https://www.scientificarchives.com/journal/journal-of-cancer-immunology

Journal of Cancer Immunology Commentary

Purinergic System and Cervical Cancer: Perspectives

Marta Schmidt Pfaffenzeller, Maria Luiza Mukai Franciosi, Adriana Fuganti Wagner, Andreia Machado Cardoso*

Medicine Course, Campus Chapecó, Federal University of Fronteira Sul, Chapecó, SC, Brazil

*Correspondence should be addressed to Andreia Machado Cardoso; andreia.cardoso@uffs.edu.br

Received date: April 02, 2020, **Accepted date:** June 02, 2020

We have recently published an article entitled "Purinergic signaling and tumor microenvironment in cervical Cancer" [1]. In this paper, we reviewed the last studies about purinergic signaling and cervical cancer, highlighting the intrinsic factors related to the inflammatory process, such as extracellular nucleotides and adenosine - components of the purinergic system. Our review focused on the role of the purinergic system in cervical cancer, especially regarding the interaction of extracellular nucleotides with their respective receptors expressed in the tumor microenvironment of cervical cancer and their role in the host immune response. Here we comment the main points of our work and suggest further basic and clinical investigations related to these key factors.

The Involvement of Purinergic Signaling in the Tumor Development

It is widely known that cervical cancer is the fourth most prevalent cancer in the female population worldwide, representing more than 300,000 deaths per year [2]. The development of this cancer occurs mainly due to the persistent infection of one of the carcinogenic types of *human papillomavirus* (HPV) [3], which leads to the disruption of mechanisms that are involved in differentiation and programmed cell death. Recently, the purinergic system has been associated with an essential signaling pathway related to tumor cell progression [4] – interfering with mechanisms such as disordered cell proliferation, promotion of angiogenesis, and failure of mechanisms controlling apoptosis [5]. These processes occur due to the nature of tumor environment that presents an unbalance in the concentrations of *adenosine triphosphate* (ATP), *adenosine diphosphate* (ADP), *adenosine monophosphate* (AMP) nucleotides and *of adenosine* (Ado) nucleoside; as well as overexpression or, in some cases, the low expression of P2 receptors [6].

Tumor microenvironment (TME) of CeCa is rich in ATP and Ado [7] – suggesting that signaling and the purinergic pathway play an important role concerning the mechanisms that control cell growth and death in cancer [8]. These molecules – which typically exhibit opposite effects – are involved in purinergic signaling in several biological processes, such as proliferation, cell differentiation and growth, apoptosis, and mediation of immune responses [6]. Nevertheless, regarding the apoptotic effect induced by these molecules, extracellular ATP has a minor effect on mechanisms that lead to programmed cell death on CeCa cancer cells when compared to Ado, formed by the degradation of ATP by ectonucleotidases. Ado acts as the main fatcor responsible for inducing apoptosis in these cells. If the inhibition of the conversion of Ado into AMP occurs, this will result in the inhibition of cytotoxic effect, indicating that Ado – originated from extracellular ATP – is the leading cause of apoptosis induction in CeCa cells [9]. Ado is also a significant determinant of the immunosuppressive tumor milieu [10].

Among the extracellular purines that are released in TME by the injured cells, extracellular ATP has different roles depending on its concentration on the tumor site and the P2 receptor subtypes expressed by the immune and cancer cells [7]. The high concentration of ATP in TME is mainly due to the regulated efflux of ATP – which can be stimulated by chronic inflammation, hypoxia and ischemia – or by injury to the plasma membrane due to necrosis, apoptosis or mechanical stress. Accumulated ATP can either trigger P2 purinergic receptors (P2XRs and P2YRs) or can be further degraded to adenosine by the sequential action of CD39 and CD73 ectonucleotidases [4]. Extracellular ATP in TME behaves as a Danger-Associated Molecular Pattern (DAMP) and promotes both innate and adaptive immune responses. Alternatively, it also stimulates the development of both endothelial and tumor

cells through interaction with P2 receptors expressed in these cells [10,11].

Concurrently, as soon as extracellular ATP accumulates in TME, it gets degraded quickly into AMP by CD39 ectonucleotidase activity. CD73 ectonucleotidase then mediates the dephosphorylation of AMP, which leads to Ado formation. The catabolism of extracellular ATP is, therefore, the leading cause of the generation and accumulation of high levels of Ado in TME [12]. High levels of extracellular Ado potentially interact with P1 receptors, which can induce tumor angiogenesis, immune suppression, and metastasis. Ado's accumulation at TME is, therefore, an essential mechanism for promoting tumor progression [11].

Purinergic signaling presents itself as an essential factor in the control of growth, survival, and tumor progression. Not only does it act directly on injured cells in TME, but it also serves as a modulator of the immune system through the mediation of interactions between cancerous cells and immune cells of the host. Therefore, the purinergic constituents involved along the development and progression of tumors constitute potential targets in the development of new antitumor therapies [11].

The development of CeCa follows several mechanisms of immune response suppression and evasion [13]. Purinergic signaling is also able to adjust and modulate the function of immune cells through cell-cell interactions, cytokine, and chymosin secretion, the release of cell surface antigens, removal of intracellular pathogens and generation of reactive oxygen species [14]. Such effects occur through the interaction of purinergic mediators – such as ATP and Ado – with specific receptors that lead to the transmission of efferent signals that affect some immune responses [15].

Ectonucleotidases control and modulate ATP and Ado levels in TME [16]. Among them, CD39 and CD73 act on the regulation of the duration, magnitude, and composition of the *purinergic pool* that involves the immune cells and, therefore, have their expressions and enzymatic activity altered according to the pathophysiological context in which they are inserted [14].

This balance between extracellular ATP and Ado is an indispensable factor in immunologic homeostasis due to the role played by these two molecules while interacting with purinergic receptors expressed on the surface of immunologic cells. As previously mentioned, ATP acts as a *danger signal* when released by injured cells or under stress, through the interaction with P2 type purinergic receptors [17] – behaving as a potent DAMP and trigger inflammatory responses. At the same time, extracellular ATP can also act as a source of the Ado immunosuppressant *via* CD39 and CD73 ectonucleotidases [17].

Recent reports indicate that the adenosinergic pathway has an essential effect on the pathogenesis of gynecological cancer. Hypoxia caused by tumor growth in the cervix, as a consequence of inadequate vascularization, promotes the degradation of adenine nucleotides, resulting in the release of extracellular Ado excess. Also, the concentration of extracellular Ado becomes much higher when associated with the hydrolysis of extracellular ATP due to the high rates of CD39 and CD73 enzymes in the TME [18].

Beyond the immune suppression effect, extracellular Ado also stimulates tumor angiogenesis through increased blood flow through vasodilation. Moreover, Ado rich TME promotes endothelial progenitor recruitment of endothelial progenitor cells in response to wound healing, it stimulates endothelial cell tube formation and promotes the release of pro-angiogenic factors such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) [19].

In contrast to the immunosuppressive effect of Ado on immune cells, where Ado has substantial immunosuppressive activities concerning the mechanisms present in the tumor cells, Ado can either stimulate or inhibit tumor growth, depending on the cell type and receptor expressed on tumor surface. Gao et al. [20] showed that extracellular Ado could inhibit the migration and invasion of CeCa cells by repressing the epithelial to mesenchymal transition progress.

Gao et al. [20] also found that extracellular Ado may lead to disturbed balance between pro and anti-apoptotic factors, causing the release of a cascade of pro-apoptotic signaling molecule (caspase-3 being the most relevant) which played the function via their proteolytic activities to induce the multiple cellular changes, and finally leading to programmed cell death. The activation of pro-apoptotic cascades shows that Ado induces the death of CeCa cells by activating the mitochondrial apoptosis pathway. This fact, accordingly, demonstrates the vital role played by Ado in the induction of cellular death of cancerous cells of the uterine cervix.

Purinergic Receptors and Future Perspectives about Purinergic System and Cervical Cancer

Most new anti-cancer drugs interfere with the cellular components involved in cell survival and the apoptosis process. As outlined in our review, extracellular nucleotides, and other components of the purinergic system can regulate proliferation, differentiation, and apoptosis of CeCa cells. The role played by purinergic signalling components in tumor microenvironment makes them important therapeutic targets.

The P2Y6R is highly selective for 5′-diphosphate derivatives; therefore, Uridine 5′-O-(2-thiodiphosphate) (UDPβS) is a potent agonist of this receptor. However, although they are weaker agonists compared to UDPβS, thymidine 5′-Omonophosphorothiolate (TMPS) and uridine 5′-O-monophosphorothiolate (UMPS) also stimulate P2Y6R-mediated cell migration from HeLa cells. Moreover, it was observed that although UMPS is a more powerful agonist, when compared to TMPS, it is more easily degraded by CD73 ectonucleotidases. It is also understood that improving the stability of TMPS and UMPS, as well as their affinity with P2Y6R, could result in specific long-term effects mediated by this receptor. Despite the understanding that P2Y6R expressed in CeCa cells facilitates the migration of cervical tumor cells [21], the potential role of UDP and P2Y6R in the regulation of tumor formation in the CeCa has not been deeply investigated. Nevertheless, the knowledge of the properties of P2Y6 agonists offers excellent potential in the development of alternative therapies, especially when inducing long-term effects provided by the activation of P2Y6R.

CeCa cells also express P2X7R, which interacts with ATP and are responsible for the control and induction of the programmed cell death. The expression of P2X7R in CeCa cells is considerably lower in cancer and pre-cancerous epithelial cells when compared to healthy cells [22], so using the expression of P2X7R as a biomarker for CeCa could be an alternative way to determine the prognosis of the disease [23].

P2X7R plays an essential role as a pro-apoptotic modulator in human cervical epithelial cells [24]. Therefore, the P2X7R could be an appealing therapeutic target for chemoprevention and in CeCa treatment. Adinolfi et al. [25] stimulated P2X7R in HeLa cells with benzoyl ATP (BzATP) - a potent P2X7R agonist - and observed the loss of mitochondrial membrane potential with subsequent fragmentation of mitochondria and cell death. Fu et al. [26], in another study, used BzATP to upregulate P2X7-mediated apoptosis and modulate the growth of epidermal neoplasia. These data suggest that the activation of P2X7R-dependent apoptosis with BzATP could be used as a chemotherapeutic treatment to avoid cell growth of pre-cancerous lesions and early cervical epithelial lesions [27].

Considering what is stated above, despite the increasing advances in the pursuit of new anti-cancer treatments, there are still few studies that seek to evaluate the real therapeutic potential of molecules (agonists or receptor antagonists; enzyme inhibitors or activators) that target the components of the purinergic system involved in the progression of CeCa specifically. Therefore, further studies are needed on this subject.

References

1. Pfaffenzeller MS, Franciosi ML, Cardoso AM. Purinergic signaling and tumor microenvironment in cervical Cancer. Purinergic Signalling. 2020 Mar 13:1-3.

2. Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical Cancer. The Lancet. 2019 Jan 12;393(10167):169-82.

3. Chen Z, Schiffman M, Herrero R, DeSalle R, Anastos K, Segondy M, et al. Classification and evolution of human papillomavirus genome variants: Alpha-5 (HPV26, 51, 69, 82), Alpha-6 (HPV30, 53, 56, 66), Alpha-11 (HPV34, 73), Alpha-13 (HPV54) and Alpha-3 (HPV61). Virology. 2018 Mar 1;516:86-101.

4. Di Virgilio F, Sarti AC, Falzoni S, De Marchi E, Adinolfi E. Extracellular ATP and P2 purinergic signalling in the tumour microenvironment. Nature Reviews Cancer. 2018 Oct;18(10):601-18.

5. Gao ZW, Dong K, Zhang HZ. The roles of CD73 in cancer. BioMed research international. 2014;2014.

6. Ferrari D, Malavasi F, Antonioli L. A purinergic trail for metastases. Trends in Pharmacological Sciences. 2017 Mar 1;38(3):277-90.

7. Di Virgilio F. Purines, purinergic receptors, and cancer. Cancer Research. 2012 Nov 1;72(21):5441-7.

8. Li X, Gong Z, Zhang L, Zhao C, Zhao X, Gu X, et al. Autophagy knocked down by high-risk HPV infection and uterine cervical carcinogenesis. International Journal of Clinical and Experimental Medicine. 2015;8(7):10304.

9. Mello PD, Filippi-Chiela EC, Nascimento J, Beckenkamp A, Santana DB, Kipper F, et al. Adenosine uptake is the major effector of extracellular ATP toxicity in human cervical cancer cells. Molecular Biology of the Cell. 2014 Oct 1;25(19):2905-18.

10. Di Virgilio F, Adinolfi E. Extracellular purines, purinergic receptors and tumor growth. Oncogene. 2017 Jan;36(3):293-303.

11. de Andrade Mello P, Coutinho-Silva R, Savio LE. Multifaceted effects of extracellular adenosine triphosphate and adenosine in the tumor–host interaction and therapeutic perspectives. Frontiers in Immunology. 2017 Nov 14;8:1526.

12. Bastid J, Cottalorda-Regairaz A, Alberici G, Bonnefoy N, Eliaou JF, Bensussan A. ENTPD1/CD39 is a promising therapeutic target in oncology. Oncogene. 2013 Apr;32(14):1743-51.

13. Cekic C, Linden J. Purinergic regulation of the immune system. Nature Reviews Immunology. 2016 Mar;16(3):177.

14. Antonioli L, Pacher P, Vizi ES, Haskó G. CD39 and CD73 in immunity and inflammation. Trends in Molecular Medicine. 2013 Jun 1;19(6):355-67.

15. Junger WG. Immune cell regulation by autocrine purinergic signalling. Nature Reviews Immunology. 2011 Mar;11(3):201-12.

16. Allard B, Longhi MS, Robson SC, Stagg J. The ectonucleotidases CD 39 and CD 73: novel checkpoint inhibitor targets. Immunological reviews. 2017 Mar;276(1):121-44.

17. Zhao H, Bo C, Kang Y, Li H. What else can CD39 tell us?. Frontiers in immunology. 2017 Jun 22;8:727.

18. Merighi S, Mirandola P, Varani K, Gessi S, Leung E, Baraldi PG, et al. A glance at adenosine receptors: novel target for antitumor therapy. Pharmacology & Therapeutics. 2003 Oct 1;100(1):31-48.

19. Allard B, Turcotte M, Spring K, Pommey S, Royal I, Stagg J. Anti-CD73 therapy impairs tumor angiogenesis. International Journal of Cancer. 2014 Mar 15;134(6):1466-73.

20. Gao ZW, Wang HP, Dong K, Lin F, Wang X, Zhang HZ. Adenosine inhibits migration, invasion and induces apoptosis of human cervical cancer cells. Neoplasma. 2016 Jan 1;63(2):201-7.

21. Gendaszewska-Darmach E, Szustak M. Thymidine 5'-O-monophosphorothioate induces HeLa cell migration by activation of the P2Y6 receptor. Purinergic Signalling. 2016 Jun 1;12(2):199-209.

22. Li X, Zhou L, Feng YH, Abdul-Karim FW, Gorodeski GI. The P2X7 receptor: a novel biomarker of uterine epithelial cancers. Cancer Epidemiology and Prevention Biomarkers. 2006 Oct 1;15(10):1906-13.

23. Wang YY, Bai ZL, He JL, Yang Y, Zhao R, Hai P, et al. Prognostic value of neutrophil-related factors in locally advanced cervical squamous cell carcinoma patients treated with cisplatin-based concurrent chemoradiotherapy. Disease markers. 2016; 2016: 3740794.

24. Gorodeski GI. Estrogen Attenuates P2X7-R—Mediated Apoptosis of Uterine Cervical Cells by Blocking Calcium Influx. Nucleosides, Nucleotides and Nucleic Acids. 2004 Dec 31;23(8-9):1287-93.

25. Adinolfi E, Callegari MG, Ferrari D, Bolognesi C, Minelli M, Wieckowski MR, et al. Basal activation of the P2X7 ATP receptor elevates mitochondrial calcium and potential, increases cellular ATP levels, and promotes serum-independent growth. Molecular Biology of the Cell. 2005 Jul;16(7):3260-72.

26. Fu W, McCormick T, Qi X, Luo L, Zhou L, Li X, et al. Activation of P2X 7-mediated apoptosis Inhibits DMBA/TPA-induced formation of skin papillomas and cancer in mice. BMC cancer. 2009 Dec;9(1):114.

27. Gorodeski GI. P2X7-mediated chemoprevention of epithelial cancers. Expert opinion on Therapeutic Targets. 2009 Nov 1;13(11):1313-32.