Therapy Induced Senescence

Subjects: Oncology

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Cellular senescence participates to fundamental processes like tissue remodeling in embryo development, wound healing and inhibition of preneoplastic cell growth. Most senescent cells display common hallmarks, among which the most characteristic is a permanent (or long lasting) arrest of cell division. However, upon senescence, different cell types acquire distinct phenotypes, which also depend on the specific inducing stimuli. Senescent cells are metabolically active and secrete a collection of growth factors, cytokines, proteases, and matrix-remodeling proteins collectively defined as senescence-associated secretory phenotype, SASP. Through SASP, senescent cells modify their microenvironment and engage in a dynamic dialog with neighbor cells. Senescence of neoplastic cells, at least temporarily, reduces tumor expansion, but SASP of senescent cancer cells as well as SASP of senescent stromal cells in the tumor microenvironment may promote the growth of more aggressive cancer subclones.

Keywords: senescence ; cancer therapy ; Senescence-Associated Secretory Phenotype (SASP) ; cancer cell ; tumor vasculature

1. Introduction

Cellular senescence is a stereotyped response to persisting cellular damages which reduce cell fitness [1]. The salient phenotypes of senescent cells are arrest of cell division and secretion of a collection of growth factors, cytokines and proteases collectively defined as Senescence-Associated Secretory Phenotype, SASP [2]. Through SASP, senescent cells modify their microenvironment and engage in a dynamic dialog with neighbor cells. Although cell senescence is generally considered irreversible, there are instances in which cells, particularly cancer cells with mutations in tumor suppressor genes, may escape senescence [3] (reviewed in [4]).

Cellular senescence requires a substantial alteration of gene expression driven by epigenetic remodeling of chromatin and is re-enforced by autocrine signaling from SASP components. Common markers of cell senescence are high expression of the cyclin-dependent kinase inhibitors p16INK4a and p21, lack of expression of the cell-cycle associated Ki67 protein, reduction of Lamin B1, and elevated level of trimethylated histone 3 lysine 9 (H3K9me3) often organized in foci, the Senescence-Associated Heterochromatin Foci, SAHF. Senescent cells show a flattened morphology with an enlarged lysosomal compartment accompanied with the expression of senescence-associated β-galactosidase (SA-β-gal) expression. Of note, not every senescent cell necessarily expresses all these markers, and many of them may be present in non-senescent cells.

Together with programmed cell death, apoptosis, senescence limits the expansion of mutated pre-neoplastic cells. The main functional difference between these two cellular programs is that apoptotic cells are disposed without affecting neighbor cells or causing inflammation, while senescent cells persist, have a vigorous metabolism and act locally, and sometimes systemically, via their SASP. The main cell-autonomous mechanisms that induce senescence of preneoplastic cells are telomeres attrition, which occurs upon clonal expansion in the absence of a functioning telomerase [5] and oncogene-induced senescence, OIS, originally described in primary cell cultures by Serrano et al. [6], and later confirmed to occur in vivo [7][8]. Both telomere dysfunctions and OIS activate the cellular DNA Damage Response, DDR. DDR involves a series of actors, the most important being the kinase ATM and the transcription regulator p53. DNA damage is sensed by a number of proteins that transmit the message of warning to different transducers and effectors of the DNA damage response machinery. These proteins coordinate checkpoint regulation and block cell cycle progression to allow sufficient time for DNA repair. If the damage is too severe, cells can be either eliminated by apoptosis or induced to an irreversible exit from cell cycle through p53/p21- and p16INK4a/Rb-regulated pathways. DDR appears to be necessary for senescence induction by both telomere attrition and OIS. Regulation of SASP by pro-senescence stimuli generally occurs through activation of NF-kB and C/EBPβ transcription factors and may be fine-tuned by other transcription regulators (for a recent review see [9].

Relevant to cancer therapy about twenty years ago, chemotherapeutic drugs and X-rays irradiation employed to kill neoplastic cells via induction of apoptosis, were shown to induce cellular senescence in several human tumor cell lines [10] and in vivo [11]. Both treatments cause massive DNA damage which overwhelms cellular mechanisms of DNA repair. The cellular choice between apoptosis and senescence generally depends on the dosage of these treatments and is modulated by the repertoire of cell mutations. The research on therapy-induced senescence has acquired growing importance especially when also targeted therapies, like those inhibiting kinases, were shown to induce senescence [12]. Chemicals and antibodies inhibiting receptor tyrosine kinases may trigger cell senescence (reviewed in [13]. The same holds true for inhibitors of serine/threonine kinase, which act downstream of growth factor receptors (e.g., the RAF/MEK/ERK cascade) or those like CDK4/6, which regulate cell cycle progression (reviewed in [14]). Importantly all these therapeutic treatments induce senescence also in non-transformed cells mirroring the effects of aging. Non transformed senescent cells have significant effects on cancer progression. Senescent stromal cells which accumulate within the tumor mass alter the growth of cancers via the SASP through a direct action on cancer cells or indirectly modifying the cancer microenvironment. As an example, modification of extracellular matrix turnover by senescent fibroblasts alters the permeability of lymphatic vasculature and changes the number and location of metastases [15]. It is important to point out that SASP may differ according to the senescence-inducing stimuli [16] and that SASP from both cancer and stromal cells may exert either pro- or anti-tumor activities, which depend on the composition of SASP. Tumor repressor functions of SASP include reinforcement of tumor cell senescence [17][18] and favoring clearance of premalignant cells via recruitment of immune cells [19]. Tumor promoting functions of SASP include stimulation of cancer cell growth, initially observed in [20], promotion of epithelial to mesenchymal transition and increased cell migration and metastasis [2], drug resistance [21][22] and stemness [23]. Recently, senescent cells persisting after cancer therapy were suggested to promote carcinogenesis [24], which may explain the increased risk of tumor formation long time after radio- or chemotherapy is a complex phenomenon starting with the angiogenic switch, which changes the ratio between pro- and anti-angiogenic factors and triggers tumor vascularization [25][26]. The process requires a series of steps: proliferation of endothelial cells, which are the principal cellular component lining the interior surface of blood vessels, extracellular matrix degradation and cellular migration toward the growing tumor mass. Angiogenesis participates in several pathways which shape the evolution of cancers: it is essential for delivering nutrients and oxygen to the tumor microenvironment and for removing waste products, it is important for cancer cell dissemination and metastasis formation [25]. On the other hand, it should be considered that a functional tumor vasculature allows the access in the tumor mass of therapeutic drugs and cells of the immune system. Additionally, hypoxia, which follows disruption of tumor vasculature, may select for more aggressive cancer subpopulations [27].

2. Cancer Response to Therapy-Induced Senescence

In glioblastoma recurring following radiotherapy, brain endothelium undergoes radiation-induced senescence eventually resulting in the impairment of tumor neo-angiogenesis. This is not sufficient to limit tumor relapse since cancer cells may adopt the endothelial trans-differentiation as a supportive mechanism to build up its vasculature and bypass senescence-dependent reduction of tumor vessels formation [28]. Targeted therapies with anti-angiogenic properties inhibit growth factors, or their tyrosine kinase receptors, which promote endothelial growth and maturation, above all members of the VEGF/VEGFR families [29]. Importantly, VEGF acts not only on endothelial cells but also on those cancer cells that express VEGFR [30], thus potentially providing a double benefit of antiangiogenic therapies. Bevacizumab, a humanized monoclonal antibody targeting VEGF, was the first angiogenic inhibitor approved by the Food and Drug Administration [31] in 2004. It was initially approved for metastatic colorectal cancer in combination with standard chemotherapy. Its indications now include metastatic breast cancer, non-small-cell lung cancer, glioblastoma, renal cell carcinoma, ovarian cancer, and cervical cancer (for an updated review, see [32]). Several molecules have been described with a VEGFR inhibitory potential, including sunitinib, sorafenib, pazopanib, vandetanib, regorafenib, axitinib, lenvatinib and regorafenib. Most of them are multikinase inhibitors, since they target a panel of kinases, although with different specificities [33][34][35][36]. Most of these drugs induce senescence through activation of the DDR pathway. Interestingly, axitinib seems to induce cancer cells senescence following two possible routes: a chronic exposure induces DNA damage, in renal cell carcinoma and in glioblastoma cell lines, eventually leading to activation of double strand break repair pathways and senescence [35][37]. Conversely, transient exposure to axitinib induces cell senescence, characterized by an increased percentage of β-galactosidase positive cells, in glioblastoma cells in vitro, with increased Reactive Oxygen Species (ROS) and modification of SASP, but with no detectable activation of DDR. We recently demonstrated that the VEGFRs inhibitor axitinib induces endothelial cell senescence through ROS accumulation and ATM activation, in the absence of DNA damage [33]. Although we also observed senescence induction and ROS increase by glioblastoma tumor cells exposed to axitinib, there is a substantial difference in the response of endothelial versus tumor cells. While endothelial cells senescence is prevented by concomitant administration of antioxidants or by ATM inhibition, the same does not hold true in cancer cells, whose commitment to senescence upon axitinib treatment is irreversible. This observation has the intrinsic

consequence that antioxidants or ATM inhibitors might be taken into consideration to protect endothelial cells from axitinib-induced senescence. This would bring a double benefit: lowering of therapy adverse effects on normal cells, and tumor vessel normalization, a requisite allowing a more effective delivery of chemotherapy to the tumor mass. These studies need to be verified in vivo since we recently found that axitinib-induced senescence of endothelial cells may be modified by factors released by glioblastoma cells [38].

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