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Mini-review

Nucleotide metabolism, oncogene-induced senescence and cancer

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ABSTRACT

Senescence is defined as a stable cell growth arrest. Oncogene-induced senescence (OIS) occurs when an activated oncogene is expressed in a normal cell. OIS acts as a bona fide tumor suppressor mechanism by driving stable growth arrest of cancer progenitor cells harboring the initial oncogenic hit. OIS is often characterized by aberrant DNA replication and the associated DNA damage response. Nucleotides, in particular deoxyribonucleotide triphosphates (dNTPs), are necessary for both DNA replication and repair. Imbalanced dNTP pools play a role in a number of human diseases, including during the early stages of cancer development. This review will highlight what is currently known about the role of decreased nucleotide metabolism in OIS, how nucleotide metabolism leads to transformation and tumor progression, and how this pathway can be targeted as a cancer therapeutic by inducing senescence of cancer cells.

1. Introduction

Nucleotides are necessary for a variety of cellular processes. It has been well characterized that imbalances in nucleotide levels lead to a variety of human diseases, including cancer [1–4], immunodeficiency [5,6], aging [7,8], kidney diseases [9,10], gout [6], and a number of mitochondrial pathologies [11,12].

1.1. Synthesis of nucleotides: The de novo pathway

Nucleotides can be synthesized through either the de novo pathway or the salvage pathway [13]. In the de novo pathway, glucose and glutamine are the main nutrients needed to synthesize nucleotides [14]. Glucose is converted to ribose-5-phosphate during the pentose phosphate pathway, which is used for both purine and pyrimidine synthesis [15]. Glutamine is necessary for supplying nitrogen [16]. Purines and pyrimidines are synthesized in two distinct ways [13,15,17]. Purines are made by directly assembling the atoms that comprise the purine ring onto ribose-5-phosphate through 11 steps. This yields inosine monophosphate (IMP), which is further modified to produce adenosine monophosphate (AMP) and guanosine monophosphate (GMP). In contrast, during pyrimidine synthesis, the pyrimidine ring is completed before addition of the ribose-5-phosphate moiety. Pyrimidines are made through a 6-step process, which produces uridine monophosphate (UMP). UMP can then be converted into cytidine triphosphate (CTP). Thymine nucleotides are synthesized after uridine diphosphate (UDP) and cytidine diphosphate (CDP) are reduced, and thymidylate synthase (TS) is necessary for dTTP synthesis [17].

1.2. Synthesis of nucleotides: The salvage pathway

In addition to the de novo pathway, a salvage pathway exists for both purine and pyrimidines [13,17,18]. Normal cells undergo turnover and degradation of cellular materials, leading to release of free purines or substrates that compose the pyrimidine ring [17]. These can be converted back into dNTPs by a variety of en-

Abbreviations: 3-AP, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone; ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3-related protein; AMP/ADP/ATP, adenosine mono-, di-, and tri-phosphate; BLM, Bloom syndrome RecQ helicase-like; BRAF, V-raf murine sarcoma viral oncogene homolog B; BRCA1/2, Breast Cancer 1/2, Early Onset; CDK, cyclin-dependent kinase; CMP/CDP/CTP, cytidine mono-, di-, and tri-phosphate; c-myc, v-myc avian myelocytomatosis viral oncogene homolog; DNA, deoxyribonucleic acid; DDR, DNA damage response; dNDP, deoxyribonucleotide diphosphate; dNTP, deoxyribonucleotide triphosphate; E2F1, E2F transcription factor 1; E2F7, E2F transcription factor 7; EOC, epithelial ovarian cancer; GMP/GDP/GTP, guanosine mono-, di-, and tri-phosphate; HRAS, Harvey Rat Sarcoma Viral Oncogene Homolog; HU, hydroxyurea; IMP, inosine monophosphate; NADPH, Nicotinamide adenine dinucleotide phosphate; NDP, ribonucleoside diphosphate; NRAS, neuroblastoma RAS viral (V-Ras) oncogene homolog; NTP, ribonucleoside triphosphate; OIS, oncogene-induced senescence; p53R2/RRM2B, Ribonucleotide Reductase M2 B (TP53 Inducible); pRb, retinoblastoma protein; R1, ribonucleotide reductase subunit 1; R1/RRM1, ribonucleotide reductase M1; R2, ribonucleotide reductase subunit 2; Rad51, Rad51 recombinase; RAS, rat sarcoma oncogene; RECQL4, RecQ Protein-Like 4; RNR, ribonucleotide reductase; RRM2, ribonucleotide reductase M2; SA-B-Gal, senescence-associated beta-galactosidase; shRNA, short hairpin RNA; TS, thymidylate synthase; TTP, thymidine triphosphate; UDP, uridine diphosphate; UMP, uridine monophosphate; WRN, Werner syndrome ATP-dependent helicase.

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zymes in both the cytosol and mitochondria [17,18]. Interestingly, pyrimidine salvage is more efficient than purine salvage [17].

1.3. Synthesis of deoxyribonucleotides

One particular type of nucleotide, 2′-deoxyribonucleoside 5′-triphosphates (dNTPs), is necessary for both DNA replication and repair [17,19]. Without the correct levels of dNTPs, cells cannot faithfully replicate either nuclear or mitochondrial DNA, and DNA damage cannot be repaired [7,20]. The rate-limiting step in dNTP synthesis is reduction of ribonucleoside di- or tri- phosphates (NDPs/NTPs) at the 2′ position of ribose sugar to deoxyribonucleotide-di- or tri- phosphates (dNDPs/dNTPS) by ribonucleotide reductase (RNR) [17,19]. During reduction of ribonucleosides, RNR is oxidized and then reduced by either thioredoxin or glutathione [19]. Nicotinamide adenine dinucleotide phosphate (NADPH) is the ultimate source of the electrons. RNR reduces all four rNDPs/rNTPs (i.e., ADP/ATP, GDP/GTP, UDP/UTP, and CDP/CTP) [17]. RNR activity is tightly regulated by allosteric regulation and enzyme specificity [19]. RNR is a tetrameric complex consisting of two large catalytic subunits (R1: ribonucleotide reductase M1, RRM1) and two small regulatory subunits (R2: ribonucleotide reductase M2, RRM2; or p53R2/RRM2B) [17,19]. RRM1 contains both the catalytic site and the allosteric regulatory sites [19]. RRM1 is expressed throughout all phases of the cell cycle [21]. The R2 subunit contains the tyrosyl radical, the site necessary for the reduction reaction [19]. RRM2 is the R2 subunit that controls reduction during S phase of the cell cycle when dNTPs are needed for DNA replication [21]. Therefore, RRM2 expression is rate-limiting for RNR activity [19]. In contrast, p53R2 is involved in supplying dNTPs for DNA repair and mitochondrial DNA synthesis in the G0/G1 phase of the cell cycle [22].

1.4. Senescence

First described in 1961 by Leonard Hayflick and Paul Moorhead, cellular senescence is defined as a stable cell growth arrest [23]. Senescence can be induced by a number of different stimuli, including critically shortened telomeres, activated oncogenes, DNA damage, and some cancer therapeutics [24]. Senescent cells have unique morphological and molecular characteristics [25]. Phenotypically, they are characterized by a large, flat morphology and increased activity of β-galactosidase (termed senescence-associated β-galactosidase or SA-β-gal) [26].

1.5. Oncogene-induced senescence

Oncogene-induced senescence (OIS) occurs when an oncogene (such as RAS or BRAF) becomes activated in a primary (normal) mammalian cell [27]. Paradoxically, expression of an activated oncogene leads to a cell cycle exit and sustained growth arrest [28]. OIS is therefore considered a bona fide tumor suppressor mechanism *in vivo* [24,29]. The hallmarks of OIS include DNA replication stress leading to a sustained DNA damage response (DDR) [30,31] and upregulation of the p53/p21 and p16/pRb pathways (Fig. 1), which contribute to the stable growth arrest [32].

This review will focus on the role of nucleotide metabolism as a newly identified pathway in OIS. In addition, we will discuss how changes in nucleotide metabolism can overcome OIS and transform cells. Finally, we will briefly outline how nucleotide metabolism is a diagnostic and prognostic biomarker for cancer and how this pathway could be targeted as a cancer therapeutic by inducing senescence of cancer cells.

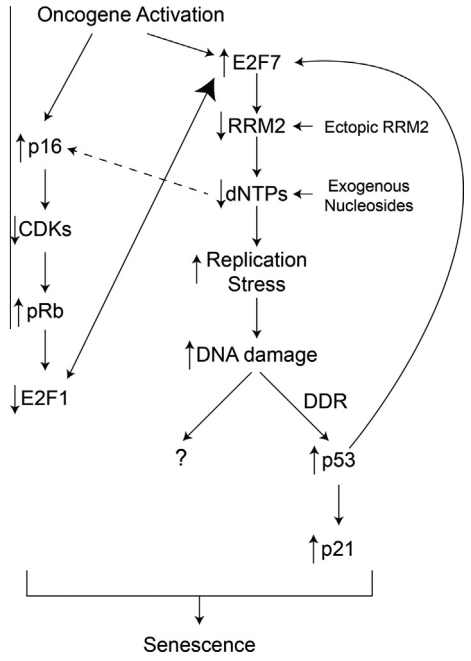

Fig. 1. Overview of the oncogene-induced senescence pathway. Oncogene-induced senescence begins with activation of an oncogene in a primary (normal) mammalian cell. This upregulates the repressive E2F7, which inhibits transcription of the *RRM2* gene. Inhibition of RRM2 leads to a significant decrease in dNTP levels. This leads to two outcomes: (1) replication stress and accumulation of DNA damage; and (2) upregulation of p16 through an unknown mechanism. Replication stress and DNA damage accumulation activates the DNA damage response (DDR), in particular p53. p53 activation positively feeds back by further increasing E2F7 activity. Sustained activation of p53 induces high expression of p21, which helps facilitate cell cycle arrest. DNA damage can also lead to cell cycle arrest independent of p53 and p21 status. Upregulation of p16 inhibits cyclin-dependent kinases (CDKs), which relieves their inhibition of pRb. pRb can then repress E2F1 activity, which leads to more E2F7 binding to repress *RRM2* transcription. Decreased E2F1 also leads to a cell cycle exit through a decrease in transcription of numerous cell cycle-related genes. These pathways all act in concert to establish and maintain the stable senescence-associated cell cycle exit.

2. Role of nucleotide metabolism in oncogene-induced senescence

Nucleotide metabolism and imbalances in dNTP pools have long been known to play a role in a variety of human pathologies [1–4]. However, until recently, there was no specific research to demonstrate that nucleotide metabolism plays a role in OIS. The following sections will review what is currently known about the role of dNTP pools in OIS, including their role in replication stress, the DNA damage response, and execution of senescence pathways after oncogene activation.

2.1. Role of decreased dNTP pools in replication stress during OIS

Senescence induced by activated oncogenes such as RAS is characterized by accumulation of cells in S-phase of the cell cycle [30]. This is due to the induction of replication stress leading to stalled and collapsed replication forks, thereby arresting cells in S-phase [30,31]. The replication stress leads to activation of either ataxia telangiectasia and Rad3-related protein (ATR) (stalled forks) or ataxia telangiectasia mutated (ATM) (collapsed forks), which effectively activates an intra-S phase checkpoint [33,34]. Indeed, suppression of S-phase progression is sufficient to block RAS-induced senescence [35]. Early studies showed that a decrease in dNTP pools by hydroxyurea (HU) leads to an S-phase arrest [36,37]. Recent evidence from our lab suggests that the replication

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