LETTER

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Senescence–associated reprogramming promotes cancer stemness

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Cellular senescence is a stress-responsive cell-cycle arrest program that terminates the further expansion of (pre-)malignant cells[1,2]. Key signalling components of the senescence machinery, such as $p16^{INK4a}$, $p21^{CIP1}$ and p53, as well as trimethylation of lysine 9 at histone H3 (H3K9me3), also operate as critical regulators of stem-cell functions (which are collectively termed 'stemness')[3]. In cancer cells, a gain of stemness may have profound implications for tumour aggressiveness and clinical outcome. Here we investigated whether chemotherapy-induced senescence could change stem-cell-related properties of malignant cells. Gene expression and functional analyses comparing senescent and non-senescent B-cell lymphomas from Eμ-*Myc* transgenic mice revealed substantial upregulation of an adult tissue stem-cell signature, activated Wnt signalling, and distinct stem-cell markers in senescence. Using genetically switchable models of senescence targeting H3K9me3 or p53 to mimic spontaneous escape from the arrested condition, we found that cells released from senescence re-entered the cell cycle with strongly enhanced and Wnt-dependent clonogenic growth potential compared to virtually identical populations that had been equally exposed to chemotherapy but had never been senescent. *In vivo*, these previously senescent cells presented with a much higher tumour initiation potential. Notably, the temporary enforcement of senescence in p53-regulatable models of acute lymphoblastic leukaemia and acute myeloid leukaemia was found to reprogram non-stem bulk leukaemia cells into self-renewing, leukaemia-initiating stem cells. Our data, which are further supported by consistent results in human cancer cell lines and primary samples of human haematological malignancies, reveal that senescence-associated stemness is an unexpected, cell-autonomous feature that exerts its detrimental, highly aggressive growth potential upon escape from cell-cycle blockade, and is enriched in relapse tumours. These findings have profound implications for cancer therapy, and provide new mechanistic insights into the plasticity of cancer cells.

Cellular senescence, which is implemented in response to severe cellular insults such as oncogenic activation or chemotherapeutic DNA damage, is a failsafe program that protects organismic integrity by excluding potentially harmful cells from further expansion[2,4], and also has a physiological function in tissue homeostasis during organ development[1]. Senescence has been shown to cancel the pro-tumorigenic potential of Ras-/Raf-driven (pre-)cancerous lesions[5–7], and to contribute to the outcome of anticancer chemotherapy *in vivo*[8,9].

Notably, stem-cell functions, collectively referred to as 'stemness'[3], and senescence seem to be co-regulated by overlapping signalling networks. Key senescence-relevant signalling molecules (for example, Bmi-1, $p16^{Ink4a}$, $p21^{Cip1}$ or p53) have critical roles in stem-cell maintenance by preventing premature exhaustion (reviewed in ref. 3). Senescence-enforcing *p53* (also known as *Trp53*)-, *Cdkn2a* (also known as *Ink4a* or *Arf*)- or *Suv39h1*-encoded gene products raise an initial barrier to the efficient conversion of normal cells into induced pluripotent stem cells (see refs 10, 11, and references therein), suggesting an underexplored interplay between senescence- and stemness-controlling signalling networks. Trimethylation of H3K9, as mediated by the H3K9 methyltransferase Suv39h1 (ref. 12), confers senescence by establishing a transcriptionally repressive heterochromatin mark in the vicinity of S-phase-relevant E2F target genes[6,9,13], and reflects an epigenetic principle linked to induced pluripotent stem cell reprogramming[14]. Using a cancer-unrelated, inducible reprogramming mouse model in which many cells primarily senesced, previous studies have shown that factors secreted from these senescent cells facilitated the reprogramming of their neighbours[15,16]. Whether the senescence condition promotes cancer stemness, especially in a cell-autonomous manner, is not known. Although a permanent senescent cell-cycle block is per se incompatible with self-renewal, we report here the senescence-evoked cell-intrinsic reprogramming of cancer cells into a stem-like state, and the acquisition of tumour-initiating potential after their forced release or spontaneous escape from a chemotherapy-induced senescent cell-cycle arrest.

As indicated by their strong senescence-associated β-galactosidase (SA-β-gal) activity and other previously demonstrated markers of senescence, primary Eμ-*Myc* transgenic *Bcl2*-overexpressing lymphomas (hereafter referred to as control;*Bcl2* lymphomas) serve as a well-established model for therapy-induced senescence (TIS)[8,9]. First, we analysed stem-cell-related transcripts in the gene expression profiles of 12 matched pairs of primary control;*Bcl2* lymphomas that either entered TIS after *in vitro* exposure to the chemotherapeutic agent Adriamycin (ADR) or remained untreated. Using gene set enrichment analysis (GSEA), a previously established adult tissue stem-cell (ATSC) signature[17] was strongly skewed towards the TIS group, but was not found to be enriched in the equally ADR-treated but senescence-incapable group of Suv39h1-deficient Eμ-*Myc*;*Bcl2* (that is, *Suv39h1*$^{-}$;*Bcl2*) lymphomas[9] (Fig. 1a and Extended Data Fig. 1a, b). Almost the entire population turned double-positive for the stem-cell antigen Sca1 and the senescence marker H3K9me3 upon senescence induction (Fig. 1b, top). Furthermore, TIS cells, unlike non-senescent

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