CANCER

Targeting nucleotide metabolism as the nexus of viral infections, cancer, and the immune response

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Virus-infected cells and cancers share metabolic commonalities that stem from their insatiable need to replicate while evading the host immune system. These similarities include hijacking signaling mechanisms that induce metabolic rewiring in the host to up-regulate nucleotide metabolism and, in parallel, suppress the immune response. In both cancer and viral infections, the host immune cells and, specifically, lymphocytes augment nucleotide synthesis to support their own proliferation and effector functions. Consequently, established treatment modalities targeting nucleotide metabolism against cancers and virally infected cells may result in restricted immune response. Encouragingly, following the introduction of immunotherapy against cancers, multiple studies improved our understanding for improving antigen presentation to the immune system. We propose here that understanding the immune consequences of targeting nucleotide metabolism against cancers may be harnessed to optimize therapy against viral infections.

INTRODUCTION

All living cells require nucleotides as building blocks for deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis for replication, transcription, and translation of genetic information, enabling cellular biomass increase. To ensure the accuracy of these processes, nucleotide metabolism is tightly regulated at all levels to maintain constant pools of pyrimidines—cytosine, uracil, and thymine (C, U, and T, respectively) and purines—adenine and guanine (A and G, respectively). Cancer cells and virus-infected cells share a metabolic dependency on nucleotide synthesis to support their unrestrained proliferation (1, 2). From the disease standpoint, a favorable outcome of uncontrolled proliferation lies in inducing mutations that further promote disease virulence and evolvability and enable survival in continuously changing environments (3, 4). Indeed, different drugs targeting nucleotide metabolism in either cancer or viral diseases constitute a shared therapeutic strategy to restrict replication.

The benefit from inhibiting nucleotide synthesis goes beyond restraining proliferation. In cancer, drugs that disrupt the balance of nucleotide pools can generate mutations that affect antigen presentation and, consequently, the immune response against the disease (5, 6). In addition, inhibition of purine synthesis can directly alleviate immune suppression, as secreted purines directly bind inhibitory receptors on immune cells (7, 8). On the other hand, targeting nucleotide metabolism could negatively affect the response of the host immune system. An early and essential step in the adaptive immune response against cancers and virally infected cells involves the rapid proliferation of lymphocytes (9), which will be disrupted by nucleotide deficiency. Therefore, any therapeutic strategy that targets nucleotide metabolism may potentially exert secondary effects on immune cells.

Despite the general characteristics discussed above, it is important to note that different viruses can impose distinct metabolic alterations of nucleotide metabolism in the infected cells. Furthermore, in cancer, metabolic heterogeneity is found among cells, locations, and at different stages of the same tumor. Here, we focus on the shared commonalities in nucleotide metabolism between cancer and virally infected cells and on the cross-talk it generates with immune cells along disease courses and during therapy. We highlight the advantages this cross-talk provides for disease progression, as well as the vulnerabilities it introduces that may constitute targets for therapy.

NUCLEOTIDE METABOLISM IN CANCERS AND VIRALLY INFECTED CELLS

Reprogramming of nucleotide metabolism to increase synthesis is regulated in cancers and virus-infected cells by similar signaling and metabolic pathways. In cancers, mutations and genomic aberrations in pro-growth and biosynthetic pathways are selected for promoting nucleotide synthesis (10). Virus-infected cells use other strategies, such as protein-protein interactions, to turn on the same biosynthetic machinery within the host cell. In both diseases, inhibition of tumor suppressors and activation of oncogenes, together with changes in expression of metabolic enzymes, lead to a shared outcome of increased nucleotide levels.

Rewiring of nucleotide metabolism via regulation of cell signaling

Several major signaling pathways and transcription regulators are commonly altered in cancers and virus-infected cells to increase nucleotide synthesis. These include MYC, RAS, P53, and mammalian target of rapamycin (mTOR). As the metabolic rewiring induced by these pathways have been extensively reviewed elsewhere (11, 12), here we will only briefly describe the most relevant metabolic alterations that augment nucleotide metabolism (Fig. 1).

MYC is considered a "master regulator" of cell proliferation, growth, and metabolism (10, 13) and is overexpressed and/or activated by oncogenic or epigenetic events in most human cancers (14). MYC directly binds and enhances expression of bottleneck enzymes in nucleotide biosynthesis, including CAD (the trifunctional enzyme carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase), thymidylate synthase, and IMPDH (inosine

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monophosphate dehydrogenase) (*15–18*). MYC further supports nucleotide synthesis by augmenting glycolysis and glutaminolysis to supply carbon and nitrogen precursors and by promoting the alternative splicing isoform of pyruvate kinase, PKM2, over PKM1. PKM2 increases the availability of glycolytic intermediates for branching the anabolic pathways: the pentose phosphate pathway (PPP) and serine synthesis (*19, 20*), which generate precursors for nucleotide synthesis. In virus-infected cells, MYC can be activated by various strategies, such as protein-protein interaction, and by induction of its transcription. For example, adenovirus-encoded protein E4ORF1 translocates to the nucleus, where it binds MYC and enhances its binding to metabolic genes (*21*). In addition, hepatitis C virus (HCV) enhances MYC transcription by activating other signaling pathways such as AKT and β-catenin (*22*).

RAS oncogene promotes nucleotide synthesis in multiple ways, which include MYC activation (*12, 23*), and by activation of extracellular signal–regulated kinase, which phosphorylates and stimulates the purine synthetic enzyme phosphoribosylformylglycinamidine synthase (*24*) and the pyrimidine synthetic enzyme CAD (*25*). Viruses also use RAS signaling to promote proliferation. In human herpesvirus–infected cells, RAS activation is induced by interaction of viral glycoproteins and cellular receptors, as reviewed in (*26*). Similarly, the HCV generates replication complexes that are composed of virally-encoded proteins and Ras-GTPase–activating protein-binding protein 1 (*27*).

Cancer and virally infected cells further promote nucleotide synthesis by inhibiting signaling of tumor suppressors such as p53. In cancers, mutated p53, or its loss of function, reprograms cell metabolism to support growth, proliferation, and macromolecule synthesis. In DNA virus–infected cells, specific inactivating proteins or viral regulatory factors form complexes to inactivate p53 (*28–30*). In addition to its direct effects on metabolic pathways such as glycolysis and the salvage nucleotide pathway, p53 is among the plethora of genes, nutrients, and stress and growth signals that regulate the mTOR pathway, which is a critical regulator of mammalian metabolism (*31*). Loss of p53 activates mTOR complex 1 (mTORC1) to stimulate de novo pyrimidine and purine synthesis through activation of CAD enzyme and induction of one-carbon metabolism, which uses serine and glycine to generate one-carbon units for thymidine and purine synthesis (*32–34*). Similarly to MYC and mutated p53, mTORC1 stimulates glycolysis, and the PPP thus increases supply of precursors for nucleotide production (*35*). Activation of mTOR signaling has been demonstrated in multiple cancers (*36*) and following viral infections (*37*) and is mediated by various oncogenic alterations and virally induced regulatory proteins, respectively.

Rewiring of nucleotide synthesis via a direct regulation of metabolic enzymes

Mutations and genomic aberrations in metabolic enzymes are selected in cancer to promote proliferation and increase nucleotide
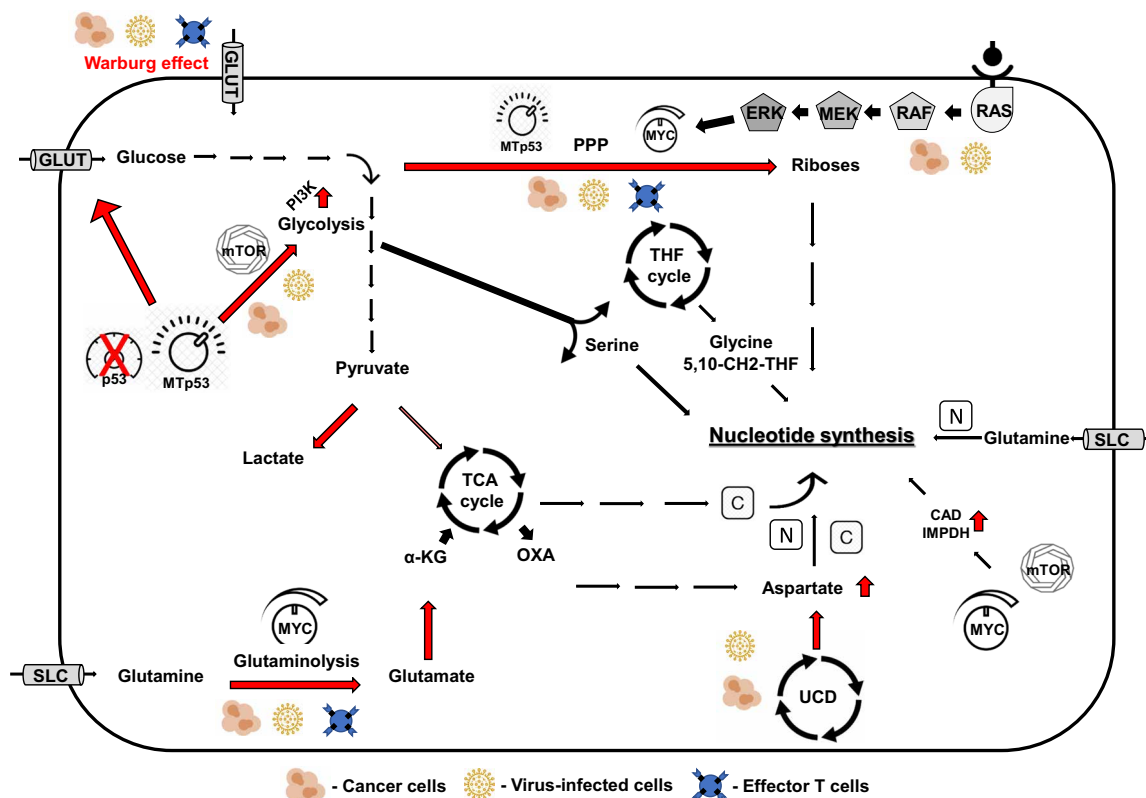

Fig. 1. Shared signaling and metabolic rewiring between virus-infected cells, cancers, and lymphocytes. Virus-infected cells, cancers, and lymphocytes rewire cell metabolism to promote anabolism in general and that of nucleic acids specifically as building blocks for their enhanced proliferation. In addition to the "Warburg effect" that enhances glycolysis, the scheme depicts the metabolic reprogramming that is promoted by major signaling pathways such as p53, MYC, mitogen-activated protein kinase (MAPK), and mTOR to increase nucleotide synthesis (red arrows) for carbon (C) and nitrogen (N) utilization. UCD, urea cycle dysregulation; SLC, solute carrier; THF, tetrahydrofolate; TCA, tricarboxylic acid, MTp53, mutated p53; and OXA, oxaloacetate.

synthesis. These include mutations that drive overexpression of enzymes that promote production of precursors for nucleotide synthesis, such as the glycolytic enzymes: hexokinase, phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase (38–41). In some cancers, such as melanoma and subtypes of breast and lung carcinomas, an amplification of a region on chromosome 1p increases the expression of phosphoglycerate dehydrogenase, the committed step in serine biosynthesis, leading to enhancement of the tetrahydrofolate cycle and purine synthesis (42, 43). In addition, amplifications of CAD enzyme that catalyzes the initiating step in pyrimidine synthesis and of several enzymes in the purinosome, the multienzyme complex that catalyzes purine synthesis, have been found in different cancers to directly increase nucleotide levels (44, 45). Similarly, some virus-infected cells induce nucleotide synthesis by directly encoding for metabolic enzymes. For example, the herpes simplex virus 1 (HSV1) encodes thymidine kinase, ribonucleotide reductase, dUTPase, and uracil-DNA glycosylase (46), all of which increase pyrimidine production.

Mechanisms used by cancers and viruses for increasing substrate levels necessary for nucleotide synthesis include not only the induction of anabolic enzymes but also interference with catabolic pathways. One example is the rewiring of the urea cycle (UC), the main pathway for disposal of excess nitrogen. Altered expression of UC enzymes is detected in many tumors (5). For example, overexpression of the UC enzyme CPS1, as seen in lung cancer, increases the availability of carbamoyl phosphate for pyrimidine synthesis (47). In addition, down-regulation of the UC enzyme argininosuccinate synthase 1 (ASS1), as seen in multiple cancers [e.g., osteosarcoma, melanoma, and mesothelioma (48, 49)], increases the availability of its substrate aspartate, thus facilitating pyrimidine synthesis and cancer proliferation (50). Aspartate is also essential for asparagine activation of mTOR-dependent nucleotide synthesis (51, 52). Likewise, down-regulation of ASS1 enhances viral genome replication and production of infectious HSV1 (53). Interestingly, such changes may also affect the immune response. For example, lymphocytic choriomeningitis virus has been demonstrated to repress the transcription of the UC enzymes OTC (Ornithine transcarbamylase) and ASS1 in the liver, consequently leading to decreased arginine and increased ornithine concentrations in the circulation, which suppress virus-specific cytotoxic T cell responses (54).

These studies highlight different mechanisms by which viruses and cancers rewire the host cellular and systemic metabolism to provide their insatiable need for nucleotides. Consequently, targeting nucleotide metabolism is an established treatment approach to restrain proliferation in both diseases.

TARGETING NUCLEOTIDE METABOLISM AGAINST CANCERS AND VIRAL INFECTIONS

Over the years, different therapeutic strategies have been used against cancers and virus-infected cells. Interestingly, while targeting nucleotide synthesis induces similar effects on cancers and virus-infected cells, attempting at hampering nucleotide metabolism by targeting metabolic master regulators can generate differential effects against each disease. For example, the mTOR inhibitors (Rapalogs), which are widely used against various cancers (55, 56), have variable effects against different viruses and have been shown to diminish cytomegalovirus and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication (57, 58) and, on the other hand, to facilitate

influenza infection and polyomavirus replication (59–61). The variability in response of different viruses to mTOR inhibitors could result from the dependence of the virus on mTOR for replication as well as from the level of effect the inhibitor has on the antiviral immune response. In contrast, similar benefits have been demonstrated against both diseases when using nucleotide analogs and agents that directly inhibit the enzymes involved in nucleotide metabolism. Surprisingly, despite the shared mechanisms of action, there are no drug agents that are approved against both cancers and viral infections (tables S1 and S2).

Nucleotide analog treatment modality

One main strategy to halt cellular replication uses modified purine and pyrimidine nucleosides that terminate DNA or RNA polymerase activity. In these synthetic nucleosides, the deoxyribose moiety is replaced with nonfunctional modifications such as azide or hydrogen, which inhibit elongation of the DNA or RNA strands. Purine and pyrimidine analogs used in cancer treatment are summarized in table S1, together with their labeled indications and mechanisms of action. Most of these drugs are incorporated into the DNA and inhibit the activity of DNA polymerase (62). New generations of nucleosides are more sophisticated and display additional metabolic consequences in their cellular cytotoxicity. For example, in addition to blocking RNA synthesis, the adenosine analog 8-amino-adenosine causes an energy crisis and induces cell death by decreasing the intracellular concentrations of adenosine triphosphate (ATP) (table S1) (63). Nucleosides are efficient drugs; however, cancers can develop mechanisms that overcome the metabolic hurdles they impose. For example, gemcitabine is a pyrimidine prodrug analog of deoxycytidine that has been approved for the treatment of non–small cell lung cancer, pancreatic cancer, bladder cancer, and breast cancer (64). However, it was recently demonstrated that tumor-infiltrating activated macrophages synthesize and release a spectrum of pyrimidine nucleosides including deoxycytidine that are consumed by the cancer cells (65). These nucleosides directly compete with gemcitabine, hindering its efficiency as a chemotherapy.

Nucleoside and nucleotide analogs represent one of the largest classes of small-molecule antiviral drugs (summarized in table S2). The mechanisms of action of these drugs include lethal mutagenesis, specific or nonspecific chain termination, and inhibition of nucleotide biosynthesis (66, 67). Indeed, for the SARS-CoV-2, one of the trialed antiviral drugs used is remdesivir, a prodrug of a nucleotide analog that is intracellularly metabolized to an ATP analog, which inhibits the activity of viral RNA polymerases (68). Another example of a nucleoside analog used as viral therapy is acyclovir and its related drugs: valacyclovir, penciclovir, and famciclovir, used against infections with HSV-1, HSV-2 and varicella zoster virus. Ganciclovir and its related drug valganciclovir are additional nucleoside analogs used against members of the family Herpesviridae (69). The mechanism of action of these drugs requires phosphorylation by a virally encoded enzyme that induces chain termination only in infected cells. Ribavirin, a Food and Drug Administration (FDA)–approved guanosine analog, is standard for care against several viruses, including respiratory syncytial virus and HCV (70). Although its mechanism of action is a matter of debate, several possibilities have been proposed, including depletion of guanine nucleotides through inhibition of IMPDH (71). Intriguingly, ribavirin's antiviral effect against HCV is associated with induction of transition mutations in the viral genome, resulting in the generation of noninfectious virions (72).

Inhibition of metabolic enzymes involved in nucleotide metabolism

Direct inhibition of enzymes involved in DNA and RNA synthesis is another known treatment strategy against both cancers and viral infections. In fact, one of the first FDA-approved molecules for cancer treatment was the purine inhibitor 6-mercaptopurine (6-MP). 6-MP inhibits the first enzyme of de novo purine synthesis, 5-phosphoribosyl-1-pyrophosphatase (73), and the purine salvage enzyme, hypoxanthine-guanine phosphoribosyltransferase (74). Merimepodib, which inhibits IMPDH, an enzyme catalyzing de novo synthesis of guanosine nucleotides, has been demonstrated to suppress replication of a variety of RNA viruses, including SARS-CoV-2 replication in vitro (75, 76).

Inhibitors of pyrimidine biosynthesis currently used for cancer treatment include 5-fluorouracil, a thymidylate synthase inhibitor, and methotrexate, which inhibits dihydrofolate reductase and causes a drop in cellular levels of thymidine (77, 78). Not surprisingly, efforts have been made to target CAD and dihydroorotate dehydrogenase (DHODH) pyrimidine synthetic proteins (79, 80). While potent CAD inhibitors are still lacking, inhibitors of DHODH, such as brequinar sodium, are currently being tested in clinical trials as cancer therapy for acute myeloid leukemia (81). DHODH inhibitors have also been tested in different models of viral infections (82). The repression in viral growth induced by these inhibitors was attributed to enhanced innate immune response in reaction to pyrimidine deprivation (83).

Targeting a specific nucleotide pathway for the synthesis of either purines or pyrimidines can generate a nucleotide pool imbalance by decreasing the levels of one pool relative to the other. Since the ratio between the two pools is tightly regulated, induced imbalance can subsequently cause genotoxic stress and increase mutagenesis (84, 85). While prompting mutations can increase fitness and survival of cancers and virally infected cells, high mutation number will ultimately generate more neoantigens that can improve the response of immune cells (86).

NUCLEOTIDE METABOLISM REGULATES THE HOST IMMUNE SYSTEM

The rationale and benefit from targeting nucleotide metabolism to inhibit replication of tumor cells or viruses during infection are clear. However, we should not overlook the potential for consequential deleterious effects on the immune response against both diseases, specifically that of T lymphocytes. T lymphocytes are the cellular arm of the adaptive immune system and can be divided into different subpopulations with distinct functions: CD8+ cytotoxic T cells kill cells that express foreign antigens including cells infected with virus and tumor cells. CD4+ T helper cells regulate the function of other immune cells, including CD8+ cytotoxic T cells. Upon activation, T cells induce anabolic metabolism to support rapid growth, proliferation, effector molecule production, and differentiation using the same signaling and metabolic pathways induced to support proliferation in cancer cells and virally infected cells (87). Signals received through the T cell receptor activate MYC and mTOR to induce transcription of multiple metabolic enzymes (88). In addition, wild-type P53 is downregulated in lymphocytes by its regulator mouse double minute 2 (MDM2) (Fig. 2) (89). Consequently, glucose uptake and glycolysis are induced, with increased serine biosynthesis and increased metabolic flux through the PPP, producing five-carbon sugars for nucleotide
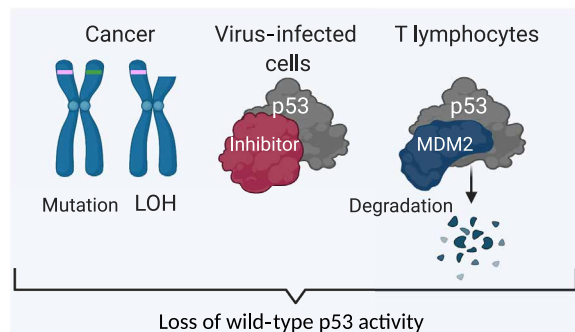

Fig. 2. p53: Engaging a common pathway by different mechanisms. Nucleotide synthesis is induced in T cells, cancers, and virally infected cells through engagement of the same signaling pathways but by different mechanisms, as nicely illustrated in the case of p53. Cancer cells select for loss of wild-type p53 activity via mutations or loss of heterozygosity (LOH). Virus-infected cells inhibit p53 through protein-protein interaction. In T lymphocytes, MDM2 facilitates p53 degradation. This figure was generated using https://biorender.com/.
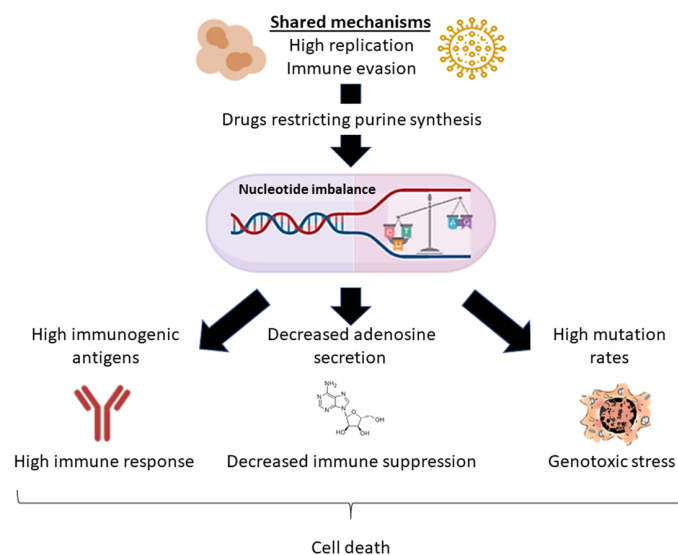

Fig. 3. Modulating nucleotide metabolism to favor pyrimidine synthesis can increase the host immune response against viral attack and cancer. Inducing a high pyrimidine-to-purine ratio may promote the immune response against cancer and virus-infected cells by inducing genotoxic stress, decreasing immune suppression, and augmenting the antigens' immunogenicity, all contributing to virally infected and cancer cells' death.

synthesis (88). In parallel to a large increase in aerobic glycolysis, T cell activation induces a robust and highly synchronized program of mitochondrial biogenesis (90, 91), giving rise to one-carbon metabolism that uses serine to generate glycine and one-carbon units for de novo purine biosynthesis (91). Mitochondrial respiration further supports nucleotide synthesis and proliferation through production of aspartate, a precursor for CAD enzyme (Fig. 1) (92, 93). Thus, T cells engage in nucleotide metabolism to support their growth, proliferation, and redox balance using the same pathways "hijacked" by tumors and virally infected cells (Fig. 2). Not surprisingly, similar to the anticancer and antiviral therapies, drugs that inhibit nucleotide synthesis have long been used to treat inflammatory autoimmune diseases such as rheumatoid arthritis, Crohn's disease, and psoriasis and to prevent host versus graft disease following organ transplantation (table S3).

Inhibition of nucleotide synthesis as a treatment for cancer and viral infections is not all bad for T cells and, under certain circumstances, could even promote a protective immune response. Cancers and virally infected cells actively generate an immunosuppressive microenvironment by secreting purines, especially adenosine, into the extracellular space (8, 94). By engaging with adenosine receptors that are expressed on most immune cells, adenosine halts immune cell differentiation and maturation, induces the expression of checkpoint molecules, such as programmed cell death protein 1 and cytotoxic T lymphocyte–associated protein-4, and interferes with secretion of chemokines and cytokines (7). Accordingly, in tumors, drugs targeting the adenosine pathway were shown to convert an immunosuppressive microenvironment to a more immuno-permissive one and to reduce metastasis and resistance to therapy (95). Such drugs are currently undergoing their first clinical trials in humans, both as single agents and in combination with other immune therapies (96).

Following the introduction of immunotherapy against cancers, multiple studies advanced our knowledge for improving the immune response via regulating antigen presentation by the cancer cells (97). One such potential strategy to increase the presentation of more immunogenic antigens on the cell surface is by promoting the presentation of more hydrophobic neoantigens (98, 99). Since presented antigens are continuously translated from the cellular mRNAs, modulating nucleotide metabolism can regulate the antigens' properties. Along these lines, we found that inducing a high pyrimidine-to-purine ratio in different cancers promotes the generation of a specific mutation signature, leading to the production of more hydrophobic, and therefore more immunogenic, neoantigens (5). Furthermore, as a proof of concept, we demonstrated that mizoribine, an inhibitor of IMPDH and consequently of purine synthesis, augmented pyrimidine-to-purine ratio and improved the response to immunotherapy in previously nonresponsive tumors (6). Of note, to avoid the potential immunosuppressive effect of mizoribine (6), it was given to cancer cells before treatment with immunotherapy. The resultant beneficial outcome exemplified by tumor growth restriction suggests the rationale that sequential drug regimens can optimize therapeutic consequences. We hence propose that better understanding of the secondary effects of drugs that target nucleotide synthesis on immune cells will minimize the inhibitory effect on T cells, enhance immunogenicity of infected or transformed cells, and thus improve existing therapies against viral infections and cancers (84, 85).

OUTLOOK

Nucleotide metabolism provides cancers and viruses with means to proliferate and evade the immune system; hence, it is a well-established therapeutic target against both diseases. Although the therapeutic strategy is shared between the two diseases, there are currently no drugs that are commonly used against both. These days, immunotherapy advances our knowledge regarding potential recruitment of the immune response as therapy against cancer. The similar cross-talks between cancers and virally-infected cells with the immune system via nucleotide metabolism offer an opportunity to take advantage of this shared vulnerability and modulate nucleotide metabolism in a way that would boost the immune response and benefit therapy. On the basis of recent insights, modulating nucleotide metabolism, for example, by generating an imbalance that favors pyrimidine synthesis, may potentially improve the immune response, may reduce tumor-induced immune suppression, and may increase genotoxic stress, consequently leading to cell death (Fig. 3).

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/21/eabg6165/DC1

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