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IMMUNOTHERAPEUTIC SIGNIFICANCE OF NTPASE 8 IN ONCOLOGY

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Annotation
This article explores the immunotherapeutic significance of the enzyme NTPDase 8 in oncology. In recent years, immunotherapy has emerged as a promising approach in the fight against cancer, highlighting the growing need to understand the complex interactions within the tumor microenvironment (TME). NTPDase 8 belongs to the family of ectonucleotidases involved in the metabolism of extracellular nucleotides and is predominantly expressed in the liver. The article discusses in detail the role of NTPDase 8 in cancer biology, its immunomodulatory functions, and its potential as a therapeutic target in cancer immunotherapy. Additionally, the pharmacological modulation of NTPDase 8 and directions for future research are considered. In conclusion, NTPDase 8 represents a promising target in cancer immunotherapy, and its further investigation may contribute to the development of novel targeted therapeutic strategies.

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Introduction. Oncology remains a major global health challenge, characterized by uncontrolled cell growth and the potential for metastasis. In recent years, immunotherapy has emerged as a revolutionary approach in the fight against cancer. The main principle of immunotherapy is to harness the body's own immune system to combat cancer cells [1]. Several types of immunotherapy exist, including immune checkpoint inhibitors, adoptive cell therapy, and cancer vaccines [1]. The success of current immunotherapies highlights the importance of understanding the complex interactions within the tumor microenvironment (TME) and the critical role of the immune system in controlling cancer.

Tumor Microenvironment (TME) and the NTPDase Family. The tumor microenvironment (TME) is a complex ecosystem composed of tumor cells, immune cells, stromal cells, and the extracellular matrix (ECM) [2]. Among the key mediators of intercellular communication within the TME are ectonucleotidases, which hydrolyze extracellular nucleotides [3]. Extracellular ATP and adenosine play important roles in modulating immune responses and tumor progression [4]. By

regulating extracellular nucleotide levels, ectonucleotidases act as central modulators of the balance between pro-inflammatory and anti-inflammatory signaling within the TME, thereby influencing the effectiveness of anti-tumor immune responses. While ATP generally promotes inflammation and immune activation, adenosine often exerts immunosuppressive effects. Ectonucleotidases such as NTPDase8, which hydrolyze ATP, can indirectly shift this balance. Understanding this shift is crucial for the development of effective immunotherapies.

The NTPDase Family. The nucleoside triphosphate diphosphohydrolase (NTPDase) enzyme family consists of eight members (NTPDase1–8), which hydrolyze nucleoside triphosphates and diphosphates [3]. Four members of this family are membrane-bound: NTPDase1/CD39, NTPDase2/CD39L1, NTPDase3/CD39L3, and NTPDase8 [3]. The remaining members — NTPDase4, 5, 6, and 7 — are located intracellularly [3]. Overall, NTPDases play a crucial role in regulating purinergic signaling by modulating the availability of extracellular nucleotides [3]. All NTPDases share five conserved "apyrase conserved regions" (ACR1–5), which are essential for catalysis [3]. Cell-surface NTPDases possess two transmembrane domains and extracellular domains that govern substrate specificity during nucleotide binding and hydrolysis [3].

The distinct localization and substrate preferences of the eight NTPDase members across various cell types indicate their specialized roles in a range of physiological and pathological processes, including cancer. Understanding these nuances is essential for designing targeted therapeutic interventions. Although all NTPDases hydrolyze nucleotides, their specific activities and localization suggest that targeting one family member may have different effects than targeting another. For instance, intracellular NTPDases may participate in different aspects of tumor cell metabolism compared to their membrane-bound counterparts that influence the TME directly.

Focus on NTPDase8. NTPDase8, also known as hepatic canalicular ecto-ATPase/hATPase, is the most recently characterized member of the NTPDase family [3]. It is primarily expressed in the liver, followed by the intestines and kidneys [3]. In the liver, it is localized to the bile canaliculi, where it plays a role in bile flow regulation and purine reuptake by hepatocytes [3]. NTPDase8 is a transmembrane protein with its active site oriented toward the extracellular space [3]. It hydrolyzes nucleoside triphosphates (NTPs) such as ATP and UTP into nucleoside diphosphates (NDPs) and subsequently into nucleoside monophosphates (NMPs) [3]. NTPDases 3 and 8 exhibit higher affinity for ATP/UTP compared to NTPDases 1 and 2 [3]. Unlike NTPDases 1 and 3, NTPDase8 may transiently release ADP and UDP during nucleotide catabolism [3]. Its enzymatic activity is typically calcium-dependent [5].

The specific expression pattern of NTPDase8, particularly its abundance in the liver—a central organ in both metabolism and immune regulation—suggests it plays a significant role in systemic immunity and inflammation, potentially influencing cancer development and progression. Its ability to transiently release ADP and UDP implies regulatory functions beyond simple nucleotide degradation. Given the liver's role in immune surveillance and metabolic homeostasis, NTPDase8 activity could be crucial in modulating systemic anti-tumor immunity. The transient accumulation of ADP/UDP, which are known to exert anti-inflammatory and pro-thrombotic effects, suggests that NTPDase8's function may be context-dependent and not purely immunosuppressive.

NTPDase8 in Cancer Biology. NTPDases, including NTPDase8, are emerging as attractive therapeutic targets due to their involvement in pathological conditions such as cancer [6]. Increased ATP hydrolysis or overexpression of NTPDases has been observed in various cancer types [7]. NTPDase8 has been implicated in the pathogenesis of diseases including cancer [5]. The role of NTPDases in regulating inflammation and immunity during tumor development is gaining increasing recognition [3].

Although the precise role of NTPDase8 in different cancer types remains under investigation, its ability to modulate levels of extracellular nucleotides—key signaling molecules in cancer—indicates a potential involvement in tumor growth, metastasis, and immune system interactions. Extracellular nucleotides like ATP can function as "danger signals" that influence immune cell recruitment and activation in the tumor context. Therefore, NTPDase8's regulation of these levels may impact the tumor's ability to evade immune surveillance or promote a pro-tumorigenic inflammatory environment.

The Immunomodulatory Role of NTPDase8 in Oncology. Membrane-bound NTPDases, including NTPDase8, can shift the extracellular microenvironment toward an anti-inflammatory state by hydrolyzing ATP to adenosine in coordination with CD73 [3]. However, the transient release of ADP and UDP by NTPDase8 may also exert anti-inflammatory effects through P2Y receptors on immune cells [3]. By modulating extracellular nucleotide levels, NTPDase8 helps regulate immune responses [5]. In the context of intestinal inflammation, NTPDase8 protects mice by limiting P2Y6 receptor activation in colonic epithelial cells, indicating its role in controlling local inflammatory responses [3]. Mice lacking NTPDase8 exhibit heightened intestinal inflammation and increased infiltration of immune cells [3].

NTPDase8 appears to play a complex immunomodulatory role in oncology. While its contribution to adenosine production may be immunosuppressive, its ability to transiently release ADP/UDP and its role in limiting inflammation in other contexts suggest a nuanced function that may vary depending on the cancer type and specific tumor microenvironment. The balance between ATP and adenosine levels in the TME is critical for immune response. NTPDase8's role in reducing ATP levels and potentially enhancing anti-inflammatory signaling via ADP/UDP requires careful consideration regarding its overall impact on anti-tumor immunity. Its protective role in intestinal inflammation points to a potential for modulating immune responses in other tissues affected by cancer.

NTPDase8 as a Target for Cancer Immunotherapy. Targeting cellular ATP metabolism pathways—including extracellular purinergic signaling regulated by NTPDases such as NTPDase8—is an active area of research in oncology [9]. Inhibiting CD39, another member of the NTPDase family, has proven an effective strategy for cancer immunotherapy by increasing immunostimulatory ATP and reducing immunosuppressive adenosine [8]. However, CD39 inhibitors must be specific to avoid undesirable toxicity due to the roles of other NTPDases like NTPDase2 and NTPDase8 [8].

Given the success of targeting other ectonucleotidases such as CD39 and CD73 in cancer immunotherapy, NTPDase8 also holds potential as a therapeutic target. However, its distinct expression pattern and physiological functions require a careful understanding of the specific contexts in which its modulation would be beneficial. The main goal of targeting CD39 is to shift the adenosine balance in the TME toward ATP. Whether a similar strategy would be effective for NTPDase8 needs further investigation, considering its different substrate affinities and potential anti-inflammatory effects. Specificity is particularly important when targeting NTPDase8 to avoid disrupting its physiological roles in the liver.

Pharmacological Modulation of NTPDase8. The study of NTPDase8 has been limited by the lack of specific tools such as antibodies [10]. However, antibodies have now been developed and characterized [10]. Commercial antibodies against NTPDase8 peptides often failed to provide specific signals [10]. The inhibitor h-NTPDase8-IN-1 is a selective inhibitor of human NTPDase8, with an IC_{50} of 0.28 μM, and its role in cancer is under investigation [11]. NTPDase-IN-2 and NTPDase-IN-3 are other selective inhibitors that also show activity against NTPDase8 [12]. The anticancer drug ceritinib has been shown to inhibit CD39 and, at higher concentrations, also exhibit inhibitory activity against NTPDase8 [13]. Heparin and its derivatives have been shown to inhibit other ectonucleotidases such as NPP1 and NTPDase1, indicating potential for modulating purinergic signaling [14].

The development of specific inhibitors for NTPDase8, such as h-NTPDase8-IN-1, provides valuable tools to explore its role in cancer and evaluate its potential as a therapeutic target. Observations that existing anticancer drugs and other compounds may modulate NTPDase activity suggest possibilities for drug repurposing or the development of multitargeted therapies. The availability of selective NTPDase8 inhibitors allows researchers to directly assess the effects of inhibiting this enzyme on tumor growth, metastasis, and immune responses in preclinical models. Understanding off-target effects of existing drugs like ceritinib on NTPDase8 may also yield insights into their mechanisms of action in cancer therapy.

Table 1.

Human NTPase family.

NTPDase number	**Alternate names**	**Location in the cell**	**Substrate preference**	**Tissue expression**	**Known or potential roles in cancer**
1	CD 39	Membrane-bound	ATF ~ADF	All tissues	Immunosuppression, tumor growth
2	CD 39 L1	Membrane-bound	ATF>ADF	All tissues	Thrombosis inflammation
3	CD 39 L3	Membrane-bound	ATF/UTF>ADF/UDF	Neurons, some epithelia	Eating, sleep-wake cycle
4		Intracellular (lysosomes, autophagic vacuoles)	ATF>ADF	All tissues	Intracellular metabolism
5		Intracellularly, in secreted from	ATF>ADF	All tissues	Intracellular metabolism, secretory functions

Conclusion and Future Perspectives. In conclusion, NTPdase 8 plays an important role in the hydrolysis of extracellular nucleotides and is expressed in key metabolic and immune organs. Its complex immunomodulatory functions include both pro-inflammatory and anti-inflammatory effects. Given its similarities with other ectonucleotidases, NTPdase 8 holds potential as a therapeutic target in cancer immunotherapy. The availability of specific inhibitors represents a promising tool for future research.

Future research directions should include the following: further investigation of the specific roles of NTPdase 8 in various cancer types and stages; preclinical studies using NTPdase 8 inhibitors and activators to evaluate their effects on tumor growth, metastasis, and anti-tumor immunity; identification of the precise mechanisms through which NTPdase 8 influences immune cell activity within the tumor microenvironment; exploration of the potential to combine NTPdase 8 modulation with existing cancer immunotherapies; evaluation of the clinical significance of NTPdase 8 expression levels in cancer patients and their correlation with treatment outcomes; development of more potent and selective NTPdase 8 inhibitors or activators for therapeutic use; understanding the role of NTPdase 8 in the context of specific immune cell populations within the tumor microenvironment; and investigation of how NTPdase 8 interacts with purinergic receptors in cancer.

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