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Emerging roles of nucleotide metabolism in cancer

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Abstract

Nucleotides are substrates for multiple anabolic pathways, most notably DNA and RNA synthesis. Since nucleotide synthesis inhibitors began to be used for cancer therapy in the 1950s, our understanding of how nucleotides function in tumor cells has evolved, prompting a resurgence of interest in targeting nucleotide metabolism for cancer therapy. In this review, we discuss recent advances that challenge the idea that nucleotides are mere building blocks for the genome and transcriptome and highlight ways that these metabolites support oncogenic signaling, stress resistance, and energy homeostasis in tumor cells. These findings point to a rich network of processes sustained by aberrant nucleotide metabolism in cancer and reveal new therapeutic opportunities.

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Declaration of interests

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Resources

History of cancer therapies targeting nucleotide synthesis

The discovery and use of chemotherapeutic agents in the 1940s and 1950s represent some of the most significant advances in the history of cancer therapy. Early seminal work by Sidney Farber established the efficacy of folate antagonists, which inhibit **nucleotide** (see Glossary) synthesis, in treating pediatric acute lymphoblastic leukemia [1,2]. Shortly thereafter, in 1951, Gertrude Elion and George Hitchings developed the purine analog 6-mercaptopurine [3,4], ushering in a new class of drugs targeting nucleotide metabolism still used to this day in oncology and multiple other areas of medicine. Elion and Hitchings received the 1988 Nobel Prize in Physiology or Medicine for their transformative work, due at least partly to the importance and lasting impact of these drugs in treating human disease [5].

Since these discoveries over seven decades ago, our knowledge surrounding nucleotide synthesis in cancer has deepened substantially. Recent findings have highlighted unique alterations in nucleotide metabolism associated with certain cancer subtypes or certain cancer-causing mutations. Critically, these patterns often confer dependencies that can be exploited therapeutically. Such discoveries have galvanized interest in repurposing classical nucleotide metabolism inhibitors and developing new agents that afford greater precision in blocking nucleotide synthesis. Moreover, new insights into context-specific reprogramming of nucleotide metabolism have nominated molecular biomarkers that hold promise for prospective identification of patients who are likely to benefit from treatment. In this review, we discuss recent advances in our understanding of nucleotide metabolism dysregulation in cancer and novel functions of nucleotides that complement their canonical roles as substrates for DNA and RNA synthesis. We group these functions on the basis of their relevance to the intersection of nucleotide metabolism and the following cellular processes: oncogenic mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) and mammalian target of rapamycin (mTOR) signaling, DNA damage repair, tumor cell state transitions, cell–cell interactions, and **central carbon metabolism**. Finally, we highlight unresolved mechanistic and translational questions in this resurgent field that merit further investigation.

Intersection of MAPK/ERK signaling and nucleotide metabolism

The MAPK/ERK pathway is a fundamental mitogenic signaling pathway that is frequently dysregulated in cancer and interfaces closely with nucleotide metabolism. In addition to the direct effects of MAPK/ERK signaling, MAPK/ERK-mediated phosphorylation of Myc can increase its stability [6], allowing Myc-driven regulation of nucleotide metabolism. Work performed 20 years ago by Graves and colleagues identified that activation of MAPK/ERK signaling leads to phosphorylation of Thr456 on the enzyme CAD (carbamoyl phosphate synthetase 2, aspartate transcarbamoylase, dihydroorotase), which catalyzes the first three steps of ***de novo* pyrimidine synthesis**, at Thr456 (Figure 1) [7]. Phosphorylation on Thr456 sensitizes CAD to allosteric activation by the *de novo* pyrimidine synthesis substrate phosphoribosyl pyrophosphate and abolishes its feedback inhibition by a terminal product of pyrimidine synthesis, uridine triphosphate (UTP). Notably, control of CAD activity by phosphorylation downstream of both MAPK and mTOR signaling (see ‘mTOR-dependent nucleotide sensing and synthesis’) appears to act additively to modulate allosteric regulation

by UTP [8]. Recently, Ali and colleagues discovered an analogous regulatory mechanism linking MAPK/ERK signaling and the ***de novo* purine synthesis** pathway [9]. ERK2 phosphorylates phosphoribosylformylglycinamidine synthase, the third of six enzymes in the *de novo* purine biosynthesis pathway [10], at Thr619. This phosphorylation event drives purine production independent of mTOR signaling [11], which stimulates pathway activity through mechanisms discussed in the following section.

Building on these fundamental discoveries, multiple groups have identified nucleotide synthesis vulnerabilities in cancers with constitutive MAPK/ERK signaling. Work from Santana-Codina and colleagues showed that supplementation of pyrimidine and purine **nucleosides** was sufficient to rescue the viability of KRAS-dependent pancreatic cancer cell lines subjected to KRAS silencing. In this context, KRAS-mediated signaling leads to MAPK-dependent upregulation of MYC, which is responsible for upregulation of the pentose phosphate pathway enzyme ribose-5-phosphate isomerase A and thus increased nucleotide biosynthesis. Furthermore, they demonstrated that inhibiting nucleotide synthesis, particularly via inhibition of the *de novo* pyrimidine synthesis pathway enzyme dihydroorotate dehydrogenase (DHODH), could overcome resistance to mitogen-activated protein kinase kinase (MEK) inhibition in KRAS-driven pancreatic cancer [12]. Reinforcing these findings, Koundinya *et al.* identified DHODH inhibitors as highly selective agents against KRAS mutant tumor cells relative to KRAS wild-type controls [13]. Subsequently, *de novo* pyrimidine synthesis has been implicated as a specific dependency in other cancers that display reliance on the MAPK/ERK signaling pathway. These include MYCN-amplified neuroblastoma [14], neuroblastomas displaying DHODH upregulation [15], and MYC-amplified medulloblastoma [16]. Intriguingly, in the context of MYC-amplified medulloblastoma, the mechanism underlying this dependency has been linked to protein glycosylation involving *O*-linked β-*N*-acetylglucosamine (O-GlcNAc) [16]. This process requires conjugation of GlcNAc to UDP, a key product of pyrimidine biosynthesis. Inhibition of pyrimidine metabolism can thus lead to impaired *O*-GlcNAcylation of Myc, a post-translational modification that promotes its stability. This regulatory modification has also been described to play a role in other tumors, both for stabilizing Myc [17] and for the nuclear accumulation of the transcription factor Sox2 [18]. These observations highlight the potential importance of sustained pyrimidine synthesis for the maintenance of oncogenic transcriptional programs. Activity of inosine monophosphate dehydrogenase (IMPDH), a purine synthetic enzyme that catalyzes the first step of guanine nucleotide synthesis, has additionally been described as a vulnerability in small cell lung cancers that harbor MYC overexpression [19].

mTORC1-dependent nucleotide sensing and synthesis

In addition to the MAPK/ERK pathway, mTOR complex 1 (mTORC1) signaling controls nucleotide synthesis via its role as a master regulator of anabolic processes in both healthy and malignant cells. Recent seminal work has characterized the molecular mechanisms by which mTORC1 signaling regulates nucleotide metabolism. Ben-Sahra *et al.* [20] and Robitaille *et al.* [21] demonstrated that S6 kinase 1 (S6K1), upon activation by mTORC1, phosphorylates CAD on Ser1859 to increase flux through the *de novo* pyrimidine synthesis pathway. In addition to direct control of pyrimidine synthesis, mTOR also promotes

production of both **purines** and **pyrimidines** through indirect mechanisms, such as by supporting **purinosome** assembly on the mitochondria during electron transport chain inhibition (Figure 2) [22]. Purinosomes are enzyme complexes composed of *de novo* purine synthesis enzymes that facilitate metabolic channeling and increased flux through this pathway. Additionally, Ben-Sahra and colleagues showed that mTOR stimulates *de novo* purine nucleotide synthesis by increasing expression of enzymes involved in the mitochondrial tetrahydrofolate cycle through an activating transcription factor 4–dependent mechanism [11]. This cycle produces one-carbon formyl groups necessary for purine ring synthesis and may also impact distal pyrimidine pools, given that conversion of deoxyuridine monophosphate to thymidine monophosphate occurs via a folate-dependent reaction. Similarly, mTORC1 stimulates the cellular entry of bicarbonate, another substrate shared by purine and pyrimidine metabolism pathways, via increased translation of the bicarbonate cotransporter SLC4A7 [23].

It is notable that the interaction between mTORC1 signaling and nucleotide synthesis pathways does not appear to be unidirectional. For example, two independent groups reported that mTORC1 activity is inhibited by purine, but not pyrimidine, nucleotide depletion [24,25]. This effect was rescued acutely by adenylates and over a longer timescale by guanylate-dependent activation of Rheb GTPase, suggesting that multiple mechanisms exist to relay nucleotide availability to mTORC1. These findings emphasize the dynamic communication between nucleotide metabolism and mTORC1. Moreover, they indicate that purines play a unique role among nucleotide species in regulating cellular macromolecule generation.

Multiple studies have leveraged these important findings to identify therapeutic vulnerabilities in cancers related to mTOR signaling and nucleotide synthesis. Valvezan *et al.* demonstrated that nucleotide pools were a key limiting factor for mTORC1-driven synthesis of rRNA and ribosomes in cells with hyperactive mTOR signaling caused by tuberous sclerosis complex inactivation [26]. A study by Lafita-Navarro and colleagues offers independent corroboration that rRNA synthesis and ribosome biogenesis are critical effectors of nucleotide synthesis pathways [27]. These cells were therefore sensitive to IMPDH inhibitor treatment.

In addition to increasing nucleotide levels to meet the demands of cell growth, mTOR signaling may also dictate the pathways cells use to supply nucleotide pools. For example, mTOR activation triggered by knockout of the NAD^+-dependent deacetylase SIRT3 causes cells to increase reliance on the *de novo* pathway for purine synthesis [28]. Phosphatase and tensin homolog (PTEN) loss also leads to hyperactive mTOR signaling, which can increase dependence on *de novo* pyrimidine synthesis through the mechanisms described earlier in this section. Mathur and colleagues showed that inhibition of *de novo* pyrimidine synthesis causes selective DNA damage and cell death in PTEN-mutant tumor cells, reflecting an oncogenotype-specific vulnerability that is now undergoing clinical testing (NCT04997993)[i] [29].

[i] clinicaltrials.gov/ct2/show/NCT04997993

Nucleotides and protection from DNA damage

Coordination between *de novo* and salvage pathways for pyrimidine and purine synthesis is critical for the provision of deoxyribonucleoside triphosphates (dNTPs) to support DNA replication during the S phase of the cell cycle [30]. Failure to harmonize the activities of these pathways causes an imbalance in nucleotide pools, leading to DNA damage in proliferating cells [31–33]. dNTP pools are proximally maintained by ribonucleotide reductase enzymes, which convert ribonucleoside diphosphates (derived from either *de novo* or salvage pathways) to deoxyribonucleoside diphosphates (dNDPs). dNDPs are then phosphorylated by nucleoside diphosphate kinases to yield dNTPs [34]. Long identified as a dependency in cancer, and inhibited by approved antimetabolite agents such as gemcitabine, ribonucleoside reductase has drawn interest as a target for development of novel inhibitors [35,36].

Although nontransformed cells may interchangeably use either or both *de novo* and salvage pathways to maintain nucleotide pools, oncogenic mechanisms can cause cancer cells to become exquisitely reliant on a single pathway or on repurposing the activity of other enzymes for nucleotide production. These insights present opportunities to develop new approaches to target nucleotide synthesis for cancer treatment with wider therapeutic windows than those associated with classical antimetabolite drugs. Specifically, the use of pathway-specific inhibitors (such as those targeting DHODH) may exploit the reliance on nucleotide synthesis programs that are inherent to tumor cells. This concept was highlighted in two recent independent studies which revealed that specific subtypes of brain cancer display hyperdependence on DHODH activity and *de novo* pyrimidine synthesis to evade genotoxic stress. Pal *et al.* showed that pediatric diffuse midline glioma (DMG) tumors display elevated flux through the pyrimidine degradation pathway, thus increasing demand for *de novo* pyrimidine synthesis to maintain nucleotide availability. Because balanced dNTP pools are required for efficient DNA replication, DMG cells treated with BAY 2402234, an inhibitor of DHODH, undergo potent replication stress, irreparable DNA damage, and cell death [37]. Work from our group revealed a similar liability in glioma brain tumors with isocitrate dehydrogenase (*IDH*) mutations [38]. In this context, continuous *de novo* pyrimidine synthesis is required to counteract mutant IDH-induced replication stress sensitivity. Indeed, emerging evidence has linked epigenetic reprogramming by *IDH* mutations to replication fork stalling and S-phase prolongation [39]. Although the underlying mechanism differs from DMG, we found that BAY 2402234 preferentially kills IDH-mutant glioma cells by inducing a similar cascade of nucleotide imbalance and DNA damage. Cancer-specific nucleotide metabolism dependencies also extend beyond canonical *de novo* and salvage pathways. For instance, KRAS/LKB1-mutant lung cancers depend on an alternative pathway to maintain nucleotide balance by co-opting the ammonia-consuming urea cycle enzyme CPS1 to drive pyrimidine synthesis [40]. In this context, inhibiting CPS1 causes DNA polymerase stalling and compromises progression through S-phase, leading to DNA damage and tumor inhibition.

These findings highlight unique dependencies in pyrimidine metabolism, and the above findings in *IDH*-mutant gliomas and DMGs expand on earlier foundational studies of *de novo* pyrimidine synthesis in *IDH* wild-type adult brain tumors. In these tumors, oncogenic

mutations that aberrantly activate MAPK/ERK or mTOR signaling drive *de novo* pyrimidine synthesis, which in turn causes resistance to MAPK/ERK and mTOR inhibitors [41]. Thus, pyrimidine synthesis antagonists can be used in concert with signaling inhibitors to target gliomas with constitutive activation of mitogenic signaling pathways or used alone to treat brain tumors with low basal levels of mitogenic signaling [42]. Although much effort has been directed toward understanding dysregulation of *de novo* pyrimidine synthesis in brain tumors, Myc-dependent upregulation of *de novo* purine synthesis is also recognized to play a key role in promoting fitness of glioma stemlike cells [41]. Importantly, inhibiting purine metabolism potently sensitizes IDH wild-type brain tumors to radiotherapy [43] and the alkylating agent temozolomide [44], providing an additional link between sustained nucleotide metabolism and prevention of genotoxic stress. Clinical testing of purine synthesis inhibition during radiation and temozolomide therapies is currently underway in glioma (NCT05236036 and NCT04477200)[ii],[iii], with promising interim results [45]. Upregulation of *de novo* pyrimidine synthesis has similarly been shown to confer resistance to the genotoxic chemotherapeutic doxorubicin in triple-negative breast cancer, which could potentially be targeted to resensitize resistant cells [46].

Collectively, these recent studies illustrate altered nucleotide metabolism in multiple cancer subtypes and outline ways to exploit these vulnerabilities to induce DNA damage and cell death. This therapeutic strategy appears particularly promising in cell contexts where DNA damage repair mechanisms are already impaired [29,38,47].

Nucleotides as mediators of cell fate and cell function

Although the importance of nucleotides in DNA synthesis and anabolic metabolism has long been appreciated, recent data indicate that an important noncanonical role of nucleotide metabolism pertains to the regulation of cell fate. In leukemia, a disease largely defined by cell state dysregulation and differentiation arrest, inhibition of nucleotide synthesis (e.g., by blocking DHODH activity) has been shown to trigger differentiation, highlighting the importance of this pathway in maintaining a stemlike leukemic phenotype [48]. The molecular mechanism underlying this effect is reported to be dependent on replication stress but independent of replication stress-induced signaling. Work from Hsu *et al.* shows that nucleotide depletion in leukemic cells triggers changes in gene expression networks that culminate in lineage differentiation [49]. Specifically, they demonstrate that replication stress alters chromatin accessibility and enables binding of transcription factors that direct lineage differentiation programs. Although DNA damage has itself been linked to cell state control [50–55], the mechanism described by Hsu *et al.* is not dependent on DNA damage signaling. These studies suggest that drugs targeting nucleotide synthesis may have utility as differentiation therapies in cancers with clearly defined differentiation blocks. Indeed, the DHODH inhibitor BAY 2402234 was recently evaluated in an early phase clinical trial for patients with myeloid malignancies (NCT03404726)[iv] [56]. Although this trial was

[ii] clinicaltrials.gov/ct2/show/NCT05236036
[iii] clinicaltrials.gov/ct2/show/NCT04477200
[iv] clinicaltrials.gov/ct2/show/NCT03404726

terminated due to lack of sufficient clinical benefit, strategies to enhance differentiation caused by nucleotide metabolism inhibitors are emerging [57].

Nucleotide metabolism has also been shown to play a potentially important role in controlling cell fate in solid cancers. As mentioned already, sustained *de novo* purine and pyrimidine synthesis is required for the fitness of stemlike cells in glioblastoma [41], suggesting the connection between nucleotide metabolism and stemness extends beyond leukemia. Studies have also implicated these pathways in the adoption of cancer-associated phenotypes, including work linking global levels of nucleotide pools to metastatic invasiveness in breast cancer [58] and data demonstrating that *de novo* pyrimidine synthesis facilitates transcriptional programs important for neural crest development in melanoma [59]. In keeping with a general association between enhanced nucleotide metabolism and tumor aggressiveness, several studies have revealed that altered nucleotide metabolism can facilitate epithelial-to-mesenchymal cell state transitions (EMT) in cancer. Shaul *et al.* identified that activity of the pyrimidine nucleotide degradation enzyme dihydropyrimidine dehydrogenase is necessary for breast cancer cells to undergo EMT [60]. Similarly, Soflaee *et al.* showed that intracellular purine nucleotide depletion causes increased serine synthesis and triggers EMT in melanoma cells [61]. These studies draw apparent connections between nucleotide synthesis and progenitor-like cell states and between nucleotide depletion and cell states related to stress resistance and/or migration. Going forward, studies of the precise molecular mechanisms underlying nucleotide metabolism-dependent regulation of tumor cell identity and behavior are warranted.

Cell–cell interactions driven by nucleotides

In addition to their cell-autonomous functions, nucleotides and nucleosides are secreted by cells to initiate paracrine and autocrine signaling and drive heterocellular metabolic networks. These interactions play key roles in regulating inflammatory processes and immune responses that ultimately shape tumor evolution [62–64]. Beyond signaling, recent evidence shows that local release of pyrimidines and purines also serves a metabolic function, enabling tumor cells to source substrates from neighboring stromal cell populations to fuel nucleotide salvage pathways. Because the literature surrounding **purinergic signaling** in cancer is particularly rich, we focus the discussion in the following section on recent studies of its role in brain cancer, though themes that emerge from the highlighted studies could hold relevance for other cancer types.

Adenosine and ATP are two purine metabolites that drive purinergic signaling between immune cells and tumor cells. In this scheme, extracellular ATP released by dying, damaged, or stressed cells in the tumor microenvironment can trigger proinflammatory signaling through type 2 purinergic (P2) G protein–coupled receptors (GPCRs) expressed on the surface of local immune cells. Alternatively, the enzyme ectonucleoside triphosphate diphosphohydrolase 1 (ENTPD1, also known as CD39), expressed by immune cells and vascular endothelial cells [62], hydrolyzes ATP to form AMP. Then, the enzyme 5’-nucleotidase (NT5E, also known as CD73) expressed on the surface of tumor cells converts AMP to adenosine. Although CD73 activity appears to be the dominant mechanism of ATP hydrolysis in the tumor microenvironment, other enzymes, including alkaline phosphatases,

nucleotide pyrophosphatases, and phosphodiesterases, can also contribute [63]. Unlike ATP, adenosine induces potent immunosuppression in the tumor microenvironment by activating type 1 purinergic (P1) GPCR signaling in immune cells. Adenosine accumulation can suppress multiple branches of the tumor immune response, including T-helper type 1 cell cytokine release, mononuclear phagocyte maturation, and effector T cell function [63].

Recent studies have extended seminal work describing the impact of purinergic signaling in the tumor microenvironment. In the context of glioblastoma brain tumors, tumor cells have been shown to drive extracellular adenosine accumulation through hyperactivation of ectonucleotidases. Interestingly, alteration of tumor metabolism to release the tryptophan-derived metabolite kynurenine activates aryl hydrocarbon receptors on tumor-associated macrophages in glioblastoma models, subsequently driving CD39 expression and dampening tumor immune responses [65]. Also in glioblastoma models, silencing CD73 expression in host tissues improved survival of mice bearing orthotopic syngeneic allografts treated with immune checkpoint inhibitors, demonstrating dual control of the immune response by tumoral and microenvironmental mechanisms [66]. A more recent study by Coy *et al.* further defined the spatial landscape of purinergic signaling in glioblastoma. They demonstrated that microglia and glioma cells predominantly express CD39 and CD73, respectively, and that enrichment of spatially restricted coexpression patterns of these enzymes correlated with signatures of immunosuppressive environments and poor clinical outcomes [67].

In contrast to the numerous roles that purines play in driving cell–cell interactions in the tumor microenvironment, pyrimidines appear less involved in such crosstalk mechanisms. Nonetheless, a recent study from Halbrook and colleagues revealed that pancreatic ductal adenocarcinoma cells induce tumor-associated macrophages to release pyrimidines as a mechanism of chemotherapy resistance [68]. Specifically, macrophages adopt the alternate M2 activation phenotype upon exposure to tumor-secreted factors and begin to release pyrimidine nucleosides, including deoxycytidine, into the tumor microenvironment. This process is dependent on *de novo* pyrimidine synthesis in tumor-associated macrophages. Local accumulation of extracellular deoxycytidine competitively inhibits tumor cell uptake of gemcitabine, a pyrimidine antinucleoside agent that is a key component of standard therapy for pancreatic cancer, thereby blunting antitumor activity [68]. These data inform a compelling model in which a heterocellular metabolic interaction dictates chemotherapeutic efficacy, raising the possibility that similar mechanisms may impact the activity of antimetabolite therapies in other cancer contexts. Indeed, a recent study by Teng *et al.* implicated the gut microbiota as a source of nucleosides that can antagonize chemotherapy and radiation efficacy in rectal cancer [69].

Ribose as a substrate for central carbon metabolism

Pyrimidine nucleotides are essential for diverse cellular processes, including protein glycosylation and phospholipid production in addition to DNA/RNA synthesis. Nucleotides consist of a nucleoside and phosphate group(s). Nucleosides contain both nitrogenous **nucleobases** and a ribose moiety. Typically, the pyrimidine nucleosides uridine, thymidine, and cytidine serve as substrates for salvage synthesis of the nucleotides uridine

monophosphate, thymidine monophosphate, and cytidine monophosphate or deoxycytidine monophosphate, respectively. Under nutrient-limiting conditions, however, recent studies have shown that tumor cells can degrade pyrimidine nucleosides, thereby liberating ribose sugars that can be used to fuel central carbon metabolism.

Studies of cancer cells expressing elevated levels of pyrimidine nucleoside phosphorylases nominated the nucleosides thymidine and uridine as alternative carbon sources during glucose restriction. Tabata and colleagues showed that thymidine breakdown into thymine and 2-deoxy-D-ribose-1-phosphate imparted resistance to the glucose metabolism inhibitor 2-deoxy-D-glucose in cells expressing thymidine phosphorylase. Ribose-derived carbon atoms from thymidine accumulated in lactate, phosphoribosyl pyrophosphate, and N-acetylamino acid pools, indicating that thymidine catabolism contributes to flux through glycolytic, pentose phosphate, and amino acid acetylation pathways when glucose catabolism is impaired [70].

More recently, two studies reported that ribose-1-phosphate, produced together with uracil through uridine catabolism, is metabolized via the nonoxidative pentose phosphate pathway to support glycolytic flux during nutrient deprivation. Jourdain and colleagues identified expression of uridine phosphorylase enzymes as a key determinant of tumor cell proliferation in glucose-free media [71]. Interestingly, supplementation with purified RNA rescued growth defects caused by sugar starvation in uridine phosphorylase-expressing cells, but not those lacking this enzyme. Like in the case of thymidine catabolism, labeled carbon atoms from the ribose moiety of uridine contributed to synthesis of intermediates in the pentose phosphate, glycolysis, and tricarboxylic acid cycle pathways. A contemporaneous study from Ward and colleagues identified uridine from among more than 175 metabolites evaluated as potential substrates for pancreatic ductal adenocarcinoma (PDAC) cell metabolism during nutrient restriction. They also tied uridine catabolism proficiency to expression of uridine phosphorylase, particularly UPP1 [72]. Importantly, they showed that knockout of uridine phosphorylase impaired the growth of pancreatic cancer xenografts, suggesting that the nutrient-poor PDAC microenvironment may provide selective pressure for tumor cells to degrade uridine. Future studies that clarify the extent of sugar depletion and nucleoside supply that are required to activate pyrimidine nucleoside catabolism will be important for assessing the generalizability of this phenotype in cancer.

Concluding remarks

Research in the past decade, conducted more than 60 years since the initial discoveries of Elion and Hitchings [3–5], has provided important insights into the reprogramming of nucleotide metabolism in cancer. Furthermore, this work has revealed previously unappreciated roles that nucleotides play in regulating a broad array of cellular processes that affect tumor cell fitness. Two broad themes emerge from recent work in this space and inform key unanswered questions that will shape future investigation (see Outstanding questions). First, nucleotides and nucleosides have critical functions outside of their canonical roles as RNA and DNA constituents. They act as extracellular signaling molecules [65,66], cell state regulators [48,60], and fuel sources during environmental stress [68,70,73]. Novel functions of nucleotide metabolism continue to emerge, including

a role for DHODH in cellular adaptation to electron transport chain impairment [74,75] and a related, albeit disputed, role in ferroptosis prevention [76,77]. Second, a historical view of pyrimidines and purines as obligate pairs of substrates for RNA and DNA synthesis is complemented by a new understanding of nucleotide class-specific activities and regulatory mechanisms. Current research provides examples of processes (such as DNA damage [37,38,43] and mTORC1 regulation [24,25]) that are distinctly tied to either pyrimidines or purines, but not both. These findings indicate that these nucleotide classes are not functionally interchangeable and, perhaps as a result, are independently controlled [11,20,21,23].

Advances in our knowledge of the intersection of nucleotide metabolism and cancer biology have revealed opportunities for direct therapeutic translation. Cancers may develop dependence on one of the two synthetic pathways (*de novo* and salvage) for nucleotide synthesis to maintain nucleotide pools and cell fitness. These dependencies may be driven by constrained substrate availability in the tumor microenvironment, constitutive activation of mitogenic signaling pathways, elevated cellular nucleotide demand, or other factors. Efforts to exploit these dependencies pharmacologically are ongoing, including many involving repurposing of classical nucleotide synthesis inhibitors (NCT04997993, NCT05236036, and NCT04477200)[i,ii,iii] [43,44]. Equipped with this newfound knowledge, translational efforts are increasingly guided by biomarkers of response to nucleotide synthesis inhibitors that have been identified through detailed mechanistic studies. Nevertheless, fundamental questions remain, including how tumor cells can adapt to nucleotide depletion and which cancer contexts harbor nucleotide metabolism dependencies that are strong enough to drive a wide therapeutic window for nucleotide-depleting treatment strategies. Answers to these questions will be vital for determining the outcomes of efforts to translate nucleotide metabolism inhibitors to the clinic for new cancer indications.

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Glossary

Central carbon metabolism
metabolic pathways, including glycolysis, gluconeogenesis, the citric acid cycle, and the pentose phosphate pathway, that transform carbon into biomolecular substrates and energy.

***De novo* pyrimidine and purine synthesis**
biosynthetic activities that incorporate carbon and nitrogen atoms from non-nucleobase metabolites to produce nucleotides.

Nucleobases (nitrogenous bases)
single- or double-ringed nitrogen-containing metabolite that can be conjugated to ribose to form a nucleoside. Canonical nucleobases are subdivided into pyrimidine (single ring) and purine (fused rings) nucleobases.

Nucleosides
metabolites comprised of a nucleobase and ribose.

Nucleotides
metabolites comprised of a nucleoside with one or more phosphate groups.

Purinergic signaling
extracellular signaling mediated by release of purine nucleotides or nucleosides.

Purines
metabolites (nucleobases, nucleosides, and/or nucleotides) containing a purine ring. Purine rings are comprised of fused five- and six-membered rings containing carbon and nitrogen.

Purinosome
a protein complex comprised of multiple *de novo* purine synthesis enzymes that facilitates metabolic flux through this pathway.

Pyrimidines
metabolites (nucleobases, nucleosides, and/or nucleotides) containing a pyrimidine ring structure. Pyrimidine rings are comprised of a single six-membered ring containing carbon and nitrogen.

Salvage nucleotide synthesis
biosynthetic activities that use a nucleobase as a starting substrate to produce nucleotides.

References

1. Farber S. et al. (1948) Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroylglutamic acid (aminopterin). N. Engl. J. Med 238, 787–793 [PubMed: 18860765]
2. Farber S. (1949) Some observations on the effect of folic acid antagonists on acute leukemia and other forms of incurable cancer. Blood 4, 160–167 [PubMed: 18107667]
3. Burchenal JH et al. (1953) Clinical evaluation of a new antimetabolite, 6-mercaptopurine, in the treatment of leukemia and allied diseases. Blood 8, 965–999 [PubMed: 13105700]
4. Burchenal JH et al. (1954) Clinical studies on 6-mercaptopurine. Ann. N.Y. Acad. Sci 60, 359–368 [PubMed: 14350539]
5. Elion GB (1989) The purine path to chemotherapy. Biosci. Rep 9, 509–529 [PubMed: 2679902]
6. Sears R. et al. (2000)Multiple Ras-dependent phosphorylation pathways regulate Myc protein stability. Genes Dev. 14, 2501–2514 [PubMed: 11018017]
7. Graves LM et al. (2000) Regulation of carbamoyl phosphate synthetase by MAP kinase. Nature 403, 328–332 [PubMed: 10659854]
8. Shin J. et al. (2023) Allosteric regulation of CAD modulates de novo pyrimidine synthesis during the cell cycle. Nat. Metab 5, 277–293 [PubMed: 36747088]
9. Ali ES et al. (2020) ERK2 phosphorylates PFAS to mediate posttranslational control of de novo purine synthesis. Mol. Cell 78, 1178–1191.e6 [PubMed: 32485148]
10. Pareek V. et al. (2021) Human de novo purine biosynthesis. Crit. Rev. Biochem. Mol. Biol 56, 1–16 [PubMed: 33179964]
11. Ben-Sahra I. et al. (2016) mTORC1 induces purine synthesis through control of the mitochondrial tetrahydrofolate cycle. Science 351, 728–733 [PubMed: 26912861]
12. Santana-Codina N. et al. (2018) Oncogenic KRAS supports pancreatic cancer through regulation of nucleotide synthesis. Nat. Commun 9, 4945 [PubMed: 30470748]

13. Koundinya M. et al. (2018) Dependence on the pyrimidine biosynthetic enzyme DHODH Is a synthetic lethal vulnerability in mutant KRAS-driven cancers. Cell Chem. Biol 25, 705–717.e11 [PubMed: 29628435]
14. Yu Y. et al. (2021) Therapeutic targeting of both dihydroorotate dehydrogenase and nucleoside transport in MYCN-amplified neuroblastoma. Cell Death Dis. 12, 821 [PubMed: 34462431]
15. Olsen TK et al. (2022) DHODH is an independent prognostic marker and potent therapeutic target in neuroblastoma. JCI Insight 7, e153836 [PubMed: 35943801]
16. Gwynne WD et al. (2022) Cancer-selective metabolic vulnerabilities in MYC-amplified medulloblastoma. Cancer Cell 40, 1488–1502.e7 [PubMed: 36368321]
17. Itkonen HM et al. (2013) O-GlcNAc transferase integrates metabolic pathways to regulate the stability of c-MYC in human prostate cancer cells. Cancer Res. 73, 5277–5287 [PubMed: 23720054]
18. Echizenya S. et al. (2020) Discovery of a new pyrimidine synthesis inhibitor eradicating glioblastoma-initiating cells. Neuro-Oncology 22, 229–239 [PubMed: 31499527]
19. Huang F. et al. (2018) Inosine monophosphate dehydrogenase dependence in a subset of small cell lung cancers. Cell Metab. 28, 369–382.e5 [PubMed: 30043754]
20. Ben-Sahra I. et al. (2013) Stimulation of de novo pyrimidine synthesis by growth signaling through mTOR and S6K1. Science 339, 1323–1328 [PubMed: 23429703]
21. Robitaille AM et al. (2013) Quantitative phosphoproteomics reveal mTORC1 activates de novo pyrimidine synthesis. Science 339, 1320–1323 [PubMed: 23429704]
22. French JB et al. (2016) Spatial colocalization and functional link of purinosomes with mitochondria. Science 351, 733–737 [PubMed: 26912862]
23. Ali ES et al. (2022) The mTORC1-SLC4A7 axis stimulates bicarbonate import to enhance de novo nucleotide synthesis. Mol. Cell 82, 3284–3298.e7 [PubMed: 35772404]
24. Emmanuel N. et al. (2017) Purine nucleotide availability regulates mTORC1 activity through the Rheb GTPase. Cell Rep. 19, 2665–2680 [PubMed: 28658616]
25. Hoxhaj G. et al. (2017) The mTORC1 signaling network senses changes in cellular purine nucleotide levels. Cell Rep. 21, 1331–1346 [PubMed: 29091770]
26. Valvezan AJ et al. (2017) mTORC1 couples nucleotide synthesis to nucleotide demand resulting in a targetable metabolic vulnerability. Cancer Cell 32, 624–638.e5 [PubMed: 29056426]
27. Lafita-Navarro MC et al. (2020) Inhibition of the de novo pyrimidine biosynthesis pathway limits ribosomal RNA transcription causing nucleolar stress in glioblastoma cells. PLoS Genet. 16, e1009117 [PubMed: 33201894]
28. Gonzalez Herrera KN et al. (2018) Small-molecule screen identifies de novo nucleotide synthesis as a vulnerability of cells lacking SIRT3. Cell Rep. 22, 1945–1955 [PubMed: 29466723]
29. Mathur D. et al. (2017) PTEN regulates glutamine flux to pyrimidine synthesis and sensitivity to dihydroorotate dehydrogenase inhibition. Cancer Discov. 7, 380–390 [PubMed: 28255082]
30. Diehl FF et al. (2022) Nucleotide imbalance decouples cell growth from cell proliferation. Nat. Cell Biol 24, 1252–1264 [PubMed: 35927450]
31. Meuth M. (1989) The molecular basis of mutations induced by deoxyribonucleoside triphosphate pool imbalances in mammalian cells. Exp. Cell Res 181, 305–316 [PubMed: 2647496]
32. Anglana M. et al. (2003) Dynamics of DNA replication in mammalian somatic cells: nucleotide pool modulates origin choice and interorigin spacing. Cell 114, 385–394 [PubMed: 12914702]
33. Poli J. et al. (2012) dNTP pools determine fork progression and origin usage under replication stress. EMBO J. 31, 883–894 [PubMed: 22234185]
34. Aye Y. et al. (2015) Ribonucleotide reductase and cancer: biological mechanisms and targeted therapies. Oncogene 34, 2011–2021 [PubMed: 24909171]
35. Huff SE et al. (2022) Inhibitors of the cancer target ribonucleotide reductase, past and present. Biomolecules 12, 815 [PubMed: 35740940]
36. Greene BL et al. (2020) Ribonucleotide reductases: structure, chemistry, and metabolism suggest new therapeutic targets. Annu. Rev. Biochem 89, 45–75 [PubMed: 32569524]
37. Pal S. et al. (2022) A druggable addiction to de novo pyrimidine biosynthesis in diffuse midline glioma. Cancer Cell 40, 957–972.e10 [PubMed: 35985342]

38. Shi DD et al. (2022) De novo pyrimidine synthesis is a targetable vulnerability in IDH mutant glioma. Cancer Cell 40, 939–956.e16 [PubMed: 35985343]
39. Schvartzman J-M et al. (2022) Oncogenic IDH mutations increase heterochromatin-related replication stress without impacting tumor mutation burden. bioRxiv Published online August 5, 2022. 10.1101/2022.08.04.502834
40. Kim J. et al. (2017) CPS1 maintains pyrimidine pools and DNA synthesis in KRAS/LKB1-mutant lung cancer cells. Nature 546, 168–172 [PubMed: 28538732]
41. Wang X. et al. (2017) Purine synthesis promotes maintenance of brain tumor initiating cells in glioma. Nat. Neurosci 20, 661–673 [PubMed: 28346452]
42. Spina R. et al. (2022) DHODH inhibition impedes glioma stem cell proliferation, induces DNA damage, and prolongs survival in orthotopic glioblastoma xenografts. Oncogene 41, 5361–5372 [PubMed: 36344676]
43. Zhou W. et al. (2020) Purine metabolism regulates DNA repair and therapy resistance in glioblastoma. Nat. Commun 11, 3811 [PubMed: 32732914]
44. Shireman JM et al. (2021) De novo purine biosynthesis is a major driver of chemoresistance in glioblastoma. Brain 144, 1230–1246 [PubMed: 33855339]
45. Umemura Y. et al. (2022) CTNI-35. Phase 0/1 trial of mycophenolate mofetil combined with chemoradiation to overcome treatment resistance in newly diagnosed and recurrent glioblastoma by targeting purine metabolism. Neuro-Oncology 24, vii79
46. Brown KK et al. (2017) Adaptive reprogramming of de novo pyrimidine synthesis is a metabolic vulnerability in triple-negative breast cancer. Cancer Discov. 7, 391–399 [PubMed: 28255083]
47. Juvekar A. et al. (2016) Phosphoinositide 3-kinase inhibitors induce DNA damage through nucleoside depletion. Proc. Natl. Acad. Sci. U. S. A 113, E4338–E4347 [PubMed: 27402769]
48. Sykes DB et al. (2016) Inhibition of dihydroorotate dehydrogenase overcomes differentiation blockade in acute myeloid leukemia. Cell 167, 171–186 e15 [PubMed: 27641501]
49. Hsu PP et al. (2022) Nucleotide depletion promotes cell fate transitions by inducing DNA replication stress. bioRxiv Published online August 16, 2022. 10.1101/2022.08.16.503984
50. Inomata K. et al. (2009) Genotoxic stress abrogates renewal of melanocyte stem cells by triggering their differentiation. Cell 137, 1088–1099 [PubMed: 19524511]
51. Wang J. et al. (2012) A differentiation checkpoint limits hematopoietic stem cell self-renewal in response to DNA damage. Cell 148, 1001–1014 [PubMed: 22385964]
52. Santos MA et al. (2014) DNA-damage-induced differentiation of leukaemic cells as an anti-cancer barrier. Nature 514, 107–111 [PubMed: 25079327]
53. Molinuevo R. et al. (2020) The DNA damage response links human squamous proliferation with differentiation. J. Cell Biol 219, e202001063 [PubMed: 33007086]
54. Kato T. et al. (2021) Dynamic stem cell selection safeguards the genomic integrity of the epidermis. Dev. Cell 56, 3309–3320.e5 [PubMed: 34932948]
55. Grow EJ et al. (2021) p53 convergently activates Dux/DUX4 in embryonic stem cells and in facioscapulohumeral muscular dystrophy cell models. Nat. Genet 53, 1207–1220 [PubMed: 34267371]
56. Christian S. et al. (2019) The novel dihydroorotate dehydrogenase (DHODH) inhibitor BAY 2402234 triggers differentiation and is effective in the treatment of myeloid malignancies. Leukemia 33, 2403–2415 [PubMed: 30940908]
57. Zee BM et al. (2021) Combined epigenetic and metabolic treatments overcome differentiation blockade in acute myeloid leukemia. iScience 24, 102651 [PubMed: 34151238]
58. Kollareddy M. et al. (2015) Regulation of nucleotide metabolism by mutant p53 contributes to its gain-of-function activities. Nat. Commun 6, 7389 [PubMed: 26067754]
59. White RM et al. (2011) DHODH modulates transcriptional elongation in the neural crest and melanoma. Nature 471, 518–522 [PubMed: 21430780]
60. Shaul YD et al. (2014) Dihydropyrimidine accumulation is required for the epithelial-mesenchymal transition. Cell 158, 1094–1109 [PubMed: 25171410]
61. Soflaee MH et al. (2022) Purine nucleotide depletion prompts cell migration by stimulating the serine synthesis pathway. Nat. Commun 13, 2698 [PubMed: 35577785]

62. Moesta AK et al. (2020) Targeting CD39 in cancer. Nat. Rev. Immunol 20, 739–755 [PubMed: 32728220]
63. Antonioli L. et al. (2013) Immunity, inflammation and cancer: a leading role for adenosine. Nat. Rev. Cancer 13, 842–857 [PubMed: 24226193]
64. Di Virgilio F and Adinolfi E (2017) Extracellular purines, purinergic receptors and tumor growth. Oncogene 36, 293–303 [PubMed: 27321181]
65. Takenaka MC et al. (2019) Control of tumor-associated macrophages and T cells in glioblastoma via AHR and CD39. Nat. Neurosci 22, 729–740 [PubMed: 30962630]
66. Goswami S. et al. (2020) Immune profiling of human tumors identifies CD73 as a combinatorial target in glioblastoma. Nat. Med 26, 39–46 [PubMed: 31873309]
67. Coy S. et al. (2022) Single cell spatial analysis reveals the topology of immunomodulatory purinergic signaling in glioblastoma. Nat. Commun 13, 4814 [PubMed: 35973991]
68. Halbrook CJ et al. (2019) Macrophage-released pyrimidines inhibit gemcitabine therapy in pancreatic cancer. Cell Metab. 29, 1390–1399.e6 [PubMed: 30827862]
69. Teng H. et al. (2023) Gut microbiota-mediated nucleotide synthesis attenuates the response to neoadjuvant chemoradiotherapy in rectal cancer. Cancer Cell 41, 124–138.e6 [PubMed: 36563680]
70. Tabata S. et al. (2017) Thymidine catabolism as a metabolic strategy for cancer survival. Cell Rep. 19, 1313–1321 [PubMed: 28514652]
71. Jourdain AA et al. (2021) Salvage of ribose from uridine or RNA Supports glycolysis when glucose is limiting. bioRxiv Published online June 10, 2021. 10.1101/2021.06.10.447789
72. Ward MH et al. (2021) Nutrient profiling reveals extracellular uridine as a fuel for pancreatic cancer through uridine phosphorylase 1. bioRxiv Published online June 8, 2021. 10.1101/2021.06.07.447448
73. Bennett CF et al. (2021) Peroxisomal-derived ether phospholipids link nucleotides to respirasome assembly. Nat. Chem. Biol 17, 703–710 [PubMed: 33723432]
74. Spinelli JB et al. (2021) Fumarate is a terminal electron acceptor in the mammalian electron transport chain. Science 374, 1227–1237 [PubMed: 34855504]
75. Bajzikova M. et al. (2019) Reactivation of dihydroorotate dehydrogenase-driven pyrimidine biosynthesis restores tumor growth of respiration-deficient cancer cells. Cell Metab. 29, 399–416.e10 [PubMed: 30449682]
76. Mao C. et al. (2021) DHODH-mediated ferroptosis defence is a targetable vulnerability in cancer. Nature 593, 586–590 [PubMed: 33981038]
77. Mishima E. et al. (2022) DHODH inhibitors sensitize cancer cells to ferroptosis via FSP1 inhibition. Res. Sq Published online October 24, 2022. 10.21203/rs.3.rs-2190326/v1

Highlights

Recent studies have broadened our understanding of oncogenic reprogramming of nucleotide metabolism, spurring renewed interest in targeting these pathways for therapy.

Mitogen-activated protein kinase/extracellular signal-regulated kinase and mammalian target of rapamycin pathways regulate and respond to cellular nucleotide pools, creating vulnerabilities in some cancers with constitutive activation of these signaling pathways.

Nucleotide imbalance and nucleoside degradation regulate cell state transitions and cell fate decisions, particularly following replication stress.

Secretion of nucleotides and nucleosides into the tumor microenvironment influences immune responses and treatment efficacy.

Ribose sugars liberated by nucleoside catabolism serve as a key fuel source for central carbon metabolism during nutrient deprivation.

Outstanding questions

mTORC1 can sense purine abundance. What are the precise molecular mechanisms underlying this sensing function? Do similar, perhaps mTOR-independent, mechanisms exist within the cell to monitor abundance of pyrimidines?

Nucleotides can be synthesized through both *de novo* and salvage pathways. What are the mechanisms that coordinate the balance between *de novo* and **salvage nucleotide synthesis** activities in nonmalignant cells? How is this balance altered during malignant transformation and tumorigenesis?

Tumor cells respond differently to nucleotide imbalance during DNA replication, based on their cell of origin, mutational profile, metabolic phenotype, and other factors. What are the molecular determinants that link each of these factors with cell fate after replication stress exposure?

Secreted nucleotides and nucleosides modulate antitumor immunity through purinergic signaling. How do cells in the tumor microenvironment modify intracellular biochemical pathways to influence the local extracellular levels of nucleotides and nucleosides?

Nucleosides can be degraded to sustain tumor cell metabolism during nutrient deprivation. What are the tumor cell–intrinsic and microenvironmental reprogramming events that are required to facilitate switching to nucleosides as fuel sources?

Many tumor cell types display unique susceptibility to nucleotide metabolism inhibitors relative to nonmalignant cells. Which of these differential vulnerabilities are robust enough to drive a wide therapeutic window necessary to support clinical testing of nucleotide metabolism inhibitors?

Mounting evidence shows that nucleotides and nucleosides function independently of their canonical roles as substrates for biomolecule synthesis. Are there additional uncharacterized roles of these metabolites that may be altered in cancer?

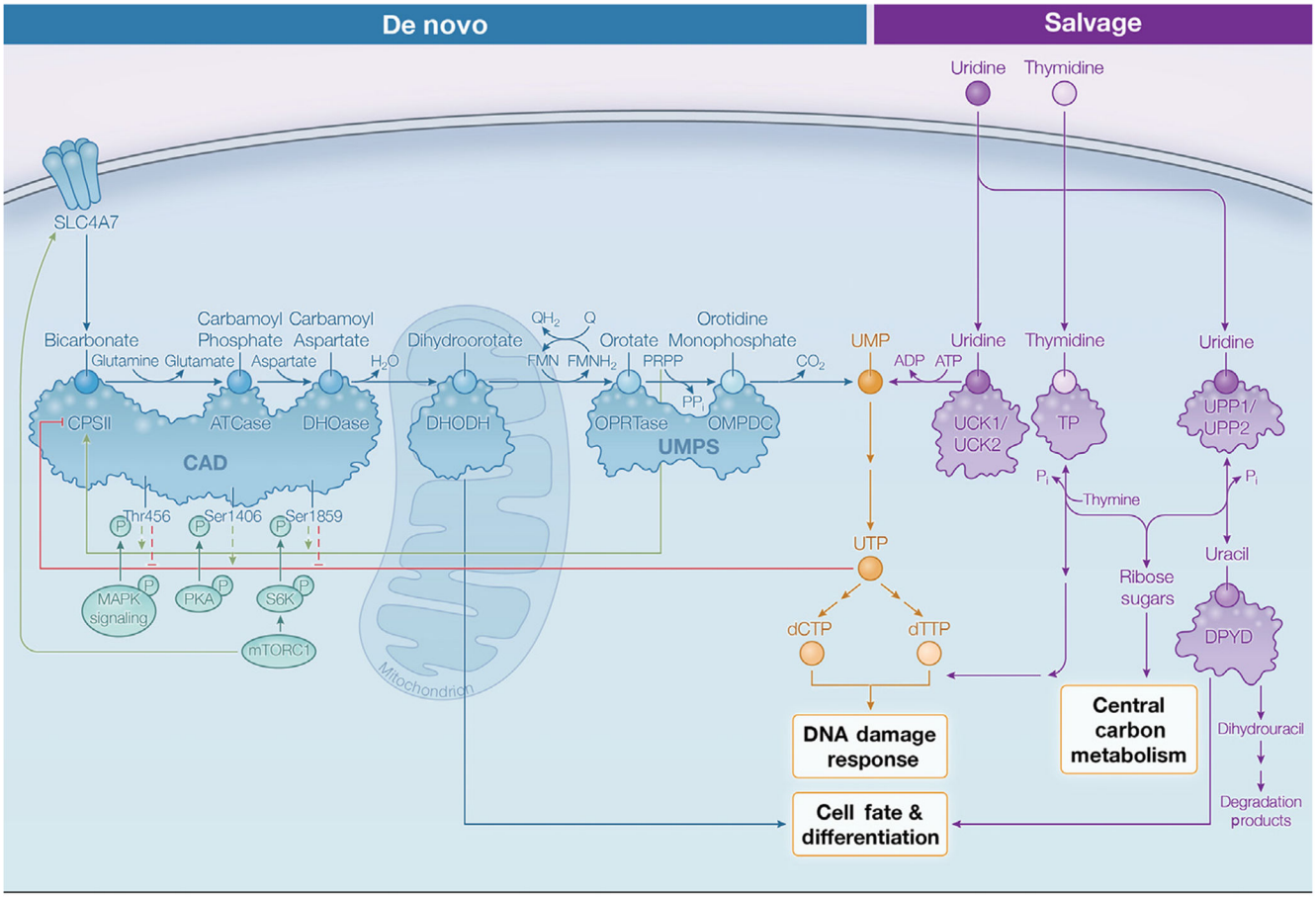

Figure 1. Pyrimidine nucleotide synthetic pathways and cellular consequences.

Pyrimidine nucleotides are synthesized by either the *de novo* or salvage pathway. *De novo* pathway (blue): The carbamoyl phosphate synthetase II (CPSII) domain of the enzyme carbamoyl phosphate synthetase II, aspartate transcarbamoylase, dihydroorotase (CAD) creates carbamoyl phosphate from bicarbonate and glutamine. Bicarbonate uptake is mediated by the transporter SLC4A7, the translation of which is regulated by mechanistic target of rapamycin complex 1 (mTORC1). Carbamoyl phosphate and aspartate undergo a condensation reaction catalyzed by the aspartate transcarbamoylase domain (ATCase) domain of CAD to form carbamoyl aspartate, which is then cyclized by dihydroorotase (DHOase) to form dihydroorotate. CAD activity is regulated by multiple phosphorylation events. Phosphorylation on Thr456 and Ser1859 of CAD, downstream of mitogen-activated protein kinase (MAPK) and mTORC1/ribosomal protein S6 kinase (S6K), respectively, increases CAD activity by enhancing its allosteric activation by phosphoribosyl pyrophosphate (PRPP) and relieving feedback inhibition by UTP. Conversely, phosphorylation on Ser1406 of CAD, downstream of protein kinase A (PKA), leads to decreased CAD activity due to enhanced feedback inhibition by UTP. Dihydroorotate dehydrogenase (DHODH) catalyzes the ubiquinone (Q)-mediated oxidation of dihydroorotate to orotate, forming ubiquinol (QH_2) and driving reduction of flavin mononucleotide (FMN) to dihydroflavin mononucleotide ($FMNH_2$). DHODH inhibitors can trigger differentiation in certain cancer contexts. The orotate phosphoribosyl transferase

(OPRTase) domain of the bifunctional enzyme uridine monophosphate synthase (UMPS) then converts orotate and PRPP to orotidine monophosphate, generating pyrophosphate (PP_i) as a by-product. Next, the orotidine-5′-monophosphate decarboxylase (OMPDC) domain of UMPS catalyzes the decarboxylation of orotidine monophosphate to form UMP, the common terminal product of the *de novo* and salvage pathways. Salvage pathway (purple): Uridine phosphorylase (UPP1/UPP2) reversibly interconverts uridine and inorganic phosphate (Pi) to uracil and ribose-1-phosphate. The ribose sugar generated by this reaction and that generated by the activity of thymidine phosphorylase (TP) on thymidine can stimulate central carbon metabolism. Uracil can be degraded by dihydropyrimidine dehydrogenase (DPYD), generating dihydrouracil. DPYD has been shown to be necessary for epithelial-to-mesenchymal transitions in cancer. Uridine cytidine kinase (UCK1/UCK2) phosphorylates uridine to form UMP, using ATP and generating ADP. Common pathways (yellow): Distal pyrimidine nucleotides are derived from UMP, including the DNA substrates dCTP and dTTP. The balance of pyrimidine and purine deoxyribonucleotides is crucial to support DNA replication and protect against DNA damage. Pyrimidine synthetic pathways and metabolic intermediates have also been linked to specific cell fates and may serve as alternative sources of fuel for central carbon metabolism.

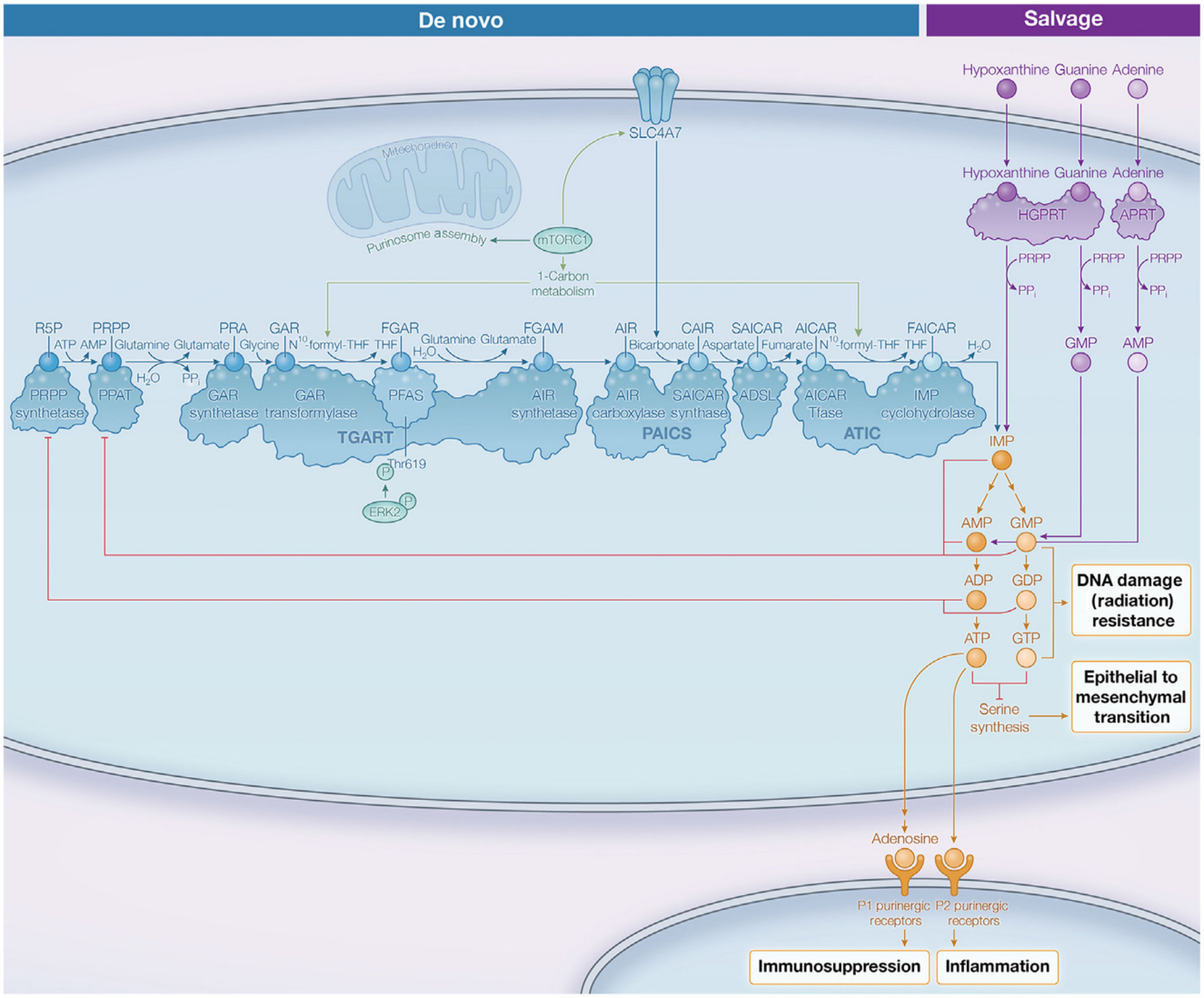

Figure 2. Purine nucleotide synthetic pathways and cellular consequences.

Purine nucleotides are synthesized by either the *de novo* or the salvage pathway. *De novo* pathway (blue): The enzyme phosphoribosyl pyrophosphate (PRPP) synthetase converts ribose-5-phosphate (R5P) and ATP to PRPP and ADP. The purines ADP and GDP inhibit PRPP synthetase activity. Amidophosphoribosyltransferase (PPAT) then converts PRPP and glutamine to phosphoribosylamine (PRA). PPAT is inhibited by the purines inosine monophosphate (IMP), AMP, and GMP. The glycinamide ribonucleotide (GAR) synthetase domain of the trifunctional GAR synthetase, GAR transformylase, aminoimidazole ribonucleotide (AIR) synthetase (TGART) enzyme uses PRA and glycine to synthesize GAR. Subsequently, the GAR transformylase domain of TGART transfers a formyl group from N^{10}-formyl-tetrahydrofolate (N^{10}-formyl-THF) to PRA, generating formylglycinamide ribonucleotide (FGAR). The enzyme phosphoribosylformylglycinamidine synthase (PFAS) then produces formylglycinamidine ribonucleotide (FGAM) from the substrates FGAR, H_2O, and glutamine. PFAS activity is positively regulated by phosphorylation on

Thr619 downstream of extracellular signal-regulated kinase 2 (ERK2). FGAM is subsequently cyclized to form aminoimidazole ribonucleotide (AIR) by the AIR synthetase domain of TGART. The AIR carboxylase domain of the bifunctional enzyme AIR carboxylase, 4-(*N*-succinyl carboxyamide) 5-aminoimidazole ribonucleotide (SAICAR) synthase (PAICS) carboxylates AIR using bicarbonate to form 4-carboxy-5-aminoimidazole ribonucleotide (CAIR). Bicarbonate is transported into cells by SLC4A7, the translation of which is regulated by mechanistic target of rapamycin complex 1 (mTORC1). CAIR and aspartate are then converted into SAICAR by the second domain of PAICS, SAICAR synthase. The enzyme adenylosuccinate lyase (ADSL) then cleaves SAICAR to form aminoimidazole-4-carboxamide ribonucleotide (AICAR) and fumarate. Next, the AICAR transformylase (AICAR Tfase) domain of the bifunctional enzyme AICAR Tfase/IMP cyclohydrolase (ATIC) transfers a formyl group from N^{10}-formyl-THF to AICAR, producing formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR). Notably, mTORC1 signaling increases expression of mitochondrial tetrahydrofolate (THF) cycle enzymes, producing one-carbon formyl groups required for the activities of GAR transformylase and AICAR Tfase. In the final step of *de novo* purine synthesis, the IMP cyclohydrolase domain of ATIC synthesizes IMP from FAICAR. Salvage pathway (purple): Hypoxanthine-guanine phosphoribosyltransferase (HGPRT) catalyzes a reaction in which the nucleobases hypoxanthine or guanine are combined with PRPP to form IMP or GMP, respectively, generating pyrophosphate (PP_i). Adenine is converted by adenine phosphoribosyltransferase (APRT) to AMP, also using PRPP as a substrate. Common pathways (yellow): Purine metabolism is vital for prevention of genotoxic stress and resistance to DNA damage. Additionally, depletion of intracellular purine nucleotides can increase serine synthesis, which triggers epithelial-to-mesenchymal transitions in certain cancer contexts. ATP from tumor cells can also interact with P2 purinergic receptors to trigger inflammatory signaling or can be converted to adenosine by nucleotidase enzymes to interact with P1 purinergic receptors and cause immunosuppressive signaling.