Ectonucleotidases in Tumor Cells and Tumor-Associated Immune Cells: An Overview

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Increasing evidence points out that genetic alteration does not guarantee the development of a tumor and indicates that complex interactions of tumor cells with the microenvironment are fundamental to tumorigenesis. Among the pathological alterations that give tumor cells invasive potential, disruption of inflammatory response and the purinergic signaling are emerging as an important component of cancer progression. Nucleotide/nucleoside receptor-mediated cell communication is orchestrated by ectonucleotidases, which efficiently hydrolyze ATP, ADP, and AMP to adenosine. ATP can act as danger signaling whereas adenosine, acts as a negative feedback mechanism to limit inflammation. Many tumors exhibit alterations in ATP-metabolizing enzymes, which may contribute to the pathological events observed in solid cancer. In this paper, the main changes occurring in the expression and activity of ectonucleotidases in tumor cells as well as in tumor-associated immune cells are discussed. Furthermore, we focus on the understanding of the purinergic signaling primarily as exemplified by research done by the group on gliomas.

1. Introduction

Nucleotide/nucleoside receptor-mediated cell communication is controlled by the action of ectonucleotidases, including the members of the ectonucleoside triphosphate diphosphohydrolases (E-NTPDases, ecto-ATPases, ectoapyrases, EC 3.6.1.5), ectonucleotide pyrophosphatase phosphodiesterases (E-NPP, EC 3.1.4.1), ectoalkaline phosphatases (ALP, EC 3.1.3.1), and ecto-5′-nucleotidase/CD73 (ecto-5′-NT/CD73, EC 3.1.3.5), which efficiently hydrolyze ATP, ADP, and AMP to adenosine (Ado) [1–3].

The E-NTPDase members differ regarding the preferences for nucleotides as substrates. While NTPDase1/CD39 hydrolyses nucleoside tri- and diphosphates almost equally well, NTPDase2/CD39L1 presents a high preference for nucleoside triphosphates and NTPDase3/CD39L3 and 8 reveal an intermediate preference for ATP over ADP [1, 4–9]. In consequence, the action of NTPDase1/CD39 produces almost directly AMP with minor amounts of free ADP in the extracellular space. This functional property implicates the participation of this enzyme in the control of specific P2Y receptors for nucleoside triphosphates. Otherwise, ADP is transiently produced by the action of NTPDase2/CD39L1, which implicates the generation of agonist for nucleoside diphosphate-sensitive receptors such as platelet P2Y1 and P2Y12 receptors [2]. The second family of ectonucleotidases is the ectonucleotide pyrophosphatase/phosphodiesterases (E-NPP). The E-NPP family is constituted by seven ectoenzymes, but only the NPP1–3 are involved in the purinergic signaling [2, 10–12]. The final step of nucleotide hydrolysis to generate adenosine is catalyzed by ecto-5′-nucleotidase/CD73 (ecto-5′-NT/CD73) [1, 13]. In addition, to constitute the major source of extracellular adenosine, other nonenzymatic functions are assigned for this protein.
Ecto-5′-NT/CD73 itself acts as a proliferative factor and is involved in the control of cell growth, cell-cell and cell-matrix interactions [14–16].

In this paper, the alterations in the ATP-metabolizing enzymes, especially the ectonucleotidases that may contribute to the physiopathological events observed in solid cancer are discussed.

2. Ectonucleotidases in Immune Cells

Extracellular nucleotides and nucleosides play an important role in inflammatory and immune responses. To date, ATP is mainly associated to proinflammatory response whereas adenosine has opposite effects limiting the inflammation by suppressing the actions of immune cells [17–20]. Moreover, the plasticity of immune cells during early phase to resolution of inflammation turns important of the control of these immunomodulatory molecules. Increasing evidence suggests the participation of ectonucleotidases in inflammatory process involving immune cells [21–26]. The ectonucleotidases are expressed in B lymphocytes, natural killers cells (NKs), monocytes, macrophages, dendritic cells (DCs) and subsets of T cells [21–26]. Although the presence of the enzymatic chain responsible for ATP hydrolysis and adenosine production was demonstrated in almost all immune cells, only recently the participation of ectonucleotidases in the control of inflammation has been shown.

The first studies to begin to elucidate the physiological role of E-NTPDases (Ecto-ATPases) in immune cells have been proposed in the early nineties [27–29]. Dombrowski et al. [27] showed evidence that Ecto-ATPase activity was required for activation of effector T cells (CD8+1) and for antigen recognition [27]. Likewise, upregulation of E-NTPDase activity on CD4+ cells has been described soon after stimulation whereas CD4+ naive cells present a negligible activity [30]. In the same study it was shown that the inhibition of E-NTPDase or ATP depletion on CD4+ diminished INF-γ and IL-2 secretion [30]. Recently the role of adenosine generated by ecto-5′-nucleotidase/CD73 in graft-versus-host disease was demonstrated. The ecto-5′-nucleotidase/CD73 deficiency led to enhanced T-cell expansion, IFN-γ and IL-6 production, and the migratory capacity of CD73+/− T cells [31].

Recent studies have shown the central role of ectonucleotidases in Foxp3+ T regulatory cells (Tregs). The NTPDase1/CD39 and the ecto-5′-NT/CD73 expressed in Tregs compose one of the immunosuppressive mechanisms associated to these immune cells [32–35]. In addition, alterations in NTPDase1/CD39 and ecto-5′-NT/CD73 machinery may produce more adenosine, which lead to severe immunodeficiency with recurrent infection [36, 37]. In accordance, recently Tang et al. [38] verified that the NTPDase1/CD39 on Foxp3+ T regulatory cells correlates with progression of hepatitis B virus infection and it can be associated with other viral infections, and autoimmune diseases [38]. Moreover, it has been reported that lupus patients express low levels of NTPDase1/CD39, and this is associated with reduced generation of adenosine [39].

In relation to the ectonucleotidases in macrophages, some advances have been done. Hyman et al. [40] have reported the importance of NTPDase1/CD39 in the trafficking of monocyte/macrophage during an ischemic process. They showed that inhibition or genetic deletion of NTPDase1/CD39 (CD39⁻/⁻) generated an increase in the ischemic area and the leukocytes number (mainly monocytes/macrophages). The data demonstrated that NTPDase1/CD39 reduces stimulation of the P2X7 receptor by modulating αMβ2 integrin expression on the surface of monocytes/macrophages, thus controlling their migration [40]. Pelegrin and Surprenant [41] have reported the participation of pyrophosphate originated from extracellular ATP hydrolysis to inhibit IL-1β release in alternative/M2 polarized macrophages. In addition, NTPDase1/CD39, the dominant ectonucleotidase on macrophages, controls the IL-1β secretion by these cells by regulating the P2X7 receptor activation [26]. Notably, we have shown that NTPDase1/CD39 and ecto-5′-NT/CD73 are differentially expressed during macrophage polarization, which results in extracellular ATP accumulation in proinflammatory/M1 phenotype while anti-inflammatory or alternative/M2 phenotype generates immunosuppressive adenosine [24].

Although the NTPDase1/CD39 expression has been reported in dendritic cells, little is known about the role of E-NTPDases and other ectonucleotidases in immune function of these cells and its subtypes [42]. For instance, skin-resident dendritic cells (Langerhans cells) in CD39⁻/⁻ mice reveal a dichotomy role in irritant versus allergic contact dermatitis [43]. In the irritant dermatitis there was an exacerbated skin inflammation in CD39⁻/⁻ mice indicating that the NTPDase1/CD39 serves as the first line of defense at the environmental interface against nucleotide-mediated inflammatory signals. On the other hand in the allergic contact dermatitis was severely attenuated in these mice by impairing the Langerhans cell with T cell communication in antigen presentation [43].

Coridden et al. [44] showed the participation of NTPDase1/CD39 chemotaxis regulation by facilitating extracellular ATP hydrolysis in human and neutrophil lineage. Corroborating this data, it has been demonstrated that NTPDase1/CD39 controls IL-8 production in human neutrophils via regulation of P2 activation [25]. Even though NTPDase1/CD39 is expressed in NK and NK-T cells, the application of this has not yet been fully elucidated.

Of note, NTPDase1/CD39 and ecto-5′-NT/CD73 expression by tumor-infiltrated immune cells can lead to adenosine generation, inducing an immune suppression around the tumors. So, these ectoenzymes might allow immune cells to adjust the outcome of the extracellular purinergic cascade in order to fine-tune their functions during the inflammatory set. Therefore, the continuing development of therapeutic strategies targeting the combat for the disordered inflammation and aberrant immune reactivity that involve ectonucleotidases could offer promising finding.

3. Ectonucleotidases in Solid Cancer

Cancer development is a multifactorial process consisting of numerous genetic alterations that controls cell proliferation
and differentiation, including the regulation in oncogenes expression (MDM2, CDK4, EGFR) and tumor suppressor genes (p53, p16, p15, and RB1) [45–48]. However, increasing evidence points that the genetic alteration does not guarantee the development of a tumor and indicates that complex interactions of tumor cells with the microenvironment are fundamental to tumorigenesis. In the tumor microenvironment, the presence of secretory products released by tumor and tumor-associated cells creates a growth factor-rich environment linked to tumor maintenance and growth [49].

A number of studies have investigated the identity of these endogenous signals, their receptors, and signaling pathways using tumor models. The most likely candidates are dying cells or extracellular matrix components, glutamate, nucleotides, and nucleosides, for example, ATP and adenosine, all of which were found to be present in the tumor environment [50–52].

Purinergic signaling involving ATP and the respective breakdown or hydrolytic products such as ADP and adenosine activate their own responses via purinergic receptor activation and modulate cross-talk with chemokines [17]. ATP has been identified as a mitogen for v-src immortalized neural progenitor cells [53]. In astrocytes, extracellular ATP regulates ERK function by activating P2Y1, P2Y2, or P2Y4 purinoceptors [54, 55] indicating the potential for cross-talk with FGF-, EGF- and PDGF- driven cell mitogenic pathways. Adenosine may accumulate in the tumor interstitium [52] where it modulates cell proliferation and angiogenesis, and suppresses anticancer immune responses [56, 57]. Purines can be released from damaged cells during tumor growing, acting as a classical danger signal for the immune system and as a proliferative stimulus to different cancer kinds. However, purines are also released from host normal cells, immune as well as cancer cells through several active mechanisms, including shear stress, hypotonic swelling, hypoxia, stretching, hydrostatic pressure, as well as in response to Ca2+- mobilizing pharmacological agonists [58–60]. As presented before, nucleotide/nucleoside receptor-mediated cell communication is orchestrated by ectonucleotidases, which efficiently hydrolyze ATP, ADP, and AMP to adenosine [61]. The presence/absence of ectonucleotidases in a variety of human tumors has been reported such as ovarian cancer [62], Walker 256 tumor [63], melanomas [64], colorectal cancer [65], glioma [66], and bladder cancer [67]. Therefore, it is tempting to propose that disruption of ectonucleotidase activity from both tumor and infiltrated cells may constitute important regulators of tumor spread and metastasis. Accordingly, it has shown that ATP accumulates in the tumor interstitium at hundreds micromolar range, while being almost undetectable in healthy tissues [51]. Extracellular ATP may be crucial for the tumor not only as a stimulus for growth but also as a source of an immunosuppressive agent such as adenosine [51].

The anti- or protumor effect target by ectonucleotidases, mainly NTPDase1/CD39, is related to tumor kind and its interaction with stromal, immune and endothelial cells. For example, a study published by Häusler et al. [62] showed aberrant NTPDase1/CD39 and ecto-5′-NT/CD73 expression in human ovarian cancer biopsies. Functional assays in ovarian cancer cell culture applying siRNA against NTPDase1/CD39 and ecto-5′- NT/CD73 or pharmacological inhibitors of A2A adenosine receptors revealed that tumor-derived adenosine inhibits the proliferation of allogeneic human CD4+ T cells as well as cytotoxic effect of T cell priming and NK cells cytotoxicity [62]. The presence of E-NTPDase and ecto-5′-NT/CD73 has been characterized in Walker 256 tumor, where the NTPDase1/CD39, NTPDase2/CD39L1, and ecto-5′-NT/CD73 were identified as the dominant enzymes expressed, which by regulating the ratio of nucleotides/nucleosides may target tumor growth [63]. On the other hand, in melanomas, an association was observed between NTPDase1/CD39 overexpression, the differentiation degree of tumor cells, and the tumor escape from immunological effectors mechanisms at early stages of tumor progression, indicating a role of purinergic signaling in cell differentiation and antitumor immune response [64]. Indeed, the deletion of NTPDase1/CD39 resulted in reduction of melanoma growth and inhibition of pulmonary metastases, associated with abrogation of angiogenesis [68]. In addition to ectonucleotidases expressed by tumor cells, the nucleotide-metabolizing enzymes present at surface of tumor-associated cells also contribute to tumor growing or inhibition. The NTPDase1/CD39 expression on Treg inhibits NK cell-mediated antitumor activity and is permissive for hepatic metastatic tumor growth, whereas vascular NTPDase1/CD39 boosts angiogenesis [69]. Extracellular ATP limits melanoma cell growth, and this antitumor effect could be overcome by intrinsic NTPDase1/CD39 expression by endothelial cells [70]. The authors suggest targeting the NTPDase1/CD39 activity or expression in combination with conventional therapy could provide a novel approach to cancer treatment [70]. In human follicular lymphoma, it has been observed that, in addition to Treg-suppressing effect, infiltrating T cells are suppressed by extracellular adenosine, which is produced by ATP-nucleotidase-adenosine system present in lymph node mononuclear cells [71]. Indeed, the selective NTPDase1/CD39 inhibitor and the A2A and A3B antagonists partially overcome T cell suppression [71]. Finally, the increased expression of NTPDase1/CD39 and ecto-5′-NT/CD73 in Treg cells of patients with head and neck cancer is related to the conversion of ATP to immunosuppressive adenosine. Elevations in adenosine levels are responsible for suppressor functions of CD4+CD39+ Treg in patients with an active disease as well as those with no evident disease after successful therapy [32].

Ecto-5′-NT/CD73, originally defined as a lymphocyte differentiation antigen, is thought to function as a co-signaling molecule on T lymphocytes and is widely expressed on many tumor cell lines and in cancerous tissues [56, 72, 73], including bladder cancer [67], glioma cell lines [74], melanoma [75], ovarian cancer [76], thyroid cancer [77], esophageal cancer [78], prostate cancer [79], breast cancer [80, 81], and lymphoma [82]. Ecto-5′-NT/CD73 upregulation is associated with a highly invasive cancer phenotype, drug resistance, and tumor-promoting functions [56]. In addition, to produce immunosuppressive adenosine from AMP hydrolysis, ecto-5′-NT/CD73 acts as an adhesive molecule and interacts with extracellular matrix glycoprotein, such as
fibronectin and laminin, to produce cancer-invasive properties [56]. Studies suggest that ecto-5'-NT/CD73 expression can enhance breast-cancer cell migration and invasion [81], and its expression has been proposed as prognostic marker to patients. Indeed, the therapy with anti-CD73 monoclonal antibody delayed the breast primary tumor growth and inhibited the development on spontaneous lung metastases [83]. These antitumor effects were dependent on an induction of an adaptive antitumor immune response. In addition, ecto-5'-NT/CD73 was involved in tumor chemotaxis, and the A2B adenosine receptor participates in this process [83]. In line with the role of ecto-5'-NT/CD73 in cancer progression, Zhi et al. [84] evaluated the participation of ecto-5'-NT/CD73 in breast cancer growth by examining the effect of ecto-5'-NT/CD73 suppression via RNA interference and ecto-5'-NT/CD73 overexpression on tumor growth in vitro and in vivo. As expected, the cell growth rate was significantly lower after ecto-5'-NT/CD73 suppression. In opposite, the ecto-5'-NT/CD73 overexpression increased cell viability and promoted cell cycle progression, depending on its enzyme activity [84]. Taken together, these studies suggest that ecto-5'-NT/CD73 play an important role in cancer growth by affecting cell cycle progression and apoptosis and by triggering adaptive antitumor immunity and inhibiting metastasis [83, 84].

Although the functions of ecto-5'-NT/CD73 in cancer cells have been investigated to some extent, the contribution of host ecto-5'-NT/CD73 activity to cancer progression has been recently addressed. In these studies, authors employed ecto-5'-NT/CD73 gene-targeted mice to investigate the role of host-derived ecto-5'-NT/CD73 in antitumor immunity, tumor cell metastasis, and carcinogenesis [85–87]. Ecto-5'-NT/CD73 deficient mice had significantly elevated ATPase and ADPase activities in T lymphocytes. In a melanoma model, the growth of primary tumors and formation of metastasis were significantly attenuated in mice lacking ecto-5'-NT/CD73. The intratumoral accumulation of Tregs and mannose receptor macrophages, which are related to tumor malignancy, was also attenuated in ecto-5'-NT/CD73-deficient mice [85]. In addition, it has been shown that the host-derived ecto-5'-NT/CD73 ablation significantly suppressed the growth of colon cancer, lymphoma, mammary tumors, and melanoma [86]. The protective effect of ecto-5'-NT/CD73 deficiency on primary tumors was dependent on CD8+ T cells and associated with an increased frequency of antigen-specific CD8+ T cells in peripheral blood and tumors [86]. Finally, recent studies suggest that host-derived ecto-5'-NT/CD73 exerts a critical oncogenic function during tumorigenesis. Ecto-5'-NT/CD73 deficiency suppressed the development of 3-methylcholanthrene- (MCA-) induced fibrosarcomas and also suppressed prostate tumorigenesis in TRAMP transgenic mice. Notably, the treatment with an anti-CD73 monoclonal antibody effectively suppressed growth of established tumors and inhibited the development of TRAMP-C1 lung metastases [87]. Taken together, these data indicate that suppression of ecto-5'-NT/CD73 activity at multiple levels, including tumor cells, Tregs and non-hematopoietic cells, may be a new tool to control tumor growing and modulate antitumor immune responses.

3.1. Ectonucleotidases in a Model of Solid Tumor: Gliomas.

Different signaling pathways, including the purinergic system, are involved in glioma progression [66].

It was previously showed that several glioma cells are resistant to cytotoxic ATP while this nucleotide promotes glioma proliferation [88, 89] and neuronal cell death [90]. We have shown that a variety of glioma cell lines (C6, U138MG, U251MG, and U87MG) exhibit diminished ATP hydrolysis (low ATPase/ADPase activities) and elevated capacity of hydrolyze AMP (high AMPase activity) when compared to astrocytes in culture [91]. According to enzymatic activity profile, glioma cells present low expression of NTP-Dase1/CD39, NTPDase2/CD39L1, and NTPDase3/CD39L3 in relation to astrocytes [66]. The same ectonucleotidases profile can be found in bladder tumor [67].

Notably, we also verified that the coinjection of apyrase (an ATP and ADP scavenger) with C6 glioma cells, in an in vivo glioma model, resulted in reduction of tumor growth, which was followed by a decreased inflammatory infiltrate, angiogenesis, and malignant characteristics [66]. NTPDase2/CD39L1 overexpression in C6 glioma cells dramatically increased tumor growth, malignant characteristics, a sizable platelet sequestration and macrophage/microglial activation in the tumor area [92]. The NTPDase2/CD39L1, by preferentially removing ATP, may favor extracellular ADP accumulation and consequent P2Y1 and P2Y12 receptor modulation on glioma-associated platelets [1, 2]. These data suggest that the ADP derived from NTPDase2/CD39L1 activity stimulates platelet migration to the tumor area and that NTPDase2/CD39L1, by regulating angiogenesis and inflammation, seems to play an important role in tumor progression [92]. In addition, to promote in vivo glioma growth, the NTPDase2/CD39L1 overexpression in tumor cells also modulated systemic inflammatory responses [93].

Likewise, previous studies have shown that C6 glioma cells exhibit NPP1 on the plasma membrane, which are responsible for the hydrolysis of low physiological extracellular ATP concentration (1–10 μM) [94]. Interestingly Aerts et al. [95] have suggested that NPP1 can be a prognostic marker to glioma tumors since high grade tumors (grade II, III and IV) have increased NPP1 expression. Moreover, the ATP accumulation can be an explanation for the induction of NPP1 expression in glioma cells [95], which is in accordance with our hypothesis that ATP being degraded very slowly results in the accumulation of this nucleotide around the tumor [66, 95].

Many studies have demonstrated that the presence of inflammatory infiltrate is involved in tumor progression [96, 97]. In gliomas, the presence of inflammatory infiltrate is directly correlated with tumor malignancy degree [98]. C6 glioma cells, in presence of ATP, release proinflammatory factors, such as MCP-1 and IL-8, important for the recruitment of monocytes and neutrophils, respectively [99]. When these immune cells reach the tumor environment, different stimuli modulate macrophage phenotype [100, 101]. Several studies show that tumor-associated macrophages (TAMs) resemble an anti-inflammatory/M2 phenotype, in contrast to the proinflammatory/M1 phenotype [102, 103]. In agreement with these results, Komohara et al. [96] showed that patients
Figure 1: Ectonucleotidases in glioma progression. Glioma cells exhibit low ATP/ADP hydrolysis and a high AMP hydrolysis activity [91]. The inversion of extracellular nucleotide metabolism may favor extracellular ATP and adenosine accumulation within the tumor [51, 52, 66]. ATP could induce neuronal toxicity [90], glioma cell proliferation [88], and recruitment of immune cells by inducing the release of proinflammatory factors by tumor cells, such as MCP-1 and IL-8 [99]. Upon reaching the tumor, different stimuli modulate macrophage to M2 phenotype [100, 101], and studies from our laboratory showed that ectonucleotidases are involved in the differentiation of macrophages [24]. The glioma cells exhibit NPP1 on the plasma membrane [94]; this enzyme generates AMP that is toxic for gliomas [74] but is the substrate for the ecto-5'-NT/CD73 which is highly expressed in glioma [74]. Ado, product of AMP hydrolysis could induce tumor cell proliferation, angiogenesis, and immunosuppression [56]. Therefore, the ATP and its hydrolytic products could be closely related to the immune responses involved in the glioma progression.

with glioblastoma multiforme have an increased infiltration of type M2 macrophages when compared to patients with lower-grade tumors. Therefore, by modulating multiple signaling pathways closely related to tumor malignancy, TAMs are considered key elements in the tumorigenesis processes. Studies are underway in our laboratory to establish if and how the ectonucleotidases would be involved in macrophage polarization in gliomas.

Hydrolysis of AMP by ecto-5'-nucleotidase/CD73 action generates adenosine [1]. Glioblastoma multiforme is characterized by extensive hypoxia areas, which exhibit increased adenosine levels [52]. Adenosine has been recognized to mediate an immunosuppressive response to protect adjacent tissues of inflammation [57]. Furthermore, this nucleoside has been reported as mediator of cell proliferation and angiogenesis and also acts in tumor progression [56].

Previous results from our laboratory showed that increasing confluences led to an increase in ecto-5'-NT/CD73 activity in glioma cell lines [74]. This event could be related to an increased ability to infiltrate the brain parenchyma, which constitutes the main cause of glioma recurrence [104, 105]. It was also shown that the inhibition of this ectoenzyme results in a decreased glioma cell proliferation. We suggested that this process is dependent on adenosine production parallel to AMP removal, a toxic molecule for gliomas [74]. Ohkubo et al. [106] have shown that adenosine inhibits cell proliferation of C6 glioma cells by its intracellular conversion to AMP. However, in our study, we showed that the stimulus of proliferation caused by adenosine is via extracellular effects instead of an adenosine uptake-dependent effect [74].

As in another solid cancer cited herein, the ecto-5'-NT/CD73 is also involved in cell-cell and cell-matrix adhesion, key processes of tumor invasion and metastasis [107]. However, few studies are found in the literature relating the ecto-5'-NT/CD73 in invasion events of gliomas. Gessi et al. [108] showed that adenosine induced an increase of metalloproteinase-9, which is responsible for an increase of glioma cells invasion [108]. In a parallel investigation, we showed that exogenous adenosine promoted an increase in glioma cell adhesion in vitro, and the addition of selective inhibitor of this enzyme prevents this effect [109]. Therefore,
this enzyme seems to play extreme importance in the glioma development. Taken together, these data indicate that suppression of ecto-5′-NT/CD73 activity at multiple levels, including tumor cells, may be a new tool to control tumor growing.

4. Concluding Remarks

Nucleotide/nucleoside receptor-mediated cell communication is orchestrated by ectonucleotidases, which efficiently hydrolyze ATP, ADP, and AMP to adenosine. The alterations in ectonucleotidases activity/expression may contribute to the physiopathological events observed in solid cancers as it has been studied in gliomas (Figure 1). In this paper, we summarized the main changes occurring in the expression/activity of ectonucleotidases in glioma cells as well in the tumor-associated immune cells. The development of therapeutic strategies targeting ectonucleotidases in tumor environment could offer promising finding.

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