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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

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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 enzymes 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, BRAF, etc…) 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 (Figure 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.

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 stress observed during OIS is specifically due to a decrease in dNTP levels (Figure 1) [38]. Specifically, we showed a transcriptional decrease in RRM2, whose expression is rate-limiting for rNDP/rNTP reduction to dNDPs/dNTPs [19]. Indeed, ectopic expression of RRM2, which is sufficient to restore cellular dNTP levels, or supplementation with exogenous nucleosides is able to overcome the replication stress observed during OIS (Figure 1) [38]. Interestingly, replication stress due to decreased dNTP pools has also been shown to play a role in longevity in yeast [39]. Additionally, patients with mutations in replication regulators such as the RecQ helicases [e.g., Werner syndrome ATP-dependent helicase (WRN), (Bloom

syndrome RecQ helicase-like (BLM), and RecQ Protein-Like 4 (RECQL4)] display replication stress, genomic instability, and accelerated aging phenotypes [40, 41]. Given that cellular senescence is thought to contribute to tissue aging, these data suggest that dNTP pools play a larger role in replication stress during senescence.

2.2. Role of decreased dNTP pools in the activation of the DNA damage response during OIS

In addition to replication stress, accumulation of DNA damage and activation of the DDR are hallmarks of OIS (Figure 1) [30, 31, 42]. Until recently, it was unclear whether replication stress or oxidative stress was the cause of the DNA damage and DDR during OIS. Results from our lab indicate that replication stress induced by a decrease in dNTP pools plays a major role in the accumulation of DNA damage observed during OIS (Figure 1) [38]. Specifically, we show that restoration of cellular dNTP levels by either addition of exogenous nucleosides or ectopic RRM2 expression is sufficient to suppress the DDR. This correlates with rescue of the oncogene-induced DNA replication stress [38]. Another report has corroborated our finding that decreased dNTP levels are the cause of the DNA damage during senescence induced by either oncogenic RAS [43] or c-myc [44]. These authors also found that thymidylate synthase (TS) and the large subunit RRM1 were also downregulated during OIS [43], suggesting a global decrease in the nucleotide metabolic pathway during OIS. However, data from our lab demonstrates that RRM2 downregulation, but not other regulators of the nucleotide metabolic pathway such as p53R2 or RRM1, occurs before the cell cycle exit [38]. This indicates that RRM2 downregulation drives the suppression of nucleotide metabolism observed during OIS, and downregulation of other regulators of the nucleotide metabolic pathway may be simply a consequence of OIS-associated cell growth arrest.

2.3. The role of decreased dNTP pools in the execution of the OIS-associated cell cycle exit

Senescence was initially defined as an irreversible cell cycle exit [45]. In contrast to what has been observed with the reversible effects of HU on the cell cycle [36, 37], cells cannot re-enter the cell cycle after a decrease in dNTP pools due to decreased RRM2 (Figure 1) [38]. Therefore, the consequence of decreased dNTP pools must go beyond replication stress and the associated DDR. It is known that numerous signaling pathways are activated during OIS, in particular the p53/p21 and p16/pRb pathways (Figure 1) [32]. Indeed, as discussed above, the decrease in dNTP pools leads to accumulation of DNA damage and a sustained induction of the DDR [38]. Additionally, the decrease in dNTP pools also leads to elevated expression of the cell cycle regulators p21 and p16 [38], which are both upregulated during OIS [32]. p16 phosphorylates cyclin-dependent kinases (CDKs), which relieves the inhibition of pRb (Figure 1) [46]. pRb inhibits activating E2Fs such as E2F1 [47–50]. Under normal growth conditions, E2F1 contributes to transcription of RRM2 [51]. Interestingly, RRM2 downregulation occurs at the transcriptional level through replacement of the transcriptional activator E2F1 with the transcriptionally repressive E2F7 at the promoter of *RRM2* gene (Figure 1) [38]. This reinforces the decrease in dNTP pools and therefore the OIS-associated cell cycle exit. This is consistent with results from a previous report demonstrating a positive feedback loop between p53 and E2F7 [52]. Downregulation of

RRM2 activates p53 [38], which then enhances E2F7 expression [52]. In addition, pRb decreases E2F1 activity [49, 50], which may allow for enhanced binding of the repressive E2F7 to the RRM2 promoter. This suggests that these pathways act in concert to maintain the stable cell growth arrest. Interestingly, the decrease in RRM2 seems to be one of the first signals in this feedback loop as it occurs while cells maintain proliferation [38]. Moreover, in RAS-infected cells, ectopic RRM2 or supplementation with exogenous nucleosides can override the activation of p53 (Figure 1). These data all point to the presence of a feedback loop whereby the decrease in RRM2 leads to a further decrease in RRM2 through activation of the p53/E2F7 pathway and pRb pathway. Interestingly, senescence of cancer cells such as melanoma and ovarian cancer cells induced by RRM2 inhibition is independent of both p53 and pRB pathway [38, 53]. However, this correlates with activation of the DDR. This suggests senescence induced by RRM2 inhibition depends upon DNA damage and the associated pathway (Figure 1). Indeed, exogenous nucleosides suppress the DDR and also inhibit senescence induced by RRM2 inhibition. Future studies are warranted to elucidate the downstream pathways activated by DNA damage that mediate senescence and the associated cell growth arrest induced by suppression of nucleotide metabolism.

3. Nucleotide metabolism in cancer

OIS is considered to be a tumor suppressor mechanism *in vivo* [29]. However, cells that have undergone OIS can accumulate additional oncogenic hits over time, which may lead to senescence bypass and transformation. For instance, benign nevi (moles) that have undergone OIS due to expression of oncogenic BRAF or NRAS can eventually become melanomas [54]. It is important to understand how cells can bypass senescence and become transformed. These observations may allow for better preventative measures or novel cancer therapeutics. The following sections will discuss the role of nucleotide metabolism in transformation, cancer progression, and cancer therapeutics.

3.1 Role of nucleotide metabolism in genomic integrity, senescence bypass, and transformation

We have already discussed the role of decreased dNTP pools in replication stress and the DDR (Section 2.1 and 2.2). It is well known that replication stress can lead to genomic instability [7], which is a hallmark of cancer cells [55, 56]. Indeed, patients with mutations in proteins necessary for genomic integrity (i.e., BRCA1/2, Rad51, etc…) are prone to cancer [57]. Notably, early studies in yeast models demonstrated that decreased dNTP levels lead to increased mutagenesis through an increase in genomic instability [58]. A number of recent publications have attempted to elucidate the mechanism of OIS bypass and transformation. Our lab demonstrated that melanocytes expressing oncogenic BRAF or NRAS can bypass senescence when exogenous nucleosides are supplemented into the cell culture medium (Figure 1) [38]. These data have been corroborated in fibroblasts expressing HRAS [38, 43]. Additionally, we found that either ectopic expression of RRM2 or exogenous nucleoside supplementation could overcome the cell cycle exit in fully senescent fibroblasts [38]. Because these cells maintain oncogene expression, it is easy to surmise that these senescent bypassed cells could become transformed. Indeed, one report demonstrates that decreased dNTP pools in E6/E7 cells is what causes transformation of these cells [59].

Although E6/E7 expression does not induce senescence, this study clearly indicates that low dNTP pools may play a role in the early phases of tumorigenesis.

Interestingly, overexpression of RRM2 or p53R2 is tumorigenic by itself (Figure 2) [60]. Increased RRM2 or p53R2 expression is mutagenic *in vitro* and leads to lung neoplasms *in vivo*. Notably, RRM2 overexpression induces a higher mutation frequency *in vitro* compared to p53R2 overexpression. In addition, RRM2 transgenic mice have larger and more malignant lung neoplasms compared to p53R2 transgenic mice. These results indicate that RRM2 may be a more potent oncogene than p53R2. Additionally, another nucleotide metabolic enzyme TS can independently transform cells and lead to tumor formation *in vivo* [61–63]. In contrast, expression of RRM1 is thought to be tumor suppressive (Figure 2). Indeed, in both *in vitro* and *in vivo* models, overexpression of RRM1 can reduce tumor formation, migration, and metastasis [64–66]. These studies indicate that nucleotide metabolism plays an important role in senescence bypass, tumor formation, and progression.

3.2 Role of nucleotide metabolism as a cancer biomarker

It is clear that changes in nucleotide metabolism can lead to transformation and tumorigenesis (discussed in Section 3.1). Therefore, many studies have sought to determine whether components of the nucleotide metabolic pathway are either prognostic or diagnostic biomarkers in a variety of cancers.

A number of studies have shown that RRM2 is both a prognostic and diagnostic biomarker. RRM2 has been shown to be a diagnostic biomarker in colon, breast and pancreas [67–69]. Recent studies from our lab have shown that RRM2 expression is both a prognostic and diagnostic biomarker for melanomas with oncogenic BRAF or NRAS [38] and epithelial ovarian cancer (EOC) [53]. In EOC, RRM2 expression positively correlates with the cell proliferation marker Ki67, tumor grade, and stage [53]. We also observed that high RRM2 expression independently predicts a shorter overall survival in both EOC and melanoma patients [53]. [38]. Interestingly, in the same cohort of melanoma patients, RRM1 did not correlate with survival [38]. This underscores the importance of RRM2 in cancer progression.

The role of the other R2 subunit, p53R2, as a biomarker is not as clear. p53R2 has been shown to be diagnostic biomarker for a variety of cancer types, including melanoma [70], non-small cell lung cancer [71], and squamous cell carcinoma [72]. In addition, high p53R2 expression is also a poor prognostic biomarker in non-small cell lung cancer [71] and squamous cell carcinoma [73]. However, some studies have indicated that high p53R2 leads to a better prognosis [74, 75]. Future studies will need to be done on larger patient cohorts to truly determine whether p53R2 expression predicts better or worse patient outcome.

The role of RRM1 in the diagnosis and prognosis of cancer is also unclear. Studies have indicated that RRM1 is a tumor suppressor (Figure 2) [65]; therefore, high RRM1 expression is a predictor of better survival [76–78]. In contrast, other studies have found that high RRM1 expression leads to poor survival [78, 79]. In fact, one study found that depending on what treatment patients received, high RRM1 expression was inconclusive for patient prognosis [80]. Our study in melanoma compared RRM1 and RRM2 expression. We

found that RRM2, but not RRM1, predicted better survival in patients with BRAF or NRAS positive tumors [38]. The conflicting results between studies make it hard to fully distinguish whether RRM1 expression is important for the diagnosis and prognosis of cancer patients.

Other enzymes further upstream in the nucleotide biosynthetic pathway have also been shown as important cancer biomarkers. For instance, high expression of thymidylate synthase, which is necessary for dTTP synthesis [17], is a predictor of poor survival in different cancer types [81, 82].

Taken together, these studies demonstrate that nucleotide pool imbalances, and in particular dNTP pool imbalances, are both diagnostic and prognostic biomarkers for a large variety of human cancers.

3.3 Inhibiting nucleotide metabolism for cancer therapeutics

Since nucleotide metabolism plays a role in transformation and tumor progression (discussed in Sections 3.1 and 3.2), inhibition of this pathway has long been considered a therapeutic strategy for cancer. As tumor cells have a higher need for dNTPs [55, 56], many of the anti-tumor therapeutics affecting nucleotide metabolism are aimed at RNR [83–87]. The first class of drugs that were found to inhibit nucleotide metabolism are free radical scavengers, including hydroxyurea [88] and trimidox (Figure 2) [89]. Because these compounds are free radical scavengers, they can inhibit RNR by inactivating the tyrosyl radical on R2 necessary for its reductive capacity [89–94]. Although hydroxyurea was initially found to be a potent anti-neoplastic agent, it has many limitations, including low affinity for RNR and a very short half-life [95, 96]. These limitations have lead to a decrease in the use of hydroxyurea in the clinic.

More recently, iron chelators have been used as a way to target RNR [97] because iron is necessary for formation of the tyrosyl radical center [19]. 3-aminopyridine-2-carboxaldehyde thiosemicarbazone (3-AP, Triapine) is the best studied iron chelator that inhibits RNR activity (Figure 2) [98]. It is 100-fold more potent than hydroxyurea in both enzyme inhibition and cancer cell growth inhibition. It can inhibit the activity of both RRM2 and p53R2 [98, 99], which disallows for compensation by the other R2 subunit. Triapine as a single agent has generally not been effective as a therapeutic for cancer patients [100, 101]. However, in combination with DNA damage agents such as cisplatin or radiation, Triapine shows some synergistic activity [102, 103], and many clinical trials are still underway (clinicaltrials.gov). One of the main issues with Triapine is the number of side effects, including dyspnea and methemoglobinemia [97, 103, 104], which limits the use of this drug.

Data from both hydroxyurea and Triapine demonstrate that more specific RNR inhibitors are needed to minimize toxic side effects. With the identification of antisense, more recent work has gone into specifically targeting RNR subunits. Currently, an antisense oligonucleotide to RRM2 (GTI-2040) is in clinical trials (clinicaltrials.gov) (Figure 2). Phase II clinical trials with published results have shown little added benefit with GTI-2040 [105–107]. It is possible that p53R2 could compensate for RRM2 when it is inhibited. Additionally, the

effect of GTI-2040 has not been fully examined in combination with cisplatin or radiation. This may lead to a synergistic effect similar to combination therapy with Triapine (discussed above). Results from our lab using a short hairpin RNA (shRNA) specific for RRM2 indicate that these cells undergo senescence [38, 53] and not cell death. Senescent cells remain metabolically active [24], which may allow for cellular changes that could overcome senescence. Therefore, finding combination strategies that would kill these senescent cells will be important for future targeting of RNR.

4. Concluding remarks

dNTPs are necessary for both DNA replication and repair. The rate-limiting step in dNTP synthesis is the reduction of rNDPs to dNDPs by RNR. Over the past year, it has become clear that the small subunit of RNR, RRM2, plays a major role in the senescence tumor suppression mechanism. Without downregulation of RRM2, cells with activated oncogenes can bypass senescence, which may ultimately lead to cell transformation and tumorigenesis (Figure 1). Because this enzyme is so important in the establishment of uncontrolled growth in cancer and the maintenance of proliferation, anti-tumor drugs have been developed that target this specific part of the nucleotide metabolic pathway (Figure 2). More work needs to be done to explore new combination strategies by targeting RNR to drive senescence of cancer cells treated with chemotherapeutics or targeted therapies that only induce a transient cell growth arrest. Further, it will be interesting to investigate ways to eliminate senescent cells induced by RNR inhibition via promoting apoptosis. Together, these approaches may ultimately lead to a sustained, long-term response to therapeutics in cancer and enhance survival of cancer patients.

Acknowledgments

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List of 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
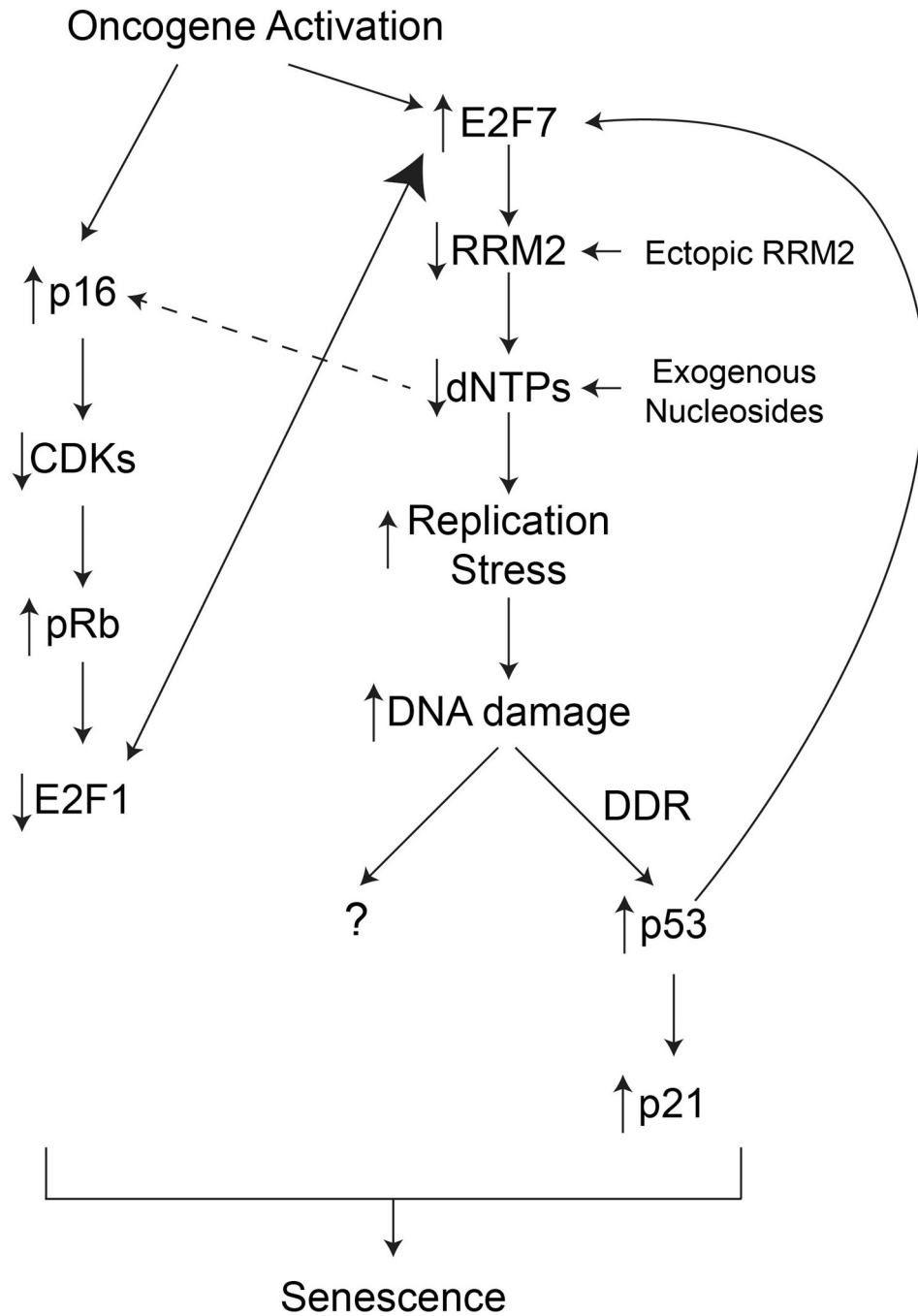
Figure 1. Overview of the oncogene-induced senescence pathway

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.
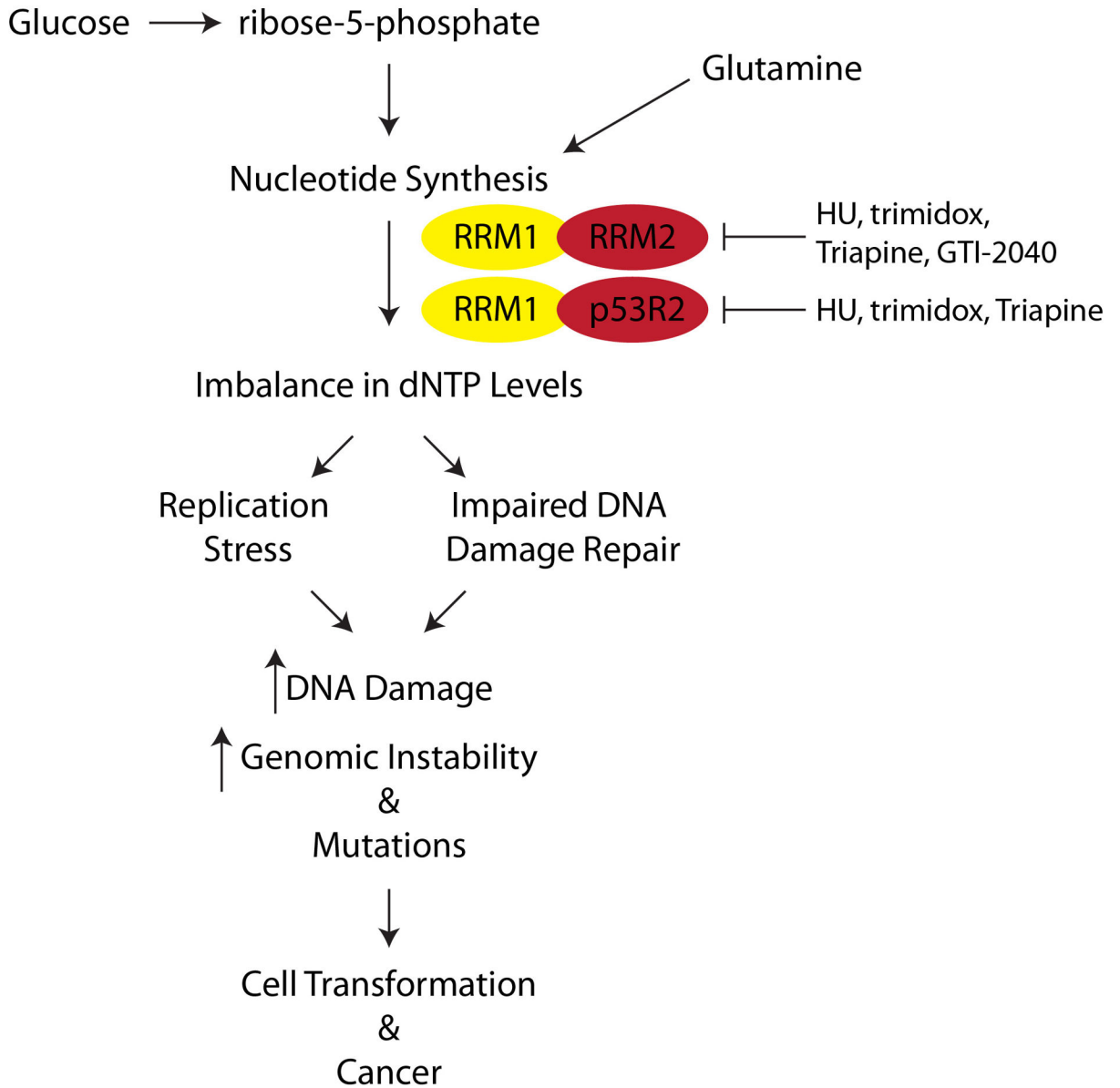
Figure 2. Overview of nucleotide metabolic pathway in cancer
Synthesis of nucleotides begins with formation of ribose-5-phosphate from glucose. Along
with glutamine, which donates the necessary nitrogen, a number of modifications lead to
formation of nucleotides. A change in RRM1, RRM2, or p53R2 expression can lead to
dNTP pool imbalances. Ultimately, this imbalance leads to replication stress and impaired
DNA damage repair, which consequently increases DNA damage, genomic instability, and
mutations. This ultimately contributes to cell transformation and cancer. Numerous

inhibitors have been developed to inhibit RRM2 and p53R2, including HU (hydroxyurea), trimidox, Triapine (3-AP), and GTI-2040.