Seminars in Cancer Biology 35 (2015) S104–S128

Contents lists available at ScienceDirect

Seminars in Cancer Biology

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

Review

Therapeutic targeting of replicative immortality

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ARTICLE INFO

Article history:
Available online 11 April 2015

Keywords:
Senescence
Telomerase
Oncogenic stress
p53
pRB

ABSTRACT

One of the hallmarks of malignant cell populations is the ability to undergo continuous proliferation. This property allows clonal lineages to acquire sequential aberrations that can fuel increasingly autonomous growth, invasiveness, and therapeutic resistance. Innate cellular mechanisms have evolved to regulate replicative potential as a hedge against malignant progression. When activated in the absence of normal terminal differentiation cues, these mechanisms can result in a state of persistent cytostasis. This state, termed "senescence," can be triggered by intrinsic cellular processes such as telomere dysfunction and oncogene expression, and by exogenous factors such as DNA damaging agents or oxidative environments. Despite differences in upstream signaling, senescence often involves convergent interdependent activation of tumor suppressors p53 and p16/pRB, but can be induced, albeit with reduced sensitivity, when these suppressors are compromised. Doses of conventional genotoxic drugs required to achieve cancer cell senescence are often much lower than doses required to achieve outright cell death. Additional therapies, such as those targeting cyclin dependent kinases or components of the PI3K signaling pathway, may

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http://dx.doi.org/10.1016/j.semcancer.2015.03.007

induce senescence specifically in cancer cells by circumventing defects in tumor suppressor pathways or exploiting cancer cells' heightened requirements for telomerase. Such treatments sufficient to induce cancer cell senescence could provide increased patient survival with fewer and less severe side effects than conventional cytotoxic regimens. This positive aspect is countered by important caveats regarding senescence reversibility, genomic instability, and paracrine effects that may increase heterogeneity and adaptive resistance of surviving cancer cells. Nevertheless, agents that effectively disrupt replicative immortality will likely be valuable components of new combinatorial approaches to cancer therapy.

1. Introduction

Among the notable feats of evolution is the remarkable protection from cancer that is enjoyed by long-lived species such as humans. Despite billions of cell divisions and trillions of cells, humans remain, on average, cancer-free for more than 50 years. One of nature's notable tumor suppressive mechanisms is cellular senescence, a response to nonlethal stress that results in persistent cytostasis. In the absence of normal growth arrest accompanying differentiation, senescence imposes limits on the proliferative capacity of clonal cell lineages. Senescence can be induced by multiple stimuli, including intrinsic cellular processes such as telomere dysfunction and oncogene expression, but also by exogenous factors such as DNA damaging agents or oxidative environments. Abundant published evidence now supports the concept that senescence is a significant impediment to malignancy, and that it is ordinarily very stringent. Indeed, as a number of investigations have shown, many cell types in which one or more senescence pathway components are functionally inactivated remain susceptible to senescence – an indication that robust compensatory mechanisms exist for this important stress response. Despite the resiliency of the senescence response, however, it is prone to failure to varying degrees, depending upon genetic/epigenetic context. Failure of senescence in cells that have undergone oncogene activation, telomere dysfunction, and/or DNA damage can result in changes favoring malignancy and drug resistance. Elucidation of mechanisms that enforce senescence has been sought in expectation that such knowledge should lead to measures that prevent or reverse its failure in susceptible pre-malignant and malignant cell populations. In this review, we focus on telomeres and other mediators of senescence induction as candidate targets for the prevention and treatment of cancers.

2. Causes of senescence

Proliferating cells can respond to genotoxic and non-genotoxic stresses in a number of ways, including transient cell-cycle arrest, senescence, and cell death. Senescence is operationally broadly defined as a viable growth arrest characterized by the inability of affected cells to resume proliferation in the presence of appropriate mitogenic factors. While multiple cellular and molecular features, including increased cell size, accumulation of lysosomes, upregulation of cell cycle inhibitors, presence of senescence-associated heterochromatic foci (SAHF), and positive staining for senescence-associated beta-galactosidase (SA-βGal) activity, have been associated with senescent cells, no single feature is a universal and specific marker of senescence. Experimental and clinical evidence indicate that an intact senescence response is important for preventing unregulated growth and malignant transformation. In addition, the ability to undergo senescence can determine the efficacy of targeted cancer therapies. As described below, however, senescence is not a discrete mechanism or pathway that can be easily classified as either intact or entirely non-functional. Instead, it is a process that can result from many different inputs with degrees of sensitivity dictated by intrinsic as well as extrinsic factors.

2.1. Telomerase repression

In the absence of externally or oncogenically induced stresses, telomerase repression may be the only physiological impediment to indefinite replication. Replicative senescence, as originally described by Hayflick in cultures of cells from non-malignant tissues, is due to natural repression of telomerase and the resulting DNA damage response that occurs when the number of telomeric TTAGGG repeat sequences on the ends of chromosomes becomes too few to support the assembly of stable telomere complexes [1,2]. Structures formed through interactions of TTAGGG repeat sequences with a protein complex referred to as shelterin function to "cap" the chromosome ends, protecting against DNA degradation, recombination, and chromosome fusion [3]. The telomeric TTAGGG repeats are replenished by telomerase [4], a ribonucleoprotein complex that consists of a catalytic reverse transcriptase protein subunit (hTERT, TERT) [5–7], an RNA template (hTR, TERC) [8–10], and other accessory proteins, including the RNA-modifying protein dyskerin [11,12]. The presence of hTERT and hTR are the minimum requirements for recapitulation of telomerase activity *in vitro*. Telomerase activity and telomere length elongation in cancer are associated with up-regulation of both hTR and hTERT, while overexpression of hTR has been shown to boost telomerase activity and more dramatically extend telomere length in cells that express endogenous or ectopic hTERT [13–15]. Thus both telomerase components restrict telomerase activity and telomere length *in vitro*, illustrating the fact that both components are required for a functional telomerase holoenzyme. Although hTERT was initially considered as the limiting component of telomerase, evidence from biochemistry, promoter studies, mouse models, and human tumors has demonstrated contexts where hTR limits telomerase enzyme levels and telomere maintenance [13–18]. At early embryonic stages, the hTERT gene and telomerase activity are expressed at high levels in many tissues [19,20]. The hTERT gene then undergoes repression as embryonic cells differentiate into adult somatic cells [21]. From the neonatal period onward, hTERT transcripts and telomerase activity are nearly or completely undetectable in most human tissues [19,22,23], except in some highly proliferative tissues, such as lymphoid cells and tissue stem and progenitor cells [24–27]. *In vitro*, attrition of TTAGGG repeats upon successive divisions in cells lacking sufficient telomerase activity ultimately results in DNA damage responses including growth arrest, followed by cell enlargement, chromatin condensation, and vacuolization – characteristic features of senescent cells. Multiple and distinct human cancer precursor lesions, but not corresponding malignant cancers, are composed of cells that display signs of telomere dysfunction-induced senescence [28]. Ectopic hTERT expression in many cell types prevents these senescent changes by stabilizing telomeres and extending replicative lifespan [29–32]. While not intrinsically essential for malignancy [33], an extended lifespan or "immortalization" permits clonal cell lineages to accumulate rare genetic and epigenetic aberrations that together can cause malignant transformation.

In the absence of a telomere maintenance mechanism, telomeres shorten with each round of cellular replication to eventually