaptophysin remained constant (Fig. 3, left). Importantly, CIS prevented the loss of insulin and Glut-2 *in vivo* (Fig. 3, right).

To demonstrate that the T_H1 -cell mediated effects on the differentiation state of RIP-Tag2 cancer cells are mediated by the T_H1 cell cytokines interferon- γ (IFN- γ) and tumor necrosis factor (TNF), we treated isolated β -cancer cells with recombinant cytokines *in vitro*. Longterm treatment of RIP-Tag2 cancer cells with a cytokine cocktail of IFN- γ and TNF indeed retained the expression of insulin and Glut-2 *in vitro* (Fig. 4) thereby mimicking the effect of T_H1 cells *in vivo*.

Conclusion Our data clearly show that immunity-induced senescence surveillance protects against dedifferentiation and loss of physiological function during development of endocrine tumors.

Fig. 1: Transfer of T_H1 cells. RIP-Tag2 is a murine tumor model. Transfer of T_H1 cells inhibits tumor growth and prolongs the life span of the mice.

Fig. 2: T_H1 cells stop tumor growth and genomic chaos. Proliferation of sham- and Tag- T_H1 -treated cancers was analyzed by Ki67 (left), and genomic stability by CGH (right).

Fig. 3: T_H1 cells stop dedifferentiation of RIP-Tag2 tumors. Staining for synaptophysin, insulin or Glut-2 during tumor development (left), and in cancers isolated from sham- or T_H1 -treated mice (right).

Fig. 4: IFN- γ and TNF keep β -cancer cells in a differentiated state. Staining for synaptophysin, insulin or Glut-2, and quantitative analysis of medium (Ctrl.) or IFN- γ + TNF-treated β -cancer cells.

References (1) Michaloglou et al, (2005) Nature 436: 720. (2) Braumüller et al, (2013) Nature 494: 361. (3) Wieder et al, (2017) Cancer Metastasis Rev 36: 357. (4) Campisi, (2013) Annu Rev Physiol 75: 685. (5) Milanovic et al, (2018) Nature 553: 96.