ENPP1 Immunobiology as a Therapeutic Target

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ABSTRACT

ENPP1 (ecto-nucleotide pyrophosphatase/phosphodiesterase) participates in the hydrolysis of different purine nucleotides in an array of physiologic processes. However, ENPP1 is frequently overexpressed in local relapses and tumor metastases, which are associated with poor prognosis and survival in a range of solid tumors. ENPP1 promotes an immunosuppressive tumor microenvironment (TME) by tilting the balance of ATP/adenosine (Ado) in conjunction with other components (CD38, CD39/ENTPD1, and CD73/NT5E). Moreover, ENPP1 intersects with the stimulator of interferon genes (STING), impairing its robust immune response through the hydrolysis of the effector 2′,3′-cyclic GMP–AMP. Thus, ENPP1 blockade emerges as a unique target eliciting immune remodeling and leveraging the STING pathway. Several ENPP1 inhibitors have shown an immunostimulatory effect, and their combination with other therapeutic modalities, such as immune-checkpoint blockade, STING activation, DNA damage response (DDR) inhibitors, and radiotherapy (RT), represents a promising avenue to boost antitumor–immune responses and to improve current clinical outcomes in several tumors. This comprehensive review summarizes the current state of the art and opens new perspectives for novel treatment strategies.

Introduction

An exceptionally wide variety of physiologic processes is triggered by purinergic signaling that is the extracellular action of purines (ATP, ADP, and Ado) and pyrimidines [uridine-5′-triphosphate (UTP) and uridine-5′diphosphate (UDP)]. These include cell proliferation, migration, apoptosis, platelet aggregation (1, 2), and muscle contraction, as well as regulating hypoxia and ischemia in tissues (3, 4).

Purine homeostasis entails the concerted activity of the mechanisms of nucleotide and nucleoside release, their extracellular metabolism mediated by several transmembrane ectoenzymes (CD38, CD39/ENTPD1, CD73/NT5E, and ENPP1; **Fig. 1A**), the intracellular signaling pathways elicited upon binding of purine metabolites to different receptors in target cells (**Fig. 1B**) and their cross-talk with other intracellular cascades.

Within this complexity, ENPP1 (CD203a/PC-1), which belongs to the family of ENPP ectonucleotidases (ENPP 1–7), is a type II transmembrane glycoprotein, also located on the endoplasmic reticulum lumen (5). ENPP1 constitutes a major purinergic signaling regulator of extracellular ATP and GTP levels that are hydrolyzed to AMP and GMP while releasing inorganic pyrophosphate (PPi). The product AMP is subsequently dephosphorylated by CD73 (Ecto-5′-nucleotidase or NT5E) to inorganic phosphate (Pi) and Ado. In addition, different membrane transporters that belong to SCL28 and SCL29 families also regulate the extracellular bioavailability of Ado (6).

ENPP1 is highly expressed in the osteochondral compartment where it displays homeostatic functions in regulating physiologic mineralization by regulating the balance between Pi, a substrate of mineral deposition, and PPi, an inhibitor of mineralization. ENPP1 deficiency has been linked to bone abnormalities (7, 8).

In tumors, ATP, AMP, and Ado play a key role in modulating immune responses. ENPP1 arises at the interphase of tumor–host immune interactions tilting the balance from the proinflammatory ATP toward Ado with an opposite anti-inflammatory role. ATP and Ado, together with other related metabolites, signal through purinergic receptors expressed in both tumor and host–immune cells (**Fig. 1B**). This array of receptors with different expression, selectivity, and affinity confers a unique fine-tuned modulation of extracellular nucleotide levels that strongly regulate tumor progression.

Emerging Roles of ENPP1

ENPP1 gene is located in the 6q22–q23 locus, a region commonly amplified in many tumors, including neural brain and breast cancers (9). High ENPP1 expression levels are also detected in many solid tumors including ovarian (10), breast (11), glioblastoma (9), and NSCLC (12) among others. Its overexpression has been linked to a more aggressive clinical course associated with poor prognosis through the induction of EMT phenotype, the acquisition of stem cell–like features and the favoring of prometastatic traits (11, 12).

ENPP1 levels increase during various stages of tumorigenesis in clinical specimens. For instance, ENPP1 expression was significantly increased in 85% of patients with high-grade ovarian serous carcinoma, compared with ∼1% in benign serous cystadenoma, suggesting a putative role in malignant tumor progression. Moreover, the higher the FIGO stage, the higher the ENPP1 levels that were detected with poorer cell differentiation (10). ENPP1 levels were also significantly elevated in human primary breast tumors relative to the normal mammary epithelium, with the highest levels observed in skeletal metastases (13). Similarly, in different animal models of lung and breast cancer, ENPP1 levels were elevated in metastatic cells as compared with the primary tumor (13, 14).

Besides tumor expression, ENPP1 is also highly expressed in several immune cells, including neutrophils and M2 macrophages promoting

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tumor progression, whereas low levels of expression were detected in natural killers (NK), DC, monocytes, T and B cells (15, 16). ENPP1 activity in concert with CD73 (NT5E) leads to the accumulation of Ado-promoting tumor–immune remodeling and robust immunosuppression (ref. 17; **Fig. 2**).

In breast cancer models, ENPP1 promotes the chemotactic infiltration of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC; ref. 18) and the inhibition of tumor-infiltrating cytotoxic T cells (19) impeding the antitumor–immune attack. This myeloid chemoattraction is elicited by the ENPP1-mediated tumor release of haptoglobin (HP), a proinflammatory acute phase reactant (20, 21) that also acts as an inducer of neutrophil extracellular traps formation (ref. 22; **Fig. 3**). Thus, inhibition of ENPP1 could revert the immunosuppressive landscape, impairing tumor progression.

ENPP1 Activity Intersects with the STING Pathway

Besides its role in immunosuppression, upregulation of ENPP1 is one of the mechanisms artfully co-opted by neoplastic cells as a resistance mechanism to impair stimulator of interferon genes (STING) activation, a major DNA-sensing antiviral pathway (23) and antitumor defense mechanism (ref. 24; reviewed elsewhere; refs. 25, 26; **Fig. 4**).

Chromosomal instability, a hallmark of cancer, is often associated with tumor progression, metastasis, and therapeutic resistance. It is a major source of cytosolic double-strand DNA sensed by cGAS (cyclic GMP–AMP synthase), which upon binding to its substrate catalyzes the formation of cyclic dinucleotide GMP–AMP (cGAMP; ref. 27) and elicits STING pathway stimulation, which activates the transcription of interferon genes and other cytokines. This tumor cell–autonomous activation is often circumvented through silencing interferon (IFN) signaling, which allows tumor progression and tumor cell dissemination (28).

Yet, cGAMP is also exported to the extracellular space where it acts in a non–cell-autonomous manner stimulating STING in host cells of the tumor microenvironment (TME). However, this STING induction is often evaded by the tumor or stromal ENPP1 transmembrane expression, which promotes the rapid degradation of exported cGAMP, which allows tumor escaping from immunosurveillance (13, 29). In contrast, inhibition of ENPP1 leads to the accumulation of cGAMP and the subsequent cGAS–STING activation, which enhances innate immune responses by inducing the production of cytokines such as IFNγ and by activating dendritic cells (DC) with antitumor–immune response.

Thus, targeting ENPP1 represents a novel opportunity to boost the cGAS–STING–IFN pathway that could be exploited in novel therapeutic approaches to immunotherapy. Furthermore, therapies that help generate cytosolic DNA such as DNA damage inhibitors, etoposide, or radiation can trigger STING activation and could synergize with ENPP1 inhibition. Of note, a delicate adjustment of STING levels may be required to achieve an optimal clinical benefit because chronic cGAS–STING engagement could lead to tumor promoter functions (30).

Exploiting ENPP1 in Cancer Therapy

Because ENPP1 is at the crossroads of several pathways, its pharmacologic blockade emerges as a promising therapeutic option in a combinatorial setting with other therapeutic strategies in a large variety of tumors (31). Inhibition of other components of the purinergic axis could be required to achieve a more salient effect. For instance, concurrent blockade of A2AR/CD73/CD39 could more efficiently abrogate the purinergic signaling, not only in the tumor but also in infiltrating immune subpopulations. Furthermore, ENPP1 inhibition could also boost the effects of STING agonists with the potential benefit of eliciting strong immune remodeling with DC activation. In addition to ENPP1 abrogation, the concomitant inhibition of purinergic pathways as well as the potential benefit of other modalities such as RT could bring about substantial benefits in the clinical setting.

A range of specific small-molecule inhibitors targeting ENPP1 have been tested in preclinical models. The ENPP1 inhibitor STF-1623/CM-3163 (Angarus) is a cell-impermeable, nontoxic specific inhibitor, which acts by chelating Zn^{2+} (32). Its lack of permeability presumably prevents toxic events with high specificity while keeping its strong STING activation. The inclusion of this ENPP1 inhibitor resulted in a decreased rate of locoregional failure of breast cancer models treated with surgery and radiation. This finding reveals not only a delay in the occurrence of locoregional failure but a net reduction in the number of failures, which may translate in a substantial benefit in the clinical setting (33). Analysis of the immune landscape posttreatment showed a decrease in MDSCs and a diminished tumor-associated macrophages (TAM) infiltration (specifically M2-polarized) as well as an enhanced antigen presentation observed by an increased DC, $CD8^+$ T cells, and NK cells. Similarly, silencing ENPP1 decreased lung metastasis formation in preclinical models (13).

Other inhibitors such as AVA-NP-695 (Avammune) display cellular permeability, oral bioavailability, and show a potent effect at nmol/L doses as monotherapy, decreasing tumor volume and showing an advantage of the oral administration in breast cancer models (34). Another orally administered ENPP1 inhibitor, TXN10128, shows synergistic growth inhibition with anti–PD-L1 in a preclinical colon cancer model with increased tumor-infiltrating lymphocytes (35).

Another broad ectonucleotidase inhibitor POM1 (Sodium polyoxotungstate) lacks specificity as it inhibits CD39 (ENTPD1, ectonucleoside triphosphate diphosphohydrolase-1) and ENPP1 at high doses. Blockade of CD39 improves antitumor immunity and decreases the metastatic burden (36).

A recently reported ENPP1 inhibitor, ZX-8177, shows marked tumor growth inhibition when used alone or in combination with anti–PD-L1, with strong immune remodeling in a colon murine model (37).

RBS2418 (Riboscience) is a potent and selective small-molecule inhibitor of ENPP1 that can be delivered orally. RBS2418 as monotherapy can potentially have an activating effect on the antitumor innate immune response and leads to antitumor responses in adult subjects with advanced or metastatic tumors. Dose escalation showed no associated toxicities. Although only one clinical trial is ongoing, prospective trials could be anticipated with different ENPP1 inhibitors combined with other therapies (Supplementary Table S1).

Recently, high-affinity and specific anti-ENPP1 antibodies and derived antibody-drug conjugates, IgG-based specific T-cell engagers, and CAR T-cells have shown potent killing activities in ENPP1-expressing cells (38).

Strategies Leveraging the Purinergic Axis

ENPP1 inhibition can be partially mirrored by inhibiting other components of the purinergic pathway such as CD39/CD73 and/or A2AR. Strategies that decrease the Ado-mediated signaling by

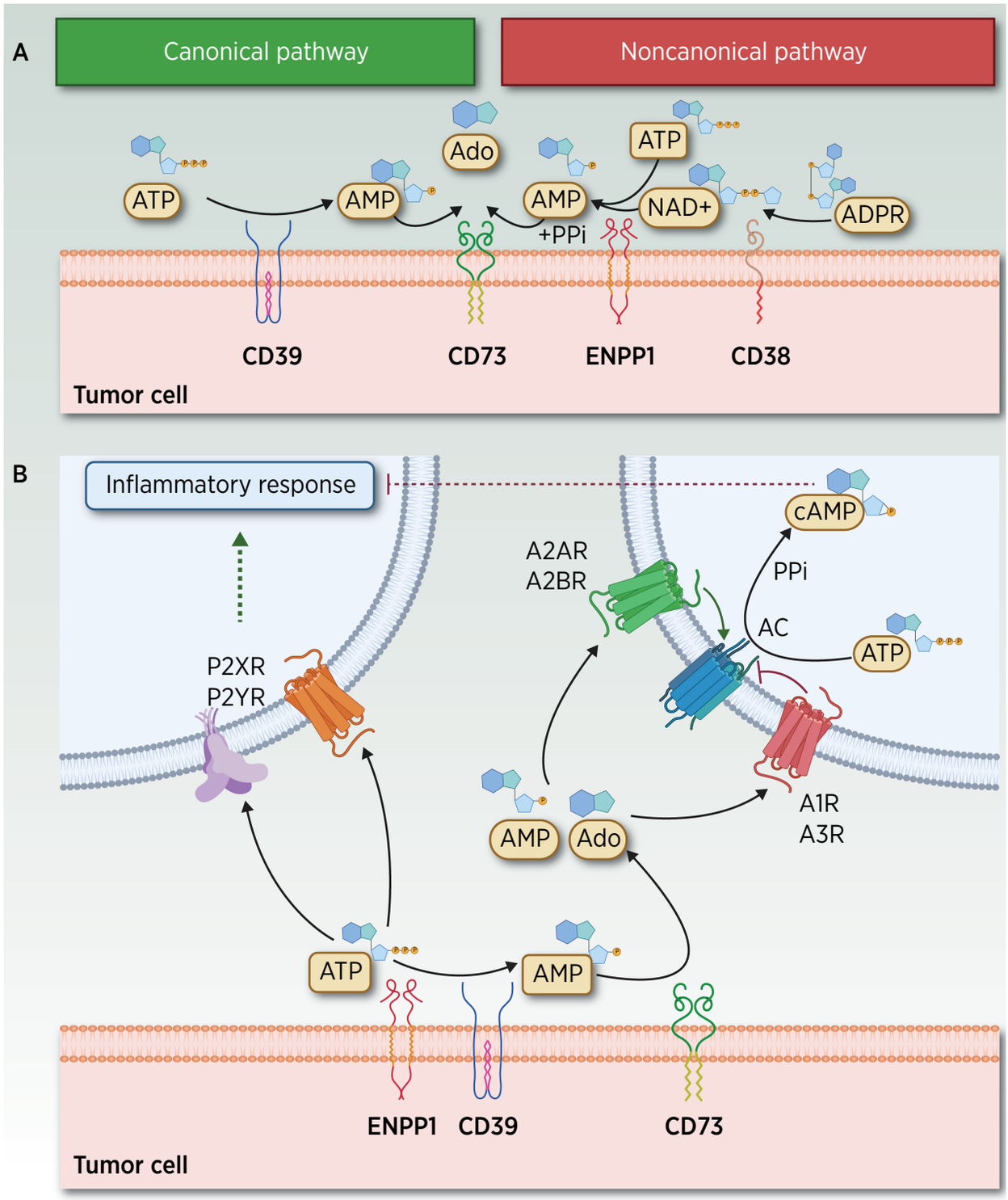

Figure 1.

ATP–Ado extracellular metabolism and elicited signaling through adenosinergic receptors. **A,** Canonical and noncanonical extracellular receptors contributing to the hydrolysis of ATP and other metabolites. In the TME, two different yet partially overlapping pathways mediate the hydrolysis of ATP to adenosine (Ado). In the noncanonical pathway, Ado can be released by CD38 (72), a cell-surface enzyme that functions as an adhesion molecule and as an ectoenzyme, expressed in T cells, neutrophils, lymphocytes and monocytes, and macrophages under inflammatory conditions. CD38 hydrolyzes nicotinamide-adenine dinucleotide (NAD^+) to ADP-ribose (ADPR), which is subsequently metabolized to AMP by ENPP1, whereas AMP is further dephosphorylated by CD73 to Ado and PPi (73, 74). (*Continued on the following page.*)

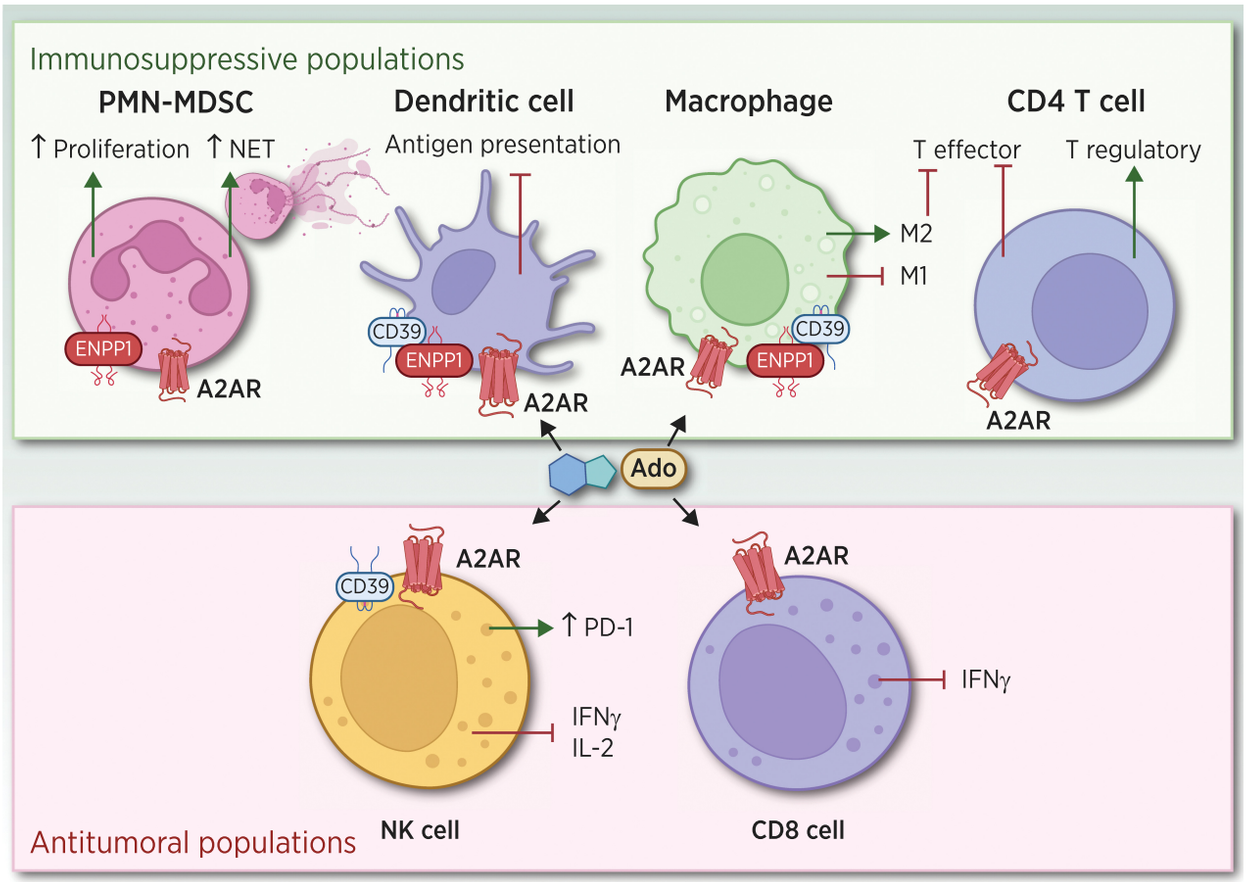

Figure 2.
Effects of Ado signaling in different immune subpopulations. ENPP1 in conjunction with CD39 and CD73 promotes the accumulation of Ado in the TME, which signals through A2AR expressed in host immune cell populations. Tumor cells with high ENPP1 promote strong tumor–immune remodeling by inducing a PMN-MDSC chemotaxis, diminishing DC infiltration, increasing M2 macrophages, and promoting CD4 regulatory T cells. In addition, the increase in Ado leads to a diminished NK and CD8 T cytotoxic activity, promoting a strong immunosuppressive milieu preventing an efficient antitumor–immune attack. (Adapted from an image created with BioRender.com.)

concomitant blockade of CD39/CD73 and A2AR (39, 40) have been developed. However, the unique effects elicited by ENPP1 inhibition on the activation of the STING pathway might represent an overt advantage when compared with the double abrogation of CD39/CD73 and A2AR. In this vein, the concurrent blockade of ENPP1 and A2AR could have more salient effects. Moreover, ENPP1 inhibition could be more advantageous in high ENPP1-expressing tumors. Other differences are related to differential expression levels of CD39/CD73 in B

(*Continued.*) ENPP1 is a Ca^{2+} and Zn^{2+}–dependent enzyme comprising two identical disulfide bonded subunits (75) involved in the regulation of hormonal (39), neurologic, immunologic, and hematologic functions (4, 76, 77) as well as in atheromatous plaque calcification (78). ENPP1 hydrolyzes its most suitable substrate ATP to AMP, and the catalysis of GTP to GMP while releasing PPi. Other substrates include dinucleotides, mainly cGAMP, diadenosine tetraphosphate, and other poor substrates (UTP and cAMP). The product AMP is subsequently dephosphorylated by CD73 (Ecto-5′-nucleotidase or NT5E) to Pi and Ado. In addition to the action of nucleotidases, different membrane transporters, which belong to SCL28 and SCL29 families, also regulate the extracellular bioavailability of Ado (6). In the canonical pathway, transmembrane or soluble ectoenzyme CD39, which converts nicotinamide-adenine dinucleotide phosphate (NADP) into NAD^+, also hydrolyzes extracellular ATP to AMP (79), a catalytic activity similar to that exerted by ENPP1. CD39 is expressed in the vasculature, in malignant cells, and in several immune subpopulations, including M2-like tumor-associated macrophages (TAM), T regulatory cells (Tregs), dendritic cells (DC), natural killers (NK), monocytes, and B cells. CD39 is expressed in MDSC and Tregs, and it is probably high on tumor-specific clonally expanded Tregs (80, 81) events associated with their immunosuppressive role in T-cell function (82). Ado results from the subsequent CD73-mediated degradation of AMP. Thus, CD73 bridges canonical and noncanonical pathways, a role currently exploited by a targeted therapy (83). **B,** ATP, AMP, and Ado signaling through adenosinergic receptors. ATP and Ado, together with other related metabolites, signal through purinergic receptors divided into two major families: Ado P1 receptors (A1R/ADORA1, A2AR/ADORA2A, A2BR/ADORA2B, and A3R/ADORA3), whose agonists include AMP and Ado, and P2 receptors, which comprise a family of P2X ionotropic receptors (P2X1–7) stimulated by ATP, and P2Y, which are G protein–coupled metabotropic receptors (P2Y 1, 2, 4, 6, 11–14) activated by nucleotides such as ATP, ADP, UTP, and UDP (84). Under hypoxic conditions, extracellular released Ado levels are further amplified by stimulated CD39–CD73, and by decreased Ado kinase activity, leading to diminished Ado degradation (73). This accumulation of AMP and Ado metabolites stimulates P1 G-protein–coupled A2AR at nanomolar range (also A1R and A3R) and A2BR Ado P1 receptors (at micromolar levels) to elicit intracellular cyclic AMP signaling, resulting in decreased production of proinflammatory cytokines and increased synthesis of anti-inflammatory cytokines (40, 85). AC, adenylyl cyclase; PPi, pyrophosphate. (Adapted from an image created with BioRender.com.)

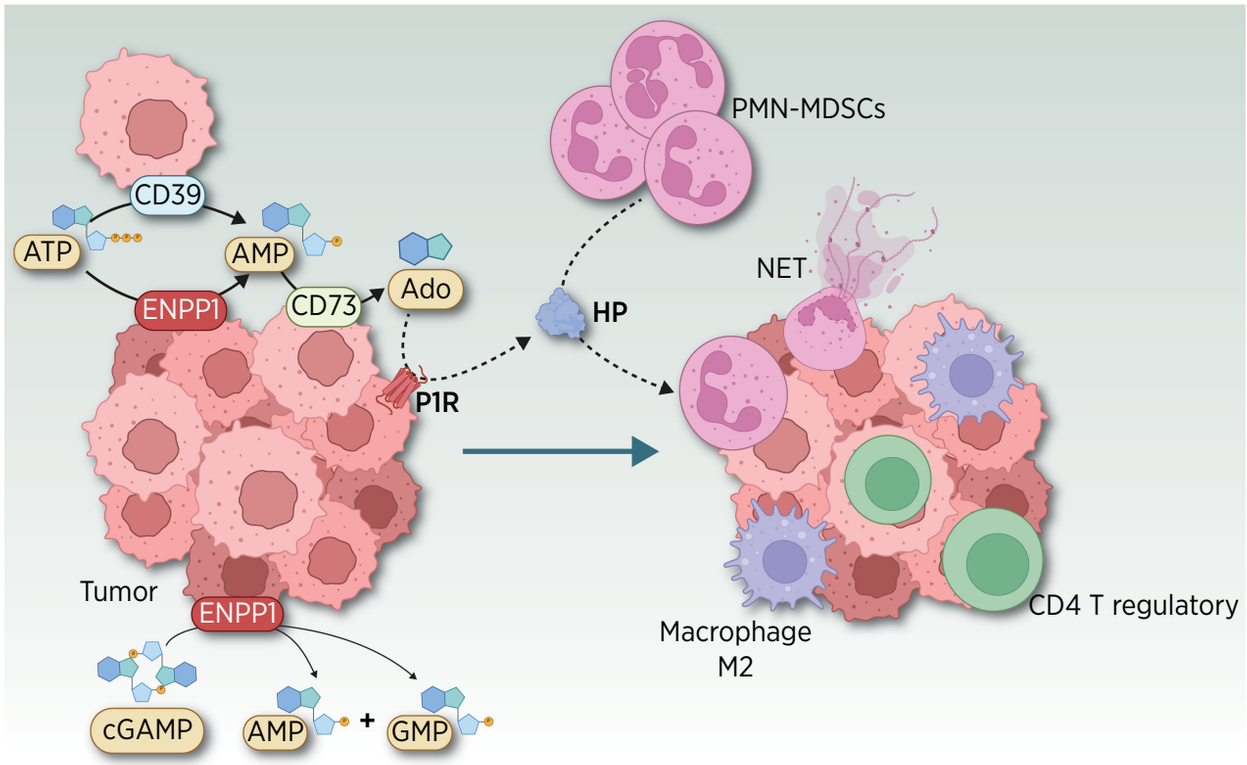

Figure 3.

Emerging role of ENPP1 in tumor cells eliciting a strong immune remodeling. ENPP1 promotes the chemotactic infiltration of PMN-MDSC (18), M2 macrophages, CD4 T regulatory cells, and the inhibition of tumor-infiltrating cytotoxic T and NK cells (19), impeding the antitumor immune attack in preclinical models (33). This myeloid chemoattraction is elicited by the ENPP1-mediated tumor release of haptoglobin (HP), a proinflammatory acute-phase reactant (20) in a manner similar to what has been shown for other chemoattractants, such as IL8 (21). AMP and Ado signaling through P1 receptors (P1R) triggers the release of HP in the TME. HP plays an unexpected double role as a chemoattractant of MDSC and as an inducer of neutrophil extracellular traps (NET), extruded DNA meshes associated with cytotoxic enzymes that promote tumor progression (22). Besides their classical role against bacteria, NETs play different roles either *in situ* or at distant sites (22, 86), promoting the trapping of circulating tumor cells (CTC) in the resected tumor bed, in preventing antitumor immune cytotoxicity, their role in priming the premetastatic niche (87), and their involvement in boosting metastasis (88). (Adapted from an image created with BioRender.com.)

and T regulatory cells among others, as compared with ENPP1 expression in immune cells, which could yield subtle differences in the immune reshaping (41, 42).

Targeting CD39

Because CD39 is expressed in macrophages, neutrophils, DC, and regulatory T cells (Treg), a marked immune remodeling was observed when inhibiting CD39, either alone or in combination with anti–PD-1, which enhanced $CD8^+$ T cells and decreased intratumor macrophages (43). Based on these findings, different phase I clinical trials are ongoing in advanced solid and hematologic tumors using anti-CD39 alone or in combination with anti–PD-1, A2AR inhibitors, and chemotherapy (Supplementary Table S2).

Targeting A2AR

P1 receptors are broadly expressed in many tumors and several immune subpopulations (ref. 44; **Fig. 2**) whereas different levels of CD39-CD73 and CD38-ENPP1 are found in tumors and fluctuate along tumor progression. These differences in tumor expression levels could have different kinds of impacts on therapeutic efficacy. For instance, a tumor growth delay was observed when tumor cells were implanted in CD73/A2AR double knockout mice, whereas the double pharmacologic systemic blockade of CD73 and A2AR powerfully impaired tumor growth and metastasis presumably related to the blockade of tumor-intrinsic functions (45). Ablating A2AR signaling promotes NK maturation and antitumor immunity, while decreasing tumor growth (46). Furthermore, blocking A2AR in combination with anti–PD-1 antibody achieved better antitumor–immune responses compared with single treatments in preclinical models (47). These effects were associated with improved immune cell infiltration, DC priming, and $CD8^+$ T-cell expansion.

Based on these findings, several clinical trials targeting Ado receptors (AZD4635 or SCH58261) are currently ongoing (Supplementary Table S3). Inhibitors of A2AR are more frequently combined with ICB, or with anti-CD73/NT5E in combination with ICB in phase I and II clinical trials in solid tumors (48). The inclusion of RT or chemotherapy combined with A2AR inhibitors and ICB is also being explored.

Phase I clinical trials of A2AR inhibitors in combination with first-in-class CD38-targeting antibody, daratumumab, currently approved for the treatment of multiple myeloma, are ongoing, although the efficacy of CD38 antibody is most likely due to antibody-dependent

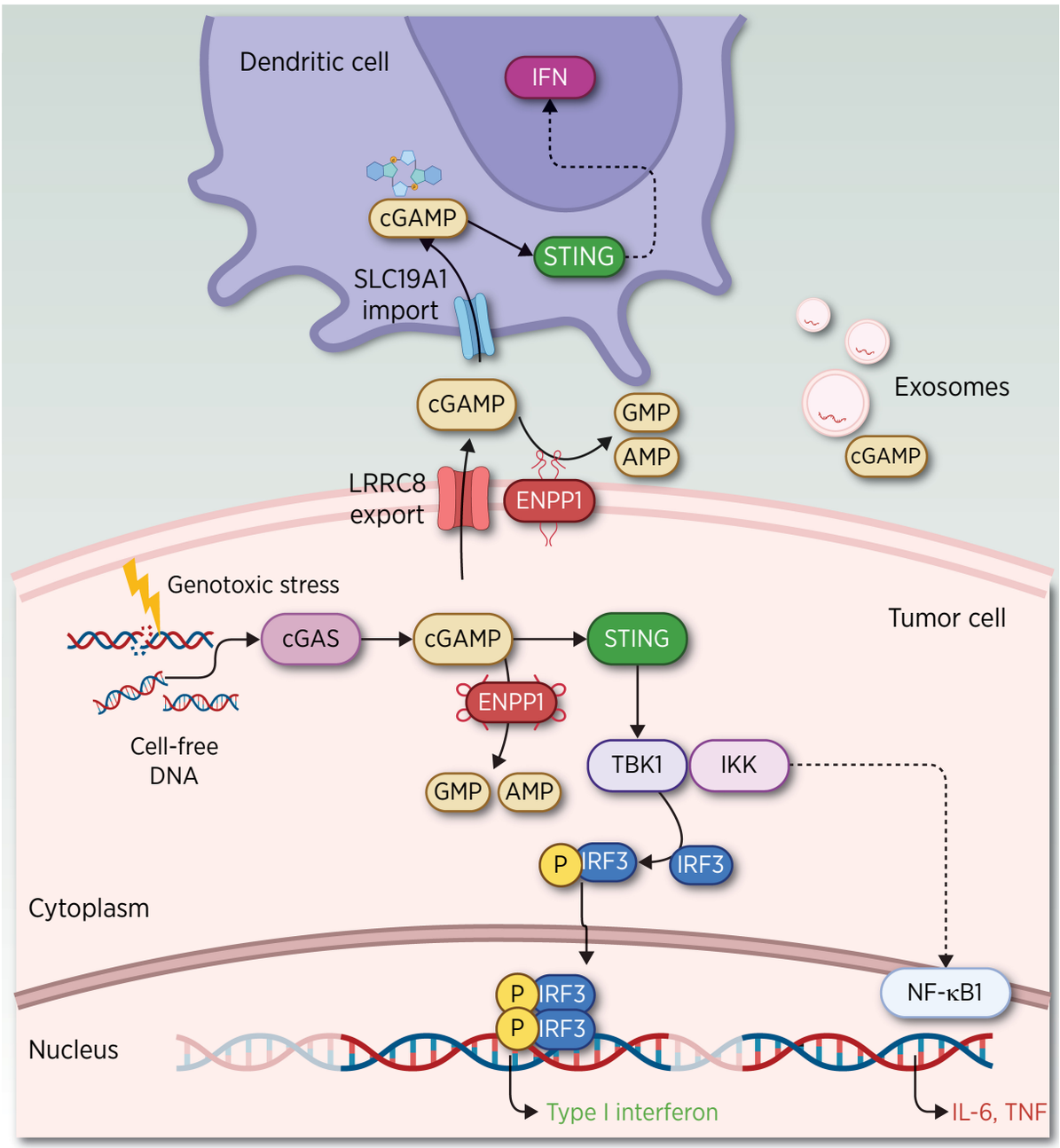

Figure 4.

ENPP1 intersects with the STING pathway. A variety of stimuli in the TME trigger the increase in cGAS (89), including the presence of ATP, GTP, and double-strand (ds) DNA generated by damage-associated molecular patterns (DAMP), pathogen-associated molecular patterns (PAMP), apoptotic cells (a common event in solid tumors), DNA breakage induced by radiotherapy (RT), or free cellular DNA as a consequence of chromosomal instability (28). The synthesis of cGAS leads to the production of cGAMP. cGAMP binds and activates STING located in the endoplasmic reticulum, inducing the transcription of interferon (IFN) genes and other cytokines (60). Intracellular cGAMP dominantly produced by cancer cells also spreads through gap junctions (90–92) to neighboring contacting cells and to the extracellular milieu in released exosomes (93) or through widely expressed cGAMP transporters, such as heteromeric channels, better known as volume-regulated anion channels (VRAC) of the LRRC8 family. Moreover, cGAMP accumulated in the extracellular space is rapidly degraded by transmembrane expression of ENPP1 in tumor or stromal cells. ENPP1 is also expressed in the lumen of the endoplasmic reticulum, which hydrolyzes cGAMP, preventing STING activation. Alternatively, extracellular accumulation of cGAMP can also be transported through the SLC19A1 importer to the cytosol of host immune cells (94, 95), where it is then sensed by STING. Activated STING through TANK-binding kinase 1 (TBK1) and IkB kinase (IKK) induces interferon regulatory factor 3 (IRF3) phosphorylation, resulting in the transcription of type I interferon genes. Activation of STING leads to increased immune infiltration of DC, in particular cross-presenting cDC1 and cytotoxic T-cell activation (29, 96). DC attracted to capture dsDNA will induce IFNβ secretion and will in turn activate $CD8\alpha^{+}$ $CD11c^{+}$ cells (97) with the release of proinflammatory cytokines (CXCL9, CXCL10, and CCL5; ref. 98). Chronic activation of the STING pathway leads to the suppression of type I IFN production and upregulation of an alternative downstream NF-κB signaling that elicits a malignant phenotype and a prometastatic program. One of the IFN-stimulated genes is indoleamine-2,3-dioxygenase-1 (IDO) released from tumors, which promotes activation of $CD4^{+}$ regulatory T cells and suppresses T-helper and effector functions (99, 100). In tumors with low antigenicity, IDO induces immune tolerance by inducing TGFβ in DC, whereas in tumors with high antigenicity, antitumor responses prevail (100–102). (Adapted from an image created with BioRender.com.)

cell-mediated cytotoxicity and not due to adenosine pathway degradation (49).

Targeting CD73/NT5E

CD73 is expressed in Treg and Breg cells of the immune compartment. CD73 is also expressed in mesenchymal stem cells, in tumor-associated stem cells, and it is highly expressed in the vast majority of solid tumors (50, 51). Because Ado accumulation is dependent on the expression and activity of CD73 in tumor cells, blocking CD73 in the TME could lead to substantial benefit impairing tumor growth, which could be more efficacious in combination with chemotherapy and ICB. Targeting CD73 (oleclumab) in combination with ICB showed better outcomes than a single ICB agent (52). Inhibition of CD73 in a preclinical model of pancreatic neuroendocrine tumors led to reduced tumor growth and metastatic potential in cancer stem cells (53). Similarly, $CD73^{-/-}$ mice also develop fewer lung metastases in preclinical models, suggesting that host CD73 also supports metastasis (54, 55). Impaired tumor growth mediated by reshaping the immune landscape with $CD8^{+}$ T-cell infiltration was revealed in models of induced fibrosarcoma and prostate tumors after anti-CD73 treatment (54). Supported by these findings, a large number of phase I and phase II clinical trials targeting CD73 inhibition in combination with ICB (anti–PD-1, anti–PD-L1, or bispecific PD-1/CTLA-4 antibody) or chemotherapy are currently ongoing in a variety of advanced solid tumors (Supplementary Table S4).

Combinatorial Blockade of ENPP1

Inhibition of ENPP1 can also be exploited in combination to heighten the effects of immunotherapy by radiotherapy (RT), agonists of STING, and the use of ICB and DDR inhibitors.

Combination with RT

Besides cytotoxic effects induced by ionizing radiation (56–58), its potent antitumor–immune response is also triggered by the cGAS/STING pathway elicited by the tumor-derived cytoplasmic DNA sensing (59–61). In this context, the combination of RT with ENPP1 inhibition (which also indirectly stimulates STING) could increase the efficacy of current treatments (60, 62). For instance, in the preclinical triple-negative breast cancer model, combined RT and ENPP1 inhibition showed a synergistic effect (33). Moreover, equi-effective fractionated RT doses resulted in a higher immune stimulation, presumably by a dual effect on wave release of tumor antigens and on the sequential STING stimulation. However, in animal models, high dose fractions (above 12–18 Gy) attenuated the immunogenicity and abscopal effects by cytosolic DNA degradation mediated by TREX1 expression (61).

These findings could be translated to the clinical setting of triple-negative breast cancer, where event-free survival is largely determined by the residual cancer burden (RCB). After neoadjuvant treatment, 5-year event survival rates range from 93% in women achieving complete or near-complete pathologic response (RCB-0) to only 41% in RCB-III cases (63). Hence, in patients with RCB-I to III, combined RT with ENPP1 inhibition would be a reasonable treatment option because locoregional failure rates are exceedingly high in this patient subset.

RT also triggers Ado release and upregulates other Ado-generating enzymes such as CD38 (64). Likewise, RT could also be used as a combined strategy with the abrogation of CD73 and Ado signaling to improve current treatments (65).

Combination with STING agonists

The use of ENPP1 inhibitors in combination with STING agonists could have the advantage of activating DC and inducing strong immunostimulatory effects. Clinical trials of STING activators either alone or in combination with ICB are currently ongoing in advanced solid and hematologic tumors (ref. 66; Supplementary Table S5). The majority of drugs tested are administered by intratumor injection, a requirement to achieve the maximum therapeutic effect (67). However, this approach may restrict optimal tumor activity at noninjected lesions, thereby jeopardizing survival rates. A first-in-human trial with a systemic intravenous administration compound targeting STING (GSK3745417) in combination with ICB is ongoing. This drug, a di-amidobenzimidazole that outcompetes cGAMP for STING activation, shows efficacy in a syngeneic model of colon tumors (68). A similar trial with another systemic STING agonist (SNX281) is also being carried out. This compound showed tumor regression and robust antitumor activity in combination with anti–PD-1 in preclinical models (69). Although these are promising, a safety profile of STING agonists should be carefully monitored in clinical trials (**Fig. 4**).

Combination with ICB

Pathways elicited by ENPP1 inhibition in immune-infiltrating cells could also be exploited by the use of ICB. In preclinical models, overexpression of ENPP1 conferred breast and colon tumors resistance to ICB, whereas abrogation of ENPP1 rendered tumors responsive to ICB therapy (13). Based on these findings, ENPP1 inhibition could result in greater immune stimulation concomitantly with anti–PD-1, anti–PD-L1, or anti–CTLA-4 currently approved in the clinical setting. In progress phase Ia/Ib study of RBS2418 as monotherapy or in combination with pembrolizumab in subjects with advanced unresectable, recurrent, or metastatic disease, is harnessing ENPP1 inhibition to unleash antitumor–immune responses (70).

Combination with DDR inhibitors

The sensing of DNA breaks by STING activation, and the exploitation of this pathway by the use of ENPP1 inhibitor could also benefit the combination with DDR inhibitors such as Poly-(ADP-ribose) polymerases (PARP) inhibitors. PARP inhibitors lead to unrepair single- and double-strand breaks and the replication fork stalls, prompting repair by other mechanisms. PARP inhibitors sensitize tumor cells to treatments that induce DNA damage such as radiation. Interestingly, PARP inhibitors stimulate STING and upregulate PD-L1 in many tumors. ENPP1 can also metabolize PAR downstream of PARP in the DDR (71). Thus, concomitant inhibition of ENPP1 and PARP may boost the antitumor effects. Furthermore, it is tempting to speculate that the triple combination with anti–PD-1 or anti–PD-L1, or RT together with PARP or other DDR inhibitors, and ENPP1 inhibitors, could reach more prominent effects than single or double treatments. Future clinical trials will probably explore the efficacy and safety of these potentially promising combinations.

In summary, ENPP1 emerges as an attractive therapeutic target to enhance the effects of RT, to improve the benefit of DNA damage inhibitors, to leverage STING agonists, and to foster mechanisms of resistance emerging with the use of ICB. Based on the array of promising preclinical models, new ongoing trials should highlight the efficacy of ENPP1 abrogation alone, or most likely in combination with other modalities, to ameliorate current clinical outcomes improving local control and ultimately increasing current survival rates in a wide variety of tumors.

Authors' Disclosures

No disclosures were reported.

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Note

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