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Review

Epigenetics and senescence: Learning from the INK4-ARF locus

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ABSTRACT

Cellular senescence is the biological consequence of aging. However, the same mechanisms that provoke senescence during aging have been proven to act in tumor suppression and thus to occur in premalignant cells. All the diverse aspects of the senescent phenotype, as are observed for many other cell fates, arise from alterations of the chromatin architecture. Relatively little is known overall about the changes in chromatin structure, and which regulatory networks are implicated in these. Major insight into the epigenetic contributions to senescence has been gained by studying the regulation of the INK4-ARF locus. Activation of the tumor suppressors encoded by this locus leads to an irreversible cell cycle exit. Importantly, epigenetic alterations at this locus have been associated with the onset of cancer. Here we discuss the recent findings that link epigenetics to the senescence pathway.

Contents

1. Introduction

More than 40 years ago, Hayflick reported that primary human fibroblasts could not be propagated infinitely in vitro. Rather, they displayed a limited proliferative lifespan under culture conditions, followed by an irreversible growth arrest. The halt of mitotic cell divisions, however, did not abrogate viability or metabolic activity, and the mere loss of proliferative potential was designated senescence [1]. Exiting the cell cycle and entering in a stage of non-division also goes in hand with changes in cell morphology. Senescent cells share several common features, such as large sizes, flattened, enlarged shapes [2], and nuclei that can display the appearance of senescence-associated heterochromatic foci (SAHF) [3].

The senescent stage is also reflected by changes in protein expression levels and activity. One of the key players involved is the tumor suppressor p53, whose activity is enhanced by progressive passaging of human fibroblasts [4]. Additionally, transgenic mice carrying an active mutant allele of p53 displayed a precipitated onset of aging and, interestingly, a decreased incidence of tumor development [5]. Microarray analyses of different cell lines have identified additional relevant genes that are differentially expressed upon entering senescence. Understanding their implications in the molecular regulation of this cell stage is of particular interest, as it will help to decipher aging and tumor suppressing mechanisms [6,7].

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In order to distinguish quiescent from senescent cells in culture, as well as to monitor senescence in vivo, several biomarkers can be used. Whether or not senescence networks have been activated can be established by the detection of several key proteins, such as p53 or the retinoblastoma protein (pRB) [8]. The most common test, however, for detecting senescent cells in tissue preparations is to measure the senescence-associated (SA) β-galactosidase activity. In senescent cells, β-galactosidase is active at pH 6 rather than at its optimum of pH 4, due to an enlarged lysosomal compartment and a consequentially higher amount of the enzyme present [9].

Many molecular pathways are involved in triggering the senescent cell response. In general, senescence is considered to be a cell-protective mechanism and to be as important as apoptosis because it irreversibly stops proliferation of stressed and damaged cells and, in this way, impedes potential further aberrations. In fact, bypassing of senescence occurs frequently in tumorigenesis.

Irreversible growth arrest can be induced by numerous factors in addition to proliferative exhaustion of cells, such as by several forms of physiologic stress (Fig. 1). Another well-characterized trigger of the senescence phenotype is telomere shortening following serial passaging of human fibroblasts [10]. The reason for the continuous shortening is that during S phase the lagging strand is synthesized only incompletely at its 5′ telomeres. Therefore, this kind of senescence is designated replicative senescence. The shortened telomeres are recognized as damaged DNA by the cellular repair machinery, which leads to activation of the CHK1 and CHK2 kinases, as well as other downstream components of the DNA repair signaling pathways [11]. In addition to telomere attrition after serial passages, there are also other stimuli that provoke the onset of the so-called premature senescence program. One important stimulus is the high expression of oncogenes, which leads to oncogene-induced senescence (OIS) in vivo. The best studied example for OIS is that induced by activated H-ras. When H-ras is expressed in primary mouse embryonic fibroblasts (MEFs), senescence is induced and the levels of the tumor suppressors p53 and p16INK4a are correspondingly elevated [12]. However, upon mutation of either p53 or p16INK4a in MEFs, senescence is circumvented by provoking cell transformation. This effect underscores the function of senescence in tumor suppression [12]. In addition, inactivation of tumor suppressors can also cause cells to go into irreversible growth arrest. The tumor suppressor PTEN is very often mutated in prostate cancer; it acts by preventing proteosomal degradation of p53 by its E3 ubiquitin-ligase Mdm2. MEFs that are deficient for PTEN show morphologic characteristics of senescence as well as elevated p16INK4a expression, and are also positive for SA β-galactosidase staining. However, when p53 was deleted in a PTEN-negative background, tumor growth was enhanced and tumors were more invasive [13].

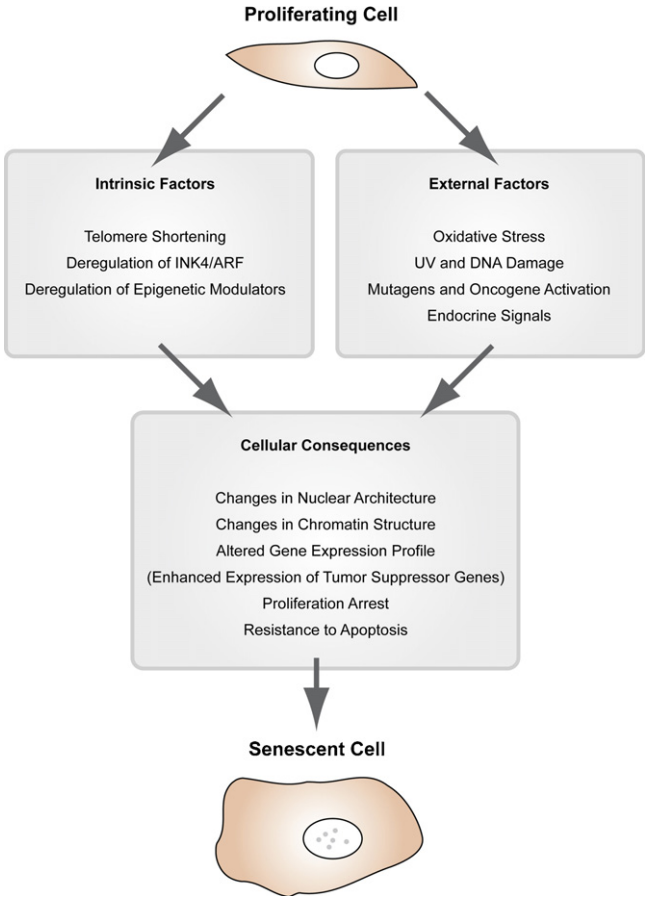

Fig. 1. Scheme of intrinsic and external factors leading to cellular senescence, including the cellular characteristics of a senescent cell.

Most of the signaling pathways in senescence converge on the activation of the tumor suppressors p53 and pRB. Transcriptional regulation of the INK4A-ARF locus plays the pivotal role here at the regulatory level, as it encodes the two unrelated tumor suppressors p16INK4a and p19ARF, both of which can induce cell cycle arrest [14]. p19ARF for instance interacts with Mdm2, the p53-associated E3 ubiquitin-ligase. Ubiquitination of p53 ends in the proteosomal degradation of p53. However, when Mdm2 is bound by p19ARF, it itself is degraded, which in turn leads to an accumulation of p53, followed by growth arrest of the cells [15].

At the transcriptional level, all of the above mentioned senescence pathways are modulated by the chromatin state. This applies equally for the DNA damage pathway as for the transcriptional control of tumor suppressors, such as that of the INK4-ARF locus. Chromatin is the functional entity of DNA made up of nucleosomes, which consist of 146 bp of DNA wrapped around histone octamers. Histone octamers are composed of an (H3/H4)2 heterotetramer and two H2A/H2B heterodimers. Chromatin is subjected to a series of highly dynamic posttranslational modifications (PTMs), such as DNA methylation at cytosine, and methylation, phosphorylation or acetylation of the histone proteins, that regulate its packaging density as well as the recruitment of further chromatin modifying enzymes. This allows the execution of a large variety of chromatin-associated processes, like regulation of gene transcription, establishment of heterochromatic transcriptional silent regions, and DNA repair. Therefore, alterations in the modification state of chromatin can lead to cancer development by affecting chromosome stability and gene expression [16]. Several key events have been reported in senescence that rely on chromatin regulation, such as telomere shortening [17], senescence-associated heterochromatic foci (SAHF) [3], and a general decline in DNA methylation [18]. Better understanding of senescence pathways will therefore be crucial to unravel their underlying mechanisms as well as the role that alterations in chromatin structure play in aging and cancer.

1.1. The INK4-ARF locus

The INK4-ARF locus is a critical regulator of senescence. Proteins encoded by the INK4-ARF locus accumulate during senescence induction and drive the cells to growth arrest. Both replicative and oncogenic stresses activate the INK4-ARF locus and lead to cellular senescence [12].

Three gene products are encoded within the INK4-ARF locus. The p16INK4a and p15INK4b proteins are cyclin-dependent kinase inhibitors (CDKi) of cyclin/cdk4 (or cdk6) complexes that prevent