Biochemical Pharmacology 126 (2017) 1–12

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Commentary

Senescence as biologic endpoint following pharmacological targeting of receptor tyrosine kinases in cancer

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ARTICLE INFO

Article history:
Received 17 June 2016
Accepted 25 August 2016
Available online 27 August 2016

Keywords:
Receptor tyrosine kinases
Premature senescence
Targeted therapies
Cancer
DNA damage response
Senescence induction

ABSTRACT

Cellular senescence was first described in 1961 in a seminal study by Hayflick and Moorhead as a limit to the replicative lifespan of somatic cells after serial cultivation. Since then, major advances in our understanding of senescence have been achieved suggesting that this mechanism is activated also by oncogenic stimuli, oxidative stress and DNA damage, giving rise to the concept of premature senescence. Regardless of the initial trigger, numerous experimental observations have been provided to support the notion that both replicative and premature senescence play pivotal roles in early stages of tumorigenesis and in response of tumor cells to anticancer treatments. Moreover, various studies have suggested that the induction of senescence by both chemo- and radiotherapy in a variety of cancer types correlates with treatment outcome. As it is widely accepted that cellular senescence may function as a fundamental barrier of tumor progression, the significance of senescence for clinical interventions that make use of novel molecular targeting-based modalities needs to be well defined. Interestingly, despite numerous studies evaluating efficacies of receptor tyrosine kinases (RTKs) targeting strategies in both preclinical and clinical settings, the relevance of RTKs inhibition-associated senescence in tumors remains less characterized. Here we review the available literature that describes premature senescence as a major mechanism following targeting of RTKs in preclinical as well as in clinical settings. Additionally, we discuss the possible role of diverse RTKs in regulating the induction of senescence following cellular stress and possible implications of this crosstalk in identification of biomarkers of inhibitor-mediated chemo- and radiosensitization approaches.

1. Introduction

Cellular senescence, defined as a state of irreversible cell cycle arrest, was first described in a seminal study by Hayflick and Moorhead in 1961 as a limit to the replicative lifespan of somatic cells after serial cultivation *in vitro* [1]. Today, we know that this phenomenon reflects one type of senescence known as replicative senescence, which is caused by shortened and/or damaged chromosome telomeres generated through repeated rounds of DNA replication coupled to cell division [2]. In the last decades, major advances in our understanding of senescence have been achieved. This mechanism was also shown to occur prematurely in response to various types of stress, such as overexpression of oncogenes, chromatin perturbation, external DNA damage and oxidative stress, with the final goal of removing damaged cells from a proliferative state [3]. Regardless of the initial trigger, both replicative and prematurely-induced senescent cells share several characteristic features that are being used for their characterization and

Abbreviations: ATIR, anti-tumor immune response; ATM, ataxia telangiectasia mutated; CDK, cyclin-dependent kinase; CGH, comparative genomic hybridization; CHK1, checkpoint kinase 1; CHK2, checkpoint kinase 2; DDAs, DNA-damaging agents; DDR, DNA damage response; DSBs, double-strand breaks; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EPHA3, EPH Receptor A3; FGFR, fibroblast growth factor receptor; FOXM1, Forkhead box protein M1; GAS6, growth arrest-specific protein 6; H3K9me3, histone 3 trimethylated on lysine 9; IR, ionizing radiation; IGF1R, insulin-like growth factor 1 receptor; MAPK, mitogen-activated protein kinase; MEFs, mouse embryonic fibroblasts; MET, mesenchymal-epithelial transition factor receptor (hepatocyte growth factor receptor); MSCs, mesenchymal stem cells; NK, natural killer; NSCLC, non-small cell lung cancer; OIS, oncogene-induced senescence; PI3K, phosphatidylinositol 3-kinase; RB, retinoblastoma; ROS, reactive oxygen species; RTKs, receptor tyrosine kinases; SAHF, senescence-associated heterochromatic foci; SASP, senescence-associated secretory phenotype; SA-βGal, senescence-associated β-galactosidase; SDFs, senescence-associated DNA damage foci; SNP, single nucleotide polymorphism; SOD1, superoxide dismutase type-1; TCGA, The Cancer Genome Atlas; TGFβ, tumor growth factor β; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; VHL, von Hippel-Lindau; VSMCs, vascular smooth muscle cells; γH2AX, Ser139-phosphorylated histone variant H2AX.

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http://dx.doi.org/10.1016/j.bcp.2016.08.022
0006-2952/

identification both *in vitro* and *in vivo* [4]. Growth arrest (usually at a stage with a DNA content typical for G1 but under certain circumstances or in the case of cancer cells also for G2 or S phases of the cell cycle (reviewed in [5])) that cannot be reversed by growth factors stimulation is one of the key features of senescent cells. The inability of senescent cells to progress through the cell cycle is further accompanied by the acquisition of typical changes in cell morphology such as increase in volume, flat shape, vacuole-rich cytoplasm and increased senescence-associated β-galactosidase (SA-βGal) activity [4,6,7]. Cellular senescence is also characterized by the presence of alterations in the chromatin structure: most senescent cells present characteristic regions with condensed heterochromatin known as senescence-associated heterochromatic foci (SAHF). SAHF are particularly enriched in histone 3 trimethylated on lysine 9 (H3K9me3), act by repressing several E2F-regulated genes involved in cell cycle progression and are induced by mechanisms involving both p53 and p16 activation (Fig. 1) [8,9]. Notably, even though senescent cells are in a state of an irreversible cell cycle arrest, they can remain metabolically active for long periods of time and secret a wide range of cytokines, chemokines and proteases to communicate their compromised state to the surrounding tissue regardless of the cell cycle phase in which they are arrested [10,11]. This complex network of secreted factors is known as senescence-associated secretory phenotype (SASP) and it has been shown to have autocrine and paracrine effects on senescent cells and tissue microenvironment, respectively [12]. Lastly, both replicative and premature cellular senescence are characterized by a robust activation of the DNA damage response (DDR) required for initiation and maintenance of the senescence phenotype (Fig. 2) [13]. In the presence of DNA damage, Ser139-phosphorylated histone variant H2AX (γH2AX) accumulates at the site of the lesion and serves as a platform for the recruitment of DNA repair proteins, resulting in the formation of so-called DDR nuclear foci. Accordingly, senescent cells are usually characterized by the presence of DDR foci, also referred to as senescence-associated DNA damage foci (SDFs) [13,14].

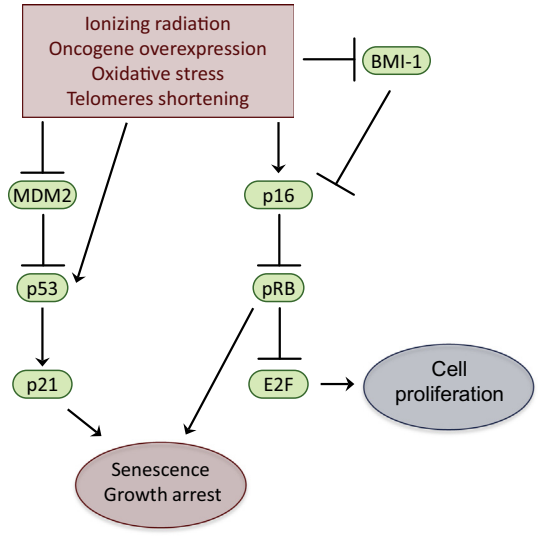

Fig. 1. Regulation of senescence by the p53 and the p16-RB signaling pathways. The senescence program is predominantly established and maintained by the p53 and the p16-RB signal transduction cascades, which converge in the activation of the CDKs inhibitors p21 and p16 with crucial functions in regulating of the onset of cellular senescence. Both p21 and p16 execute a stable cell cycle arrest by keeping RB in an active, hypophosphorylated form, thereby preventing E2F from transcribing genes that are required for cell cycle progression. As a consequence, high levels of p21 and/or p16 are typically found in senescence cells as a result of increased p53 stability and downregulation of the BMI-1 member of the polycomb family responsible for p16 suppression, respectively. *(Figure composed using Motifolio Inc. diagrams.).*

2. Molecular mechanisms in premature senescence

Over the past several years, the mechanisms responsible for the induction and the maintenance of premature senescence have attracted considerable interest as experimental evidence supports the notion that this type of senescence plays an important role in early stages of tumorigenesis as well as in the response of tumor cells to anticancer therapies. In the following subsections, we will review main molecular mechanisms that were shown to induce premature senescence and discuss their contribution to tumor cells response to both chemo- and radiotherapy.

2.1. Oncogene-induced senescence

Oncogene-induced senescence (OIS) is a robust and sustained antiproliferative response triggered by oncogenic signaling resulting from overexpression or mutation of an oncogene or the inactivation of a tumor suppressor gene. The pathways mediating OIS are complex and incompletely elucidated but the proliferative arrest involves activation of both the RB and p53 pathways (Fig. 1) [15,16]. Importantly, OIS is one of the best-known examples where premature senescence serves as a barrier to tumorigenesis. Indeed, expression of high levels of an oncogene in normal cells often results in the induction of senescence rather than in cell transformation [17]. Ruth Sager and colleagues were among the first to show that normal human fibroblasts are resistant to tumorigenesis induced by viral infection through senescence induction [18]. Years later, it was reported that overexpression of an oncogenic version of HRAS (HRASG12V) failed to transform normal cells grown *in vitro* but induced instead a transient increase in cell proliferation followed by a stable cell cycle arrest [19]. Soon after this observation, overexpression or expression of oncogenic versions of other members of the mitogen-activated protein kinase (MAPK)/ERK pathway located downstream of RTKs such as RAF, MEK and BRAF, was analogously shown to induce senescence *in vitro*, highlighting a role for cellular senescence in the defense against neoplastic transformation when this signaling cascade is inappropriately active [20–22]. Even though the molecular mechanisms of oncogene-induced senescence were mostly investigated *in vitro*, they were also validated in *in vivo* scenarios using mouse models with inducible endogenous oncogenes. Collado and colleagues showed that premalignant lung adenomas induced by oncogenic KRAS (KRASG12V) are, unlike malignant adenocarcinomas, positive for markers of senescence, confirming the hypothesis of senescence induction during the early stages of tumorigenesis driven by an oncogene [23]. Similar observations were made in a subsequent study by Morton et al. in a model of human pancreatic ductal adenocarcinoma driven by an endogenous oncogenic KRAS, where mutation of one of the most important mediators of the senescence program, the *TP53* gene, led to an escape from KRAS-induced growth arrest [24]. In addition to the proximal downstream kinases of RAS, also distal effectors of the MAPK/ERK pathway such as the E2F family of transcription factors were shown to induce senescence. For instance, upon deregulation of the E2F3 activity, sustained abnormal proliferation and tumor formation was prevented by engagement of the senescence response *in vivo* [25]. Concerning the molecular mechanisms responsible for induction of senescence following oncogene activation, it has been suggested that multiple processes cooperate to promote and maintain this type of senescence response, including replicative stress and accumulation of reactive oxygen species (ROS) that characterize the initial response of normal cells to