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TESE DE DOUTORADO

# Intersecções entre a plasticidade epitélio-mesenquimal e a via adenosinérgica no câncer

# Intersections between epithelial-mesenchymal plasticity and adenosinergic pathway in cancer

Samlai Vedovatto
Orientador: Guido Lenz
Coorientadora: Márcia Rosângela Wink

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## SUMMARY

# ABBREVIATIONS

ADP - Adenosine diphosphate

ATP - Adenosine triphosphate

CRISPR - Clustered regularly interspaced short palindromic repeats

EMP - Epithelial-mesenchymal plasticity

EMT - Epithelial-mesenchymal transition

MET - Mesenchymal-epithelial transition

PTC - Papillary thyroid carcinoma

ZEB1 - Zinc finger E-box-binding homoeobox 1

CD73 - Ecto-5′-nucleotidase/cluster of differentiation 73

*NT5E* - Ecto-5′-nucleotidase/CD73 encoding gene

TC - Thyroid carcinoma

DTC - Differentiated thyroid carcinoma

PTC - Papillary thyroid carcinoma

FTC - Follicular thyroid carcinoma

# ABSTRACT

Epithelial-mesenchymal transition (EMT) represents an essential process associated with the advancement of tumors, resistance to therapy, and unfavorable prognosis in various cancer types. Nevertheless, the endeavor to focus on EMT or partial-EMT has persisted as a formidable challenge. The CD73 enzyme, a crucial ectonucleotidase in the adenosinergic signaling cascade, has been associated with EMT. Additionally, CD73 has been associated with cancer progression and the invasive front of tumors just as ZEB1, a key transcription factor in EMT. The present work provides an overview of the interplay between the adenosinergic pathway and the EMT program, and its implications for the advancement of cancer cells. Firstly, we present an in silico analysis of RNAseq datasets which reveals that numerous tumor types exhibit a noteworthy association between the expression of *NT5E* (CD73) and an EMT score. Moreover, it is apparent that the collaboration between EMT and the adenosinergic pathway in the advancement of tumors is reliant on the specific cellular context and type of tumor. Emerging evidence indicates a significant association between EMT and the adenosinergic pathway, as so, we centered the next steps of our investigation on examining the associations between ZEB1, a pivotal constituent of EMT, and CD73, a vital element of the adenosinergic pathway. The post-transcriptional regulation of ZEB1 expression was measured in cell lines derived from papillary thyroid carcinoma (PTC) upon silencing of CD73 expression. Cells lacking CD73 exhibited a reduction in the expression of ZEB1. Furthermore, a correlation was observed

between the expressions of CD73 and ZEB1 and alterations in cellular morphological features that are implicated in cellular migration, including cell polarity index and cell migration speed. Collectively, our findings suggest that the post-transcriptional control of ZEB1 could be affected by CD73, experiencing suppression in its absence in PTC. Additional research is required to clarify the mechanisms of association between CD73 and ZEB1, with the objective of identifying them as potential therapeutic options for cancer treatment in the foreseeable future.

# INTRODUCTION

## 1. Papillary Thyroid Carcinoma

Thyroid carcinoma (TC) is the most common endocrine malignancy (ULISSE *et al.*, 2021). The main types of TC are differentiated, anaplastic and medullary, with differentiated thyroid carcinoma (DTC) representing the majority of TC cases (DIAS LOPES *et al.*, 2020). DTC has its origins in epithelial follicular cells and includes two histological types, papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC) (ABOELNAGA; AHMED, 2015). Between them, PTC is the most frequent one, accounting for approximately 85% of all cancers at this site (ALI; CIBAS, 2017). Although PTC has a 10-years-survival rate of approximately 90%, recurrence is a major adverse event, since it leads to surgical procedures and radioactive iodine ablation (BERGDORF *et al.*, 2019; GHARIB *et al.*, 2016; HAUGEN *et al.*, 2016).

Papillary thyroid carcinoma is distinguished histologically by the presence of aggregated, large, ovoid nuclei with a ground glass appearance and, in some cases, by the presence of nuclear fissures (LAM, 2022). Nuclear pseudoinclusion can also be found and refers to intranuclear cytoplasmic herniations in the absence or rarity of mitotic figures (LIVOLSI, 2011). In a subset of papillary carcinomas, the nuclear characteristics are not as well developed or are limited to focal regions. Some papillary carcinomas can be circumscribed or encapsulated. Ordinarily arboriform,

the papillae have a fine fibrovascular cluster and follicles are frequently present and differ in size and shape, but they are typically elongated, irregular, and filled with thick colloids (AL-BRAHIM; ASA, 2006; BARNES, 2019).

Even though papillary thyroid carcinoma is characterized by moderate growth and favorable prognosis, PTC recurrence is, however, a major adverse event after initial treatment, and ranges widely from 1% to 40% depending on the clinicopathological features and molecular signature, particularly when harboring mutation $BRAF^{V600E}$ in combination with TERT promoter (HAUGEN *et al.*, 2016; JEONG *et al.*, 2020). In addition to the clinical-pathological characteristics considered to contribute to a higher or lower risk of disease recurrence, the American Thyroid Association guideline suggests to include the presence or absence and degree of vascular invasion, multifocality, number and size of cervical lymph nodes with metastases, and the presence or absence of capsular invasion (HAUGEN *et al.*, 2016). The collection of these information is important since to enhance the therapeutic approach, it is crucial to identify new risk factors for relapse.

PTC treatment involves, following a complete thyroidectomy, the administration of radioactive iodine to treat any remaining or metastatic illness or to ablate any remaining tissue (CHAN; KWONG, 2022). Metastases in PTC occur through blood vessels from thyroid lymph nodes to regional lymph nodes and, less often, for the lungs (SCHLUMBERGER; BAUDIN; TRAVAGLI, 1998). In PTC, metastases outside the region of the

neck are less frequent (5-7%) (LIVOLSI, 2011). Among the clinical-pathological characteristics considered at high risk are advanced age at diagnosis, size of the primary tumor, extrathyroidal invasion, lymph node metastasis, distant metastasis and advanced stages of the disease (JARZAB; HANDKIEWICZ-JUNAK, 2007; MAO *et al.*, 2020).

## 2. Epithelial-mesenchymal plasticity

The process a cell with epithelial phenotype suffers when becoming a mesenchymal cell is called epithelial-mesenchymal transition (EMT) or epithelial-mesenchymal plasticity (EMP). EMT was first described in 1982 by Greenberg and Hay, who showed that the cell microenvironment changes cell morphology and migratory abilities in vitro (GREENBURG; HAY, 1982). In 1994, the first evidence of EMT in vivo showed that EMT activation was essential for mesoderm and neural crest formation (NIETO *et al.*, 1994). Since then, EMT has been shown to be involved in embryonic development, wound healing and cancer metastasis, gaining substantial relevance as a matter of study for tumor progression.

Furthermore, functional characteristics of EMT transition states are high proliferation, invasion, plasticity, stemness and metastatic capacity (PASTUSHENKO *et al.*, 2018). These characteristics are regulated by the bidirectional crosstalk existent between the stromal microenvironment and carcinoma cells undergoing EMT operating within individual tumors (DONGRE; WEINBERG, 2019). Moreover, EMT regulation involves transcription factors that execute roles from cancer initiation to resistance to

therapy, being able to maintain the mesenchymal phenotype and increasing tumorigenicity (PUISIEUX; BRABLETZ; CARAMEL, 2014).

The first molecular transformation switch to the mesenchymal state is the decrease in epithelial cell adhesion molecule (EpCAM) and CDH1 (Cadherin 1/E-cadherin) expression levels and concomitant increase in vimentin (PASTUSHENKO *et al.*, 2018). After EMT activation, E-cadherin (CDH1) expression is repressed, leading to loss of polygonal epithelial cell morphology and acquisition of spindle-shaped morphology. Mesenchymal markers begin to emerge, with emphasis on N-cadherin, vimentin, fibronectin and β1 and β3 integrins (DONGRE; WEINBERG, 2019). These indicators of occurring EMT are increasingly associated with cell characteristics that suggest more hostile phenotypes.

EMT markers may represent chemoresistant and/or more invasive cell states, consequently presenting major imputations to cancer development (BRABLETZ *et al.*, 2018). Additionally, EMT protein markers are used by pathologists as very specific indicators of high-grade malignancy (KALLURI, 2009). The various combinations of mesenchymal and epithelial markers may indicate cell states crucial for the spread of cancerous cells, but they are still an incipient subject with very little knowledge about its different configurations in specific tumor types and host sites.

Besides the binary definition of EMT, various cell states are found in the EMT spectrum, presenting mesenchymal and epithelial markers concurrently (STEFANIA; VERGARA, 2017). These intermediate states

have been referred to as partial, incomplete, or hybrid EMT states and have been linked as major contributors to chemoresistance (PASTUSHENKO *et al.*, 2018). Often, hybrid EMT cells present increased invasion and migration, while coexpressing mesenchymal and epithelial markers. They can be divided into different EMT states, as epithelial, early hybrid EMT, late hybrid EMT and full EMT(PASTUSHENKO; BLANPAIN, 2019). The distinction of hybrid cell states can be very helpful when evaluating EMT because of its complex network of regulators, as it is very rare that carcinoma cells in spontaneously arising tumors advance into a completely mesenchymal state (DONGRE; WEINBERG, 2019).

Transcription factors such as ZEB1, Twist1/2 and Snai1 are characteristic of early hybrid to full EMT transition states, while Trp63, Klf4 and Ovol1 configure the epithelial state (PASTUSHENKO; BLANPAIN, 2019). Consisting on a crucial component of EMT, Zinc finger E-box binding homeobox 1 (ZEB1) is a robust repressor of epithelial genes, being correlated with poor outcomes in cancer, including chemotherapy resistance, immune suppression, and metastasis (SMITA *et al.*, 2018; SPADERNA *et al.*, 2008; WANG *et al.*, 2017). It was also demonstrated that ZEB1 is required for efficient invasion and metastasis in a mouse model (KREBS *et al.*, 2017).

ZEB1 centrality in EMP has diffusive motifs, being involved with RAS/ERK (BAE *et al.*, 2013), TGFβ (GREGORY *et al.*, 2011), PI3K/Akt (CHEN; PLEBANSKI, 2020), and NFκB (CHUA *et al.*, 2007) pathways, contributing to propel tumorigenesis and metastasis (PEREZ-OQUENDO;

GIBBONS, 2022). The transcriptional regulation of ZEB1 is, in many cases, context- or tumor-specific, and its dynamic chromatin modifications can be responsible for ZEB1 expression variance (GUENTHER *et al.*, 2007; KHAN; LEE; ROH, 2015; MUELLER *et al.*, 2007). Due to its complexity and importance when studying many types of cancer, ZEB1 is a relevant selection amongst EMT transcription factors as a matter of investigation.

It is important to give notice to the post-transcriptional regulation of ZEB1, since it can play a major part when it comes to tumor progression. It mainly involves a feedback loop named ZEB1/miR200 axis that functions both ways: miR200 repressing ZEB1 mRNA in the 3'-untranslated region (3'-UTR) and ZEB1 repressing the miR-200 family via binding to its promoter regions (GREGORY *et al.*, 2008; KORPAL *et al.*, 2008). If a double feedback loop like ZEB1/miR200 is disrupted, it can lead to ZEB1-mediated tumor progression, promoting cell invasive and chemoresistant phenotypes, since it consists of a regulation mechanism of cellular plasticity, (de)differentiation, and EMT machinery (GREGORY *et al.*, 2008; KORPAL *et al.*, 2011; PARK *et al.*, 2008; TIAN *et al.*, 2014; ZHANG *et al.*, 2019).

The miR-200 family elicits a cell migration inhibitory effect by targeting ZEB1, but it can also inhibit metastasis in a ZEB1-independent manner via protein kinase Cα (PKCα) (HUMPHRIES *et al.*, 2014). Besides the independent effect, the interplay between ZEB1 and miRNA200 have an important role in leading the cell in and out of EMT (HUMPHRIES; YANG, 2015), and the comprehension about how the members of miR-200

family influence cancer development in a positive or negative manner is of utmost relevance for therapeutic advancement.

## 3. Adenosinergic signaling

The action of adenosine as a cellular signaling agent in relation to its receptors and enzymes constitutes the adenosinergic signaling, which is a critical physiological process, being involved with immune and cardiovascular functions and neurotransmission (CAMPOS-CONTRERAS; DÍAZ-MUÑOZ; VÁZQUEZ-CUEVAS, 2020). Various components of the adenosinergic pathway, from enzymes to receptors and metabolites, play key roles in tumor progression. Amongst the adenosinergic signaling receptors are A1R, A2AR, A2BR, and A3R, which have different parts in the tumor microenvironment.

A1R is primarily located in the brain, regulating neuronal excitability, while A2AR is in the brain tissue, immune cells and blood vessels, regulating dopamine release, inflammation, and vasodilation. Dual-faced as other players of the adenosinergic pathway, A1Rs was suggested to promote breast cancer cell propagation and melanoma cell chemotaxis, but its levels are minimal in advanced prostate cancer (GESSI *et al.*, 2011; LIN *et al.*, 2010; MIRZA *et al.*, 2005; MOUSAVI *et al.*, 2015). It also has a protective effect in endometrial carcinoma through stimulating actin polymerization, enhancing cell–cell adhesion, and maintaining epithelial structure (BOWSER *et al.*, 2016). A2BR is found in many tissues, including immune cells, and plays a role in inflammation, angiogenesis, and cell

proliferation, while A3R is involved in inflammation regulation, cell proliferation, and apoptosis, and its activation has been demonstrated to have anti-inflammatory and anti-tumor effects (BOREA *et al.*, 2018; GORAIN *et al.*, 2019; VAN CALKER *et al.*, 2019).

Research on A2AR and A2BR also demonstrated controversial findings. Induction of tumor cell A2ARs may increase proliferation of breast cancer cells, or induce cell demise in melanoma cells (FLAMENT, 2009; MERIGHI *et al.*, 2002). Nevertheless, there is indication that A2BR activation inhibits proliferation via the ERK pathway (GESSI *et al.*, 2011). Moreover, the receptor is overexpressed in oral squamous cell carcinoma, and its suppression seems to reduce tumor growth (KASAMA *et al.*, 2015). A2BR may also play a significant part in promoting invasion and metastasis, since its activation has been shown to result in a buildup of non-prenylated Rap 1B, a small GTPase that regulates cell adhesion (NTANTIE *et al.*, 2013). Additionally, the inhibition of A2BR inhibits the growth of bladder and breast tumors in mice, most likely by enhancing T-cell-mediated antitumor response (CEKIC *et al.*, 2012).

The function of A3Rs in cancer has been studied more extensively, also presenting contradictory results. A3Rs are expressed by numerous human tumor cell lines, as well as by a number of primary human tumors, where its expression could be associated with the development of the disease (GESSI *et al.*, 2004, 2011). Besides, stimulation of the A3R inhibits the proliferation of various cancer cells, but promotes the proliferation of colon cancer cells (GESSI *et al.*, 2011). Considering that the A3R couples

with the glycogen synthase kinase (GSK)-3 WNT/catenin pathway, it was proposed to prevent proliferation via this route (FISHMAN *et al.*, 2004). Regardless of the contradictory findings on A3R, it is the most extensively disseminated and pharmacologically accessible adenosine receptor in cancer cells.

Amongst the adenosinergic enzymes are the ectonucleotidases, which can regulate cell behavior by controlling the level of extracellular nucleotides, since they are in charge of hydrolyzing nucleotides into different nucleosides (HÄUSLER *et al.*, 2011). Cell proliferation and metastasis in ovarian cancer, for instance, are mainly caused by the overexpression of these enzymes, which are present in nearly all cells (LUPIA *et al.*, 2018). Ectonucleotidases are divided into four main families, including alkaline phosphatases (APs), ecto-5′-nucleotidase (e5NT)/CD73, ectonucleoside triphosphate diphosphohydrolases (NTPDases), and ectonucleotide pyrophosphatase/phosphodiesterases (ENPPs) (AL-RASHIDA *et al.*, 2017).

Important ectonucleotidases for controlling immunological homeostasis in cancer cells include CD39, encoded by ENTPD1 gene, and CD73, encoded by *NT5E* gene. While CD73 dephosphorylates AMP to produce adenosine, CD39 is responsible for the catalytic conversion of ATP to AMP (ALLARD *et al.*, 2016; ANTONIOLI *et al.*, 2013). Since adenosine has been the focus of cancer research for several years, its function is more widely recognized than that of ATP. The product of CD73, adenosine, is one of the most multifaceted biochemical components of the tumor microenvironment (TME), influencing both host and tumor responses.

Moreover, it is well-known for its potent immunosuppressive and anti-inflammatory effects on the host.

However, the effect adenosine has on the tumor itself is dependent on the specific receptors exhibited by tumor cells, so stimulation and inhibition of tumor growth have been reported. Due to the effect of transcription factors such as Sp1, Stat3, and Gfi-1, tumor-associated macrophages express higher levels of CD39 and CD73 under hypoxic conditions, which causes a large-scale production of immunosuppressive adenosine (BONO *et al.*, 2015). While in murine tumor models of breast cancer and prostate cancer as well as in xenograft models of human breast cancer, *NT5E* has been shown to support tumor angiogenesis (ALLARD *et al.*, 2014; WANG *et al.*, 2013; ZHOU *et al.*, 2007). Similarly, human breast cancer cells, as well as melanoma cells from mice and humans, were encouraged to invade and metastasize by *NT5E* expression (BURGHOFF *et al.*, 2014; WANG *et al.*, 2008).

Diverse cancer cohorts have associated poor patient outcomes with CD73 overexpression (JEONG *et al.*, 2020; LECLERC *et al.*, 2016; PARK *et al.*, 2018; TURCOTTE *et al.*, 2015). One of the reasons for this association is that adenosine from CD73 promotes immune escape via lymphocytes immunosuppression while increasing tumor cell migration (STAGG *et al.*, 2010). Additionally, CD73 participation in metastasis goes beyond its enzymatic role in generating adenosine, since its enzyme-independent function also contributes to the spread of cancer cells through extracellular

matrix proteins interaction and adhesion (SADEJ *et al.*, 2008; SADEJ; SPYCHALA; SKLADANOWSKI, 2006).

As a metabolic regulator, adenosine is a relevant target to immune therapies. CD39 and CD73 are known to break down extracellular ATP into adenosine, subsequently activating adenosine receptors with antitumor and protumor repercussions (YEGUTKIN; BOISON, 2022). In addition, the purinome of different cancers is of great variance due to its complex and integrated network, but the potential of attacking its participants is promising. As previously summarized, the effectiveness of adenosine-based therapeutic depends on overcoming major gaps in its investigation, as ignorance of redundant pathways controlling ATP and adenosine levels, as unawareness of the differences between receptor-dependent and -independent effects of adenosine and as attention centralization in extracellular adenosine without considering intracellular metabolism and compartmentalization (BOISON; YEGUTKIN, 2019).

Interventions directioning CD73 and adenosinergic signaling in cancer are mainly a combination of A2AR blockers and anti-CD73 antibodies, with additional benefits when using an integrated strategy with both A2AR blockers and anti-CD73 antibodies (YOUNG *et al.*, 2016). Interestingly, several anti-CD73 antibodies and inhibitors are in clinical trials, with suggestions that merging this approach together with tumor-reactive immune cells and CAR-T cells can be potentially beneficial (BEAVIS *et al.*, 2017; LEONE; EMENS, 2018; SITKOVSKY *et al.*, 2014; VIJAYAN *et al.*, 2017). Gene silencing via shRNA and siRNA have also been demonstrated

to significantly block tumor angiogenesis, supporting its clinical development for tumor therapeutics (ALLARD *et al.*, 2014; KORDAS; OSEN; EICHMÜLLER, 2018).

## 4. Interconnections between EMT and adenosinergic signaling

Purinergic signaling and the epithelial-mesenchymal plasticity (EMP) are both essential for the initiation and development of cancer. Epithelial cells lose their apico-basal polarity through EMT, changing into mesenchymal cells with the ability to invade and infiltrate tissue (BRABLETZ *et al.*, 2018). Similarly, adenosinergic signaling governs how certain receptors interact with extracellular purines like adenosine and ATP to control cell functions as migration, also contributing to tumor cell invasiveness (JEONG *et al.*, 2020). Growing data suggest that EMT and purinergic signaling are intertwined and that this connection is essential for the initiation and development of cancer (ISER *et al.*, 2022). Due to this relevance, there is now more interest in examining how these two biological processes interact in order to create novel treatments.

Interestingly, changes in the expression of CD73/adenosine pathway members are correlated with EMT and have been shown to contribute to the mesenchymal phenotype in different types of cancer (LUPIA *et al.*, 2018; NGUYEN *et al.*, 2020; PETRUK *et al.*, 2021). A study with primary ovarian cancer cells demonstrated the co-regulation of CD73 with EMT-associated factors such as SNAI1, TWIST1 and ZEB1, promoting a mesenchymal-like phenotype (LUPIA *et al.*, 2018). Additionally, nanoparticles encapsulating

CD73/ZEB-1 siRNA molecules were shown to reduce migration and proliferation of cancer cells in vitro and in vivo (ALZAMELY *et al.*, 2021).

CD73 expression is also associated with decreased survival and an unfavorable prognosis in cancer (BUISSERET *et al.*, 2018; CAO *et al.*, 2021; SCIARRA *et al.*, 2019; SHRESTHA *et al.*, 2018; XIONG *et al.*, 2014; XU *et al.*, 2020). Recently, low *NT5E* methylation at various sites within the gene and elevated CD73 mRNA levels were associated with a poor prognosis (VOGT *et al.*, 2018). CD73/*NT5E* protein levels and mRNA levels were also linked to poor prognosis in head and neck squamous cell carcinoma, as it promoted tumor progression and metastasis (CD73 is associated with poor prognosis in HNSCCREN *et al.*, 2016a). In addition, the expression of critical tumor-associated macrophage (TAM) targets that represent emerging biomarkers for immune checkpoints, such as CD73, is positively correlated with the immune risk score and was upregulated in high-risk patients (YANG *et al.*, 2021). These findings are supported by additional research demonstrating that the upregulation of CD73 in cancer cells correlates with tumor progression and aggressiveness, which are EMT-related variables (MA *et al.*, 2019; MUÑÓZ-GODÍNEZ *et al.*, 2020; PIETROBONO *et al.*, 2020; CD73 is associated with poor prognosis in HNSCCREN *et al.*, 2016b).

In papillary thyroid carcinoma specifically, EMT influences tumor progression by inducing thyroid follicular cells to lose cohesion, which in turn makes them more migratory and invasive (LOPEZ-CAMPISTROUS *et al.*, 2021). Also, activation of EMT plays a key role in thyroid cancer progression

by promoting capsular invasion, extrathyroidal extension and local and distant metastasis (SHAKIB *et al.*, 2019). CD73 is also an important player in PTC, since it has increased expression in cell lines and biopsies, positively influencing cell migration and proliferation and being associated with cells from the invasive front of PTC (BERTONI *et al.*, 2018, 2019; JEONG *et al.*, 2020; KONDO *et al.*, 2006; MONTEIRO *et al.*, 2021).

Poor survival may be attributable to the immunosuppressive microenvironment created by ADO in a diverse set of cancers, including PTC, protecting tumor cells from chemotherapy (DE LOURDES MORA-GARCÍA *et al.*, 2019; GARCÍA-ROCHA *et al.*, 2019; LI *et al.*, 2017). But CD73 is not always upregulated in cancer cells and is not always positively associated with EMT and cancer progression. There is a link between adenosine and inhibition of cervical cancer cell migration, invasion, and induction of apoptosis (GAO *et al.*, 2016). Similarly, a study on endometrial carcinoma indicates that CD73 expression is lower in profoundly invasive tumors compared to non-invasive tumors. The authors reported that the absence of CD73 shifted TGFβ1 from tumor suppressor to tumor promoter, primarily by inducing the growth of stress fibers, migration, and invasion (KURNIT *et al.*, 2021). Moreover, ovarian carcinoma cells treated with adenosine exhibited decreased migration and induction of E-cadherin (MARTÍNEZ-RAMÍREZ *et al.*, 2017), emphasizing the context-dependent nature of CD73's effect on EMT progression and tumor growth.

# GOALS

## Main goal

To evaluate the role of CD73 in ZEB1 post-transcriptional regulation via its 3'UTR in cancer.

## Specific goals

I. To understand CD73 impact on the mesenchymal phenotype;

II. To establish ZEB1 expression impact on cell morphological changes;

III. To identify how CD73 influences ZEB1 expression impact on cellular parameters.

# CHAPTER I

## The crossroads of adenosinergic pathway and epithelial-mesenchymal plasticity in cancer

Article published in the journal Seminars in Cancer Biology

The article presented in this first chapter was a collective effort to review the impacts of adenosinergic signaling participants over epithelial-mesenchymal markers in various tumors, since the unknown links between these two processes may represent new possibilities for cancer therapeutic treatment. My contribution reflected on a shared co-first authorship, being essential for the review development, from topics structure to rationale.

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# The crossroads of adenosinergic pathway and epithelial-mesenchymal plasticity in cancer

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ABSTRACT

Epithelial-mesenchymal transition (EMT) is a key mechanism related to tumor progression, invasion, metastasis, resistance to therapy and poor prognosis in several types of cancer. However, targeting EMT or partial-EMT, as well as the molecules involved in this process, has remained a challenge. Recently, the CD73 enzyme, which hydrolyzes AMP to produce adenosine (ADO), has been linked to the EMT process. This relationship is not only due to the production of the immunosuppressant ADO but also to its role as a receptor for extracellular matrix proteins, being involved in cell adhesion and migration. This article reviews the crosstalk between the adenosinergic pathway and the EMT program and the impact of this interrelation on cancer development and progression. An *in silico* analysis of RNAseq datasets showed that several tumor types have a significant correlation between an EMT score and *NT5E* (CD73) and *ENTPD1* (CD39) expressions, with the strongest correlations being in prostate adenocarcinoma. Furthermore, it is evident that the cooperation between EMT and the adenosinergic pathway in tumor progression is context and tumor-dependent. The increased knowledge about this topic will help broaden the view to explore new treatments and therapies for different types of cancer.

## 1. The adenosinergic pathway is a part of the purinergic cascade

Adenosine 5′-triphosphate (ATP) is a nucleotide that, besides acting as an intracellular molecule, also functions as an extracellular messenger [1]. Similarly, other extracellular functions were proposed for nucleotides and nucleosides of purines and pyrimidines, which would later be recognized as important players for communication between cells, influencing a variety of physiological processes such as endocrine and exocrine secretions, neurotransmission, neuromodulation, immune responses, proliferation, differentiation, migration, and cell death [2].

Two main components are important for the regulation of this signaling pathway: 1) the presence of enzymes responsible for the hydrolysis of nucleotides, which will determine the type of nucleotide/nucleoside generated, and 2) the expression of specific receptors for each nucleotide/nucleoside [3]. There are different families of ectonucleotidases, with varied hydrolysis capacities and affinities for purines and pyrimidines. Among them, E-NTPDases 1–8 (Ecto-Nucleoside Triphosphate-Diphosphohydrolase), E-NPPs (Ecto-Nucleotide Pyrophosphatase/ Phosphodiesterases), Alkaline Phosphatase, Ecto-5′-Nucleotidase (CD73) and Adenosine Deaminase (ADA) [1]. Likewise, a wide range of receptors were characterized. The P2 type receptors are subdivided into ionotropic ($P_2X$ 1–7) and metabotropic ($P_2Y$ 1,2,4,6, 11–14) receptors, and exhibit different ranges of responsiveness to ATP, UTP, and its dephosphorylated analogs. On the other hand, the P1 type receptors ($A_1$, $A_2A$, $A_2B$, and $A_3$) are responsive mainly to adenosine (ADO) [4]. ADO can cross the cell membrane through nucleoside transporters, such as Equilibrative Nucleoside Transporters (ENT1, ENT2, ENT3 and ENT4) and Concentrative Transporters (CNT1, CNT2 and CNT3), which can regulate ADO bioavailability [5,6].

The sequential hydrolysis of ATP involves ecto-nucleotidases, such as NTPDase1 (CD39), NTPDase2, or NPPs, which together degrade this nucleotide to adenosine diphosphate (ADP), and adenosine monophosphate (AMP), which, in turn, is hydrolyzed to ADO. ADO is a purine nucleoside, which can be present in the extracellular milieu via direct

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release of endogenous nucleoside or as a result of a sequential breakdown of extracellular ATP (eATP) or $NAD^+$ via ectoenzymes [1]. Two different adenosinergic pathways for ADO production have been characterized, namely canonical and non-canonical pathways. The canonical pathway involves the production of AMP from ATP by CD39 (NTPDase 1), while the non canonical pathway involves the production of AMP from $NAD^+$ by CD38 and CD203a (E-NPP1). Both converge to the production of AMP, which is metabolized by ecto-5′-nucleotidase (CD73) to ADO [7,8]. Other enzymes, such as tissue-specific alkaline phosphatases and tissue-nonspecific alkaline phosphatases (TNAPs) are also able to generate ADO from AMP [8]. Lastly, ADO can be degraded to inosine by adenosine deaminase (ADA) or uptaken into the intracellular environment through nucleoside transporters [9] (Fig. 1).

The adenosine formed in the extracellular space can signal through specific G protein-coupled adenosine receptors. These receptors are broadly expressed on different tissues and are involved in a diversity of physiological and pathological functions [1].

## 2. Adenosinergic signaling in tumor microenvironment

### 2.1. Immune regulation and adenosinergic signaling

CD73 and CD39 are the major nucleotides metabolizing enzymes expressed in the tumor microenvironment (TME), in immune, endothelial, mesenchymal, and epithelial cells, as well as tumor cells and their released exosomes [10–13]. Under physiological conditions, eATP and ADO are in the nanomolar range, but these molecules can reach the millimolar range under pathological states such as injury, hypoxia, inflammation, and chemo- and radiotherapy [14]. Consequently, under high concentrations of eATP, ectonucleotidases are essential for reducing elevated levels of nucleotides and directing purinergic receptors' response involved in a proper reaction to injury [15]. The TME produces an inflammatory state that promotes tumor growth and favors immune evasion [16]. In this context, as practically all neoplastic and non-neoplastic cells express surface receptors for extracellular nucleotides and nucleosides, the purinergic signaling can play multifaceted roles, with anti-inflammatory or pro-inflammatory responses, depending on the enzymes present, the nucleotide concentrations generated in the TME and the types of receptors activated [17].

The presence of eATP in the TME acts as a damage-associated molecular pattern (DAMP), being a "danger signal" to immune cells. This leads to increased chemotaxis and activation of dendritic cells, neutrophils, macrophages, T cells, natural killers (NK), among others, promoting tumor antigen presentation and antitumor immunity. However, CD39 is abundantly expressed by cells in the TME of most cancer types, thereby favoring the depletion of eATP [18,19]. Antibodies blocking CD39 trigger an eATP-P2×7-inflammasome-IL18 pathway, reducing intratumor macrophage number, enhancing intratumor T-cell effector function, and overcoming anti-PD-1 resistance [20]. On the other hand, ADO modulates cancer growth and dissemination by direct actions on tumor cell migration, invasion, and proliferation [21,22]. In addition, ADA is downregulated in tumor cells, contributing to ADO accumulation and favoring immunosuppression [23–26].

Furthermore, ADO contributes to the immunosuppressive

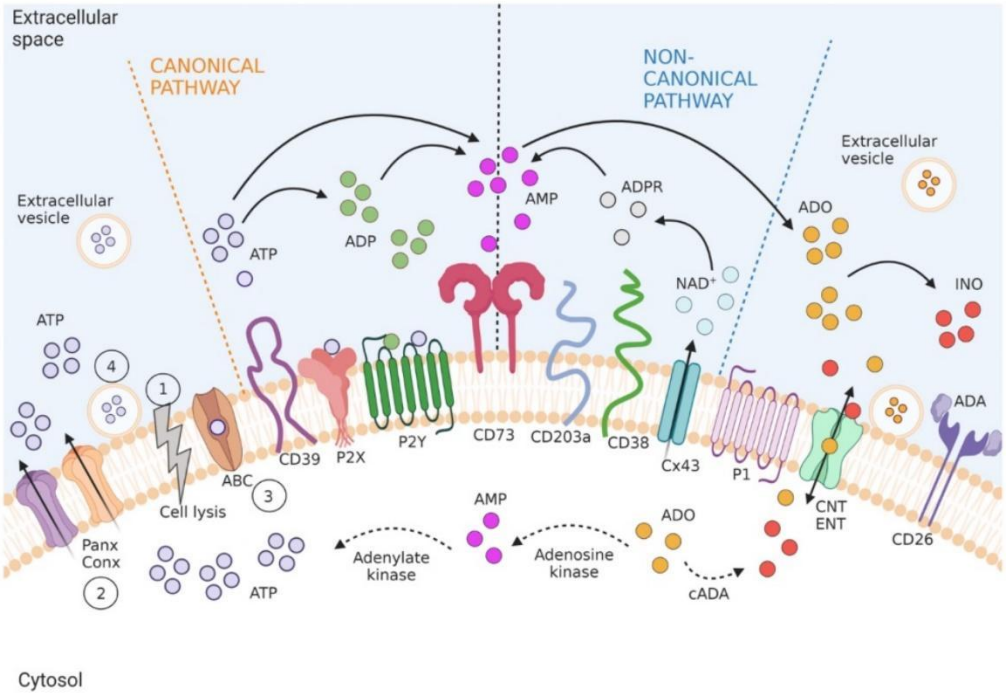


**Fig. 1.** Cellular pathways regulating adenosinergic signaling. ATP can be released into the extracellular space through four mechanisms: (1) cell lysis; (2) transport via connexin (Conx) and pannexin (Panx) channels; (3) ATP-binding cassette transporters (ABC) or (4) vesicular exocytosis. Once in the extracellular milieu, ATP can activate $P_2X$ and $P_2Y$ receptors or be enzymatically hydrolyzed to ADP and AMP by ectonucleotidases. The canonical pathway involves the sequential hydrolysis of ATP to AMP by CD39 and the hydrolysis of AMP by CD73, producing extracellular ADO. In the non-canonical adenosinergic cascade, CD38 and/or CD203a hydrolyze $NAD^+$, generating adenosine diphosphate ribose (ADPR), which, in turn, is hydrolyzed by CD203a, producing AMP. AMP is then metabolized by CD73 to ADO. Extracellular ADO activates the type 1 purinergic (P1) receptors ($A_1$, $A_2A$, $A_2B$, and $A_3$) and is catabolized to inosine by extracellular adenosine deaminase (ADA), or it can be transported into cells by both equilibrative (ENT1/2) and concentrative (CNT1/2) nucleoside transporters. Once in the cytosol, ADO can be degraded to inosine (INO) by cytosolic adenosine deaminase (ADA) or re-phosphorylated to AMP by adenosine kinase. The cytosolic AMP or ADP can next be phosphorylated by an adenylate kinase to generate intracellular ATP. Cx43, connexin 43.

environment by inhibiting CD4 and CD8 T cells, NK cells, and antigen-presenting cells, while inducing Treg cells, myeloid derived suppressor cells (MDSC), and tumor-associated macrophages (TAM) [21]. This mechanism involves mainly the action of ADO through $A_2A$ and $A_2B$ receptors, which can stimulate to release of several pro-angiogenic, pro-inflammatory, and immunosuppressive mediators by these immune cells, including VEGF, IL-8, IL-6, IL-10, cyclooxygenase-2, TGF-β, and indoleamine 2,3-dioxygenase (IDO) [27]. Therefore, the $A_2A$ and $A_2B$ inhibition have shown promising anti-tumor response in several tumor models, which is enhanced when also targeting CD73 [28,29]. Similarly, inhibition of the CD39/CD73 axis, which is co-expressed in many immune cells in the TME, can improve some immune functions, such as the increase in proliferation of T cells [30] and suppression of the function of MDSCs [31]. Analogous results were observed for CD39, showing that this enzyme converts ATP-driven pro-inflammatory milieu to an anti-inflammatory state mediated by adenosine [32].

Interestingly, CD73 levels on immune cells are found to be negligible in healthy individuals. However, within tumors, its expression is upregulated in several immune cells, further contributing to the increase of the immunosuppressive ADO levels and tumor immune evasion and progression [28,33]. Indeed, Buisseret et al. observed, in triple-negative breast cancer (TNBC) patients, that higher CD73 expression in tumor and immune cells was observed in patients with significant lymph node invasion and worse prognosis [34]. Researchers have sought to understand the mechanisms behind this increased CD73 expression on the surface of immune cells. Chambers et al., for example, recently showed that the high expression of CD73 in cancer cells is likely a prerequisite for the induction of CD73 on the surface of NK cells, which adopt a dysfunctional role leading to the tumor progression and metastasis [35]. Similarly, soluble factors released by other cell types, such as mesenchymal stromal cells (MSC), could stimulate the expression of CD73 in immune cells [36]. Taken together, these findings make CD73 an emerging immune checkpoint.

Since the immunosuppressive properties of ADO are well known, targeting adenosine receptors has emerged as a tool to induce anti-tumor activity and inhibit immunosuppressive and pro-angiogenic functions. In particular, the $A_2$ receptors have received great attention [37]. Both $A_2A$ and $A_2B$ receptors mediated signaling through cAMP/protein kinase A (PKA)-dependent mechanisms. Interestingly, cAMP-PKA signaling has been recognized as an effective negative regulator of immune cells, suppressing effector T cells [38]. There are several studies reporting that $A_2A$ and $A_2B$ antagonists can promote antitumor immunity [39–42]. Despite seeming redundant, blocking both CD73 and $A_2A$ receptors presents a more potent anti-tumor activity than blockade of CD73 or $A_2A$ alone [43]. $A_2A$ antagonists, as well as anti-CD73 drugs, were also combined with either anti PD-1 or anti CTLA-4 mAbs, with promising results [23,38].

The non-canonical pathway of ADO generation has also been investigated as a target in antitumor studies. In particular, CD38 has attracted attention. In melanoma cell lines obtained from patients, CD38, CD39, CD203a and CD73 expression was detected, besides production of ADO from AMP and $NAD^+$. ADO production was related to inhibition of CD4 + and CD8 + T cells and this effect was correlated with different patterns of expression of $A_2A$ and $A_2B$ receptors [44]. Daratumumab, the first class of antibody targeting CD38, was approved as a single agent and in combination with standard treatments for multiple myeloma. CD38 antibodies improved anti-tumor immune response by reducing immune suppressor cells, including regulatory T cells, regulatory B cells, and MDSCs. In addition, CD38-targeting antibodies had synergistic activity with other anticancer agents, such as PD1/PD-L1 inhibitors [45,46].

### 2.2. Regulation of CD73 expression

A better understanding of how CD73 expression is altered in cancer cells, thus culminating in immune escape, is provided by the knowledge about CD73 *(NT5E)* regulation on the transcriptional and post transcriptional levels. CD73 expression is regulated by transcription factors such as hypoxia inducible factor-1 (HIF-1), specificity protein 1 (SP1), SMAD, and c-Jun. Several proinflammatory signaling molecules, such as TGF-β, interferons (IFNs), tumor necrosis factor (TNF), interleukin (IL)− 1β, prostaglandin E2 and Wnt/β-catenin signaling pathway can induce CD73 expression [47–49]. The expression of CD73 in cancer cells can be regulated at different levels and the upregulation at the mRNA level does not automatically lead to increased protein expression or increased enzymatic activity [49]. In cervical cancer, for example, CD73 upregulation can promote pro-tumor effects independently of CD73 enzymatic activity [50].

*NT5E* expression can also be regulated at the post transcriptional level in head and neck squamous cell carcinoma, colorectal and lung cancers, respectively by miR-422a, miR-187, miR-30a, and miR-30a-5p, which directly target CD73 mRNA [51–54]. Interestingly, a novel tumor-promoting non-coding circular RNA (circRNA) derived from *NT5E* was described, which acts as a sponge for tumor-suppressor microRNAs (miRNAs), including miR-422a and miR-502–5p in glioblastoma and in bladder cancer, respectively [55,56]. The oncogenic activity of circRNA is related to its ability to directly bind to these miRNAs, inhibiting them and consequently promoting cancer cell proliferation and migration [56]. *NT5E* mRNA levels can also be regulated by hypermethylation in the cytosine-phosphate-guanine (CpG) island located in the regulatory region of the *NT5E* gene in breast cancer [57], melanoma [58], and cervical cancer [59]. In addition, *NT5E* can be downregulated in cancer via alternative splicing of exon 7 to produce a shorter intracellular protein isoform (CD73S), which is enzymatically inactive and promotes proteasome-dependent CD73 degradation [60]. Post-translational modifications at the protein level can also affect localization and activity of CD73. For example, CD73 can be released from the membrane by cleavage to form a soluble enzyme and also can be N-glycosylated at four different residues [49].

### 2.3. Enzymatic and non-enzymatic activity of CD73

The importance of the enzymatic activity of CD73 and the production of ADO in cancer is well established [1,7,17]. Studies with enzymatic inhibitors and monoclonal antibodies (mAb) have reinforced the contribution of the adenosinergic pathway in tumor growth. For example, the use of adenosine 5′-(α,β-methylene)diphosphate (APCP), a competitive CD73 inhibitor, produced antitumor activity in different tumors. In human breast cancer cells, the downregulation of CD73 by small interfering RNA (siRNA) or the treatment of cancer cells with APCP significantly reduced breast cancer growth *in vivo* and *in vitro*, while CD73 overexpression increased cancer cell viability and induced cell cycle progression [61]. In glioma, APCP treatment reduced by 30% the rate of glioma cell proliferation while ADO treatment increased it by a similar percentage [62]. Azambuja et al. showed that CD73 downregulation by siRNA or APCP treatment decreased glioma cell migration, invasion, and proliferation *in vitro,* as well as rat glioblastoma progression *in vivo* [63,64]. In agreement, therapy using anti-CD73 monoclonal antibodies in a model of mouse breast cancer induced an adaptive anti-tumor immunity, leading to inhibition of tumor growth and metastasis [65]. Similar results were observed in a model of melanoma [31]. In ovarian cancer patients, high CD39/CD73 expression is correlated with poor outcome and impaired lymphocyte effector function [31,66].

The influence of CD73 in tumor growth is mediated by both enzymatic and non-enzymatic activity. The protein structure of the enzyme can be involved in the regulation of cell-cell and cell-extracellular matrix (ECM) adhesion properties, through interaction with laminin and fibronectin, thus mediating cancer invasion, migration, and metastasis [67,68]. In melanoma cells, the enzymatic function of CD73 was related to the cancer invasion process, whereas its non-enzymatic action promoted cell migration and ECM adhesion through activation of focal

adhesion kinase (FAK) [69]. In glioma stem-like cells the enzymatic activity of CD73 was crucial only for invasive properties, while the protein structure was related to suppression of cell viability, proliferation, and clonogenicity [70]. Enzymatically active CD73 has also been observed in exosomes isolated from the serum of patients with melanoma, where it was related to suppression of T-cell function and ineffectiveness of anti-PD1 immune checkpoint inhibitor therapy [71]. Thus, it is not surprising that this enzyme has been found to be overexpressed in many cancers. A high expression of CD73 was found in rectal adenocarcinoma [72], glioblastoma [11,73], papillary thyroid carcinoma [74], bladder cancer [75], gastric carcinoma [76], renal carcinoma [77], prostate cancer [78], pancreatic cancer [79,80], melanoma [81], breast cancer [82], leukemia [83] and lung cancer [84].

### 2.4. The dual face of the adenosinergic signaling in TME

The CD73/ADO axis is involved with tumor development in a context-dependent way [85]. Cappellari et al. demonstrated in a xenograft model of medulloblastoma that cells overexpressing CD73 developed smaller tumors, with reduced vascularization and enhanced apoptosis rates [86]. Similar results were observed by the activation of $A_3$ receptors in animal models of melanoma [86,87], colon [88,89], prostate [90] and hepatocellular carcinomas [91]. Some studies have shown that ADO, through its receptors, can promote tumor cell death [92,93], reduction of tumor cell proliferation [94–97] and inhibition of cell invasion and migration [98,99].

CD73 is less expressed in high-grade endometrial human carcinoma in comparison to normal endometrium and low-grade tumors, and treatment with APCP promoted an increase in cell migration and invasion in endometrial carcinoma cells *in vitro*. ADO, via $A_1$ receptor, was shown to be essential to prevent migration, invasion and metastasis *in vivo*, by promoting epithelial integrity protection [100]. It was also reported that CD73 expression levels are lower in ovarian carcinoma and advanced breast cancer when compared with normal tissue [65,101]. In ovarian carcinoma cell lines, the treatment with ADO and $A_2B$ adenosine receptor agonist (NECA) promoted reduction of cell migration and cell viability, respectively [102,103]. Another study with ovarian cancer cells demonstrated that the treatment with ADO before cisplatin therapy enhanced chemotherapy-induced cytotoxicity, leading to an increase in apoptosis rate [104]. The dual role of the adenosinergic signaling in tumor growth and response to therapy adds complexity to predicting the outcome of reducing its activity or expression, but the majority of studies performed in cancer models or patients suggests a dominant pro-tumor activity.

### 2.5. Emerging opportunities targeting the adenosinergic pathway in cancer therapy

Due to the possibility of targeting the adenosinergic pathway to inhibit tumor progression, different drugs focusing on its components are being developed and tested, as ectonucleotidase inhibitors and receptors antagonists and agonists. Several clinical trials are underway to evaluate the use of anti-CD73 mAb for tumors, such as MEDI9447 (NCT02503774, NCT03381274, NCT03267589, NCT03611556 and NCT03616886) and BMS-986179 (NCT02754141) for advanced solid tumors; NZV930 (NCT03549000) to ovarian, lung, prostate, colorectal, renal, breast and pancreatic cancer; and CPI-006 (NCT03454451) to cervical, lung, breast, ovarian, pancreatic, endometrial, sarcoma, head and neck, prostate, lymphoma and bladder cancer [105,106]. Unfortunately, their results are not available yet, but preclinical results for MEDI9447, a human monoclonal antibody for CD73, showed that it is a potent inhibitor of CD73 ectonucleotidase activity. The antibody relieves lymphocyte suppression mediated by AMP *in vitro* and inhibits mouse syngeneic tumor growth *in vivo* [107]. MEDI9447 also increased CD8C effector cells subpopulation and macrophage activation in the TME of mouse models. MEDI9447 ability to inhibit conversion of AMP to adenosine in an *in vitro* assay is linked to internalization of CD73 from the cell surface, relief from inhibition of T cell proliferation mediated by AMP and direct cellular enzyme inhibition [107]. Based on these preclinical results, a Phase I study was initiated to assess tolerability, safety, and clinical activity of MEDI9447 (NCT02503774) [107].

A few clinical trials focusing on adenosine receptors in cancer therapy were completed. CF102, an $A_3$AR agonist, causes a dose response inhibition of tumor growth *in vivo*, via de-regulation of the NF-kappaB and the Wnt signal transduction pathways, inducing tumor cells apoptosis [91]. In patients with advanced unresectable hepatocellular carcinoma, CF102 was proven safe and well-tolerated [108]. A clinical trial to evaluate the safety, tolerability, feasibility and preliminary efficacy of PBF-509, an $A_2$AR antagonist, is currently completed with no results posted (NCT02403193). CPI-444, an $A_2$AR antagonist, showed antitumor efficacy *in vivo*, indicating that CPI-444 induced systemic antitumor immune memory [109]. Phase I studies focusing on lung cancer therapy with PBF-1129, an $A_2$BR antagonist, are still recruiting (NCT03274479 and NCT05234307). As well as ADO receptors, nucleoside transporters can also be an interesting target, since they appear to mediate absorption and distribution of purine antimetabolite drugs used in chemotherapy [110], but their study is even more scarce. The research on possible targets of the adenosinergic pathway in cancer is still premature. Studies in progress need to be completed and new studies have to be initiated before we can assert the importance and contextual preconditions of these targets.

## 3. The crosstalk between EMT and CD73

The epithelial-to-mesenchymal transition (EMT), as well as its inverse process (MET), are important cellular programs that contribute to physiological and pathological processes such as embryogenesis, morphogenesis, tissue homeostasis, wound healing, tissue fibrosis and cancer. EMT has a wide range of intermediate phenotypic states, consisting of cells in complete, partial, mixed or hybrid EMT states. The phenotypic plasticity that EMT offers to cancer cells is one of the key factors of cancer pathogenesis, which allows tumor cells to evade from the completely differentiated state usually found in normal cells, contributing to the proliferative state required for neoplasia. This phenotypic instability influences tumor heterogeneity, thus changing oncogenic signaling networks, apoptotic features, and immune cell functions [111–113].

In general, epithelial cells are organized into adherent sheets to delimitate tissues and organs, exhibiting epithelial intercellular junctions, apical-basal polarity and interaction with the basement membrane. When EMT is triggered, epithelial characteristics are repressed and mesenchymal characteristics are induced, such as lack of apical-basal polarity and enhanced cell motility and invasive capacity [114]. During this process, the most well-known markers for the epithelial trait, such as E-cadherin, occludins, and cytokeratins are downregulated, while the mesenchymal markers N-cadherin, fibronectin, and vimentin are induced [115]. This coordinated repression of epithelial genes and induction of mesenchymal genes is centered on the expression of EMT-inducing transcription factors (EMT-TFs), including *SNAI1*, *SNAI2*, *ZEB1*, *ZEB1*, and *TWIST1* [116]. The mesenchymal phenotype prevalence, inherent to EMT, links this process to aggressiveness, metastasis, invasion and poor prognosis in many tumors, such as gallbladder cancer [117], breast carcinoma [118], prostate cancer [118,119], pancreatic cancer [120], lung carcinoma [121,122], colorectal carcinoma [123], hepatocellular carcinoma [124], gastric carcinoma [125], bladder cancer [126], glioblastoma [127] and cervical cancer [128]. The main mechanisms include induction of cellular migration, resistance to anoikis and apoptosis, survival, genomic instability, cancer stem cell (CSC) activity, resistance to therapy, immune evasion and metabolic changes [129,130].

Only a small number of tumor cells in primary carcinoma present mesenchymal characteristics [131,132]. Nevertheless, circulating tumor

cells (CTCs) present an enrichment of EMT markers compared to cells in the tumor of origin in breast cancer patients, which has been supporting the contribution of this process in early metastasis [130]. CTCs from patients with breast cancer [133,134], lung cancer [135], endometrial cancer [136], gastric cancer [137], pancreatic cancer [138] and prostate cancer [139] present a mesenchymal enriched phenotype often correlated with poor prognosis. On the other hand, CTCs co-expressing both epithelial and mesenchymal (E/M) genes have also been observed, and these cells with hybrid phenotype are more aggressive than cells with a full mesenchymal phenotype (complete EMT) [140,141].

Members of the purinergic system, such as CD73 and CD39, are among the genes whose expression is altered during EMT. Many studies reinforce a potential link between the adenosinergic pathway and EMT-like phenotype in different tumors. To contribute to the understanding of the relation between CD73 and CD39 with EMT, we explored RNA-seq expression data from the TCGA PanCancer Atlas dataset. Of the 21 types of cancer analyzed, 11 showed statistically significant correlations between an EMT score and *NT5E* (CD73) expression, of which six presented a strong correlation ($r > 0.4$) (Fig. 2). We observed the strongest positive correlations in prostate adenocarcinoma, breast carcinoma, ovarian adenocarcinoma, and bladder urothelial carcinoma, in agreement with other data analyses for these types of cancer [65,142]. There were also statistically significant correlations between EMT score and *NT5E* expression in cervical carcinoma, head and neck squamous cell carcinoma (HNSCC), kidney renal clear cell carcinoma, lung adenocarcinoma, lung squamous cell carcinoma, and skin cutaneous melanoma (Fig. 2B), which are also in accordance with other reports [143–146].

Furthermore, the expression of *ENTPD1* (CD39) also correlated strongly with the EMT score in several cancer types, with 15 within the 21 cancer types analyzed displaying strong correlations ($p < 0.0001$, $r > 0.4$) (Fig. 2). The cancer types with the strongest correlations between EMT score and CD73 expression also presented correlations with CD39 expression, and prostate adenocarcinoma was the one with the highest correlation in both analyses. CD39 and CD73 expressions correlated strongly with each other in prostate and breast cancers, although there were cancer types such as cervical and head and neck carcinomas that also presented significant correlations with the EMT score for both genes but not between their expressions (Fig. 2B). In both cases, we noticed that the expression of the two genes contributes to the correlations observed with the EMT score (Fig. 3A and B), which does not occur for the tumors that presented a significant correlation with the EMT score for only one of the genes (Fig. 3C).

The majority of studies with CD73 suggest that this enzyme is related to the mesenchymal phenotype either through interference using ADO or inhibiting/overexpressing its enzymatic activity. It is important to notice that the existing links between CD73 and EMT are context dependent, as shown in Table 1. Inhibition of CD73 associated immunosuppression is the main principle behind the initiatives regarding cancer therapies that target the adenosinergic pathways. However, there are suggestions that anti-CD73 therapy suppresses progression of cancer cells independently of the immune effect, via EMT inhibition [150]. Lack of CD73 was also suggested to inhibit angiogenesis, preventing tumor growth, in which case both the enzymatic and the non-enzymatic functions of CD73 contributed to pro-angiogenic effects [151]. The mechanistic understanding of these links is still not clear. Beyond the immunosuppressive effect mediated by ADO, there is evidence that CD73 suppression also inhibits elongation of cellular protrusions and that its inhibitory effects are hypoxia-dependent when evaluating cell viability, while independent of hypoxia when evaluating cell migration [150]. ADO receptors, as $A_2B$ and $A_3$, may also modulate these processes, inducing EMT progression through the balance of cAMP/PKA and MAPK/ERK pathway activation and through induction of ZEB1 expression, respectively [70,152]. The cAMP-PKA signaling pathway can directly promote the production of TGF-β1 [153], which is also able to activate PKA through the interaction of an activated Smad3-Smad4 complex [154]. Although these receptors may represent an interesting target to cancer therapy, further studies are needed to comprehend in which circumstances agonists or antagonists are advantageous.

Similarly to EMT, CD73 expression is also associated with reduced survival and poor prognosis in cancer [34,162,164,165,169,171]. In head and neck squamous cell carcinoma (HNSCC), patients allocated in an HPV-positive subgroup showed low *NT5E* methylation and high CD73 mRNA levels in association with an adverse outcome [175]. CD73 was also associated with poor prognosis in HNSCC, promoting tumor progression and metastasis [167]. In addition, expression of critical tumor-associated macrophage targets, such as CD73, positively correlates with immune risk score and were upregulated in high-risk patients [176]. Furthermore, *NT5E* was the most increased gene in a study with gallbladder carcinoma cell line after treatment with TGFβ [164], an EMT inductor. This finding is accompanied by other studies showing that CD73 increase in cancer cells is related to tumor progression and aggressiveness [157,166–168], variables related to EMT. CD73 overexpression was also observed in human samples of hepatocellular carcinoma when compared to normal tissue and in metastasis foci. In accordance, CD73 knockdown dramatically inhibited metastasis and/or the mesenchymal phenotype in melanoma, breast, gastric, pancreatic, ovarian and glioma cancer cells, *in vitro* and *in vivo* [70,81,150,165,173,174].

Poor survival can be due to the immunosuppressive microenvironment created by ADO, conferring protection from chemotherapy for tumor cells [143,159,160]. It is important to reinforce that CD73 is not always upregulated in cancer cells and positively associated with EMT and cancer progression. There is evidence relating ADO to inhibition of migration, invasion, and induction of apoptosis in cervical cancer cells [161]. In addition, there is a study with endometrial carcinoma showing lower expression of CD73 in deeply invasive tumors when compared to non-invasive ones. The authors described that the loss of CD73 shifted TGFβ1 from tumor suppressor to tumor promoter, mainly by inducing the development of stress fibers, migration, and invasion [163]. Also, in this line of work, a study with ovarian carcinoma cells and ADO treatment resulted in reduced migration and induction of E-cadherin [102], highlighting the context dependency of CD73 influence in EMT progress and tumor promotion.

As well as ADO, TGFβ has also been associated with immunosuppression in the cervical tumor microenvironment by inhibiting cytotoxic T cells [159]. Treatment with anti-hTGF-β neutralizing antibodies decreased CD73 [158] and it was already found a positive correlation of CD73 high expression in precursor lesions and high levels of TGFβ in patients serum samples [157,160]. Cells present in the TME, such as MSCs, can also contribute to TGF-β production and, consequently, induce CD73 expression by tumor cells [158]. The contribution of MSC to tumor progression and EMT has also been indicated in glioblastoma cells [127,166]. These findings suggest a cooperative function of TGFβ1 and ADO in cancer cells that favors tumor progression [177].

Other studies contributed to understanding EMT and CD73 relationship through the expression of their main markers. EMT induction by TNF-α or TGFβ caused an increase in CD73 that was accompanied by EMT markers in breast cancer cells and hepatocellular cancer cells [155,156,170]. Bioinformatic analysis of gene-expression data showed a positive correlation of CD73 expression with ECM organization, TGFβ genes and EMT-TFs [155]. N-cadherin and Vimentin were increased while E-cadherin was decreased after CD73 overexpression in lung cancer cells [172]. Similar results were observed in glioblastoma cells, in which ADO treatment induced cell migration and enhanced the expression of Snail and ZEB1, while reducing the expression of E-cadherin [166,172]. In primary ovarian cancer cells and head and neck squamous cell carcinoma, the inhibition of CD73 by shRNA and/or APCP treatment resulted in decreased cell mobility and invasibility, inhibiting the expression of EMT-related markers [167,173]. Lung tumor cells with silenced CD73 expression formed smaller and less invasive 3D organoids *in vitro* and inhibited tumor growth and metastasis *in vivo* [150]. CD73 has also been associated with EMT in

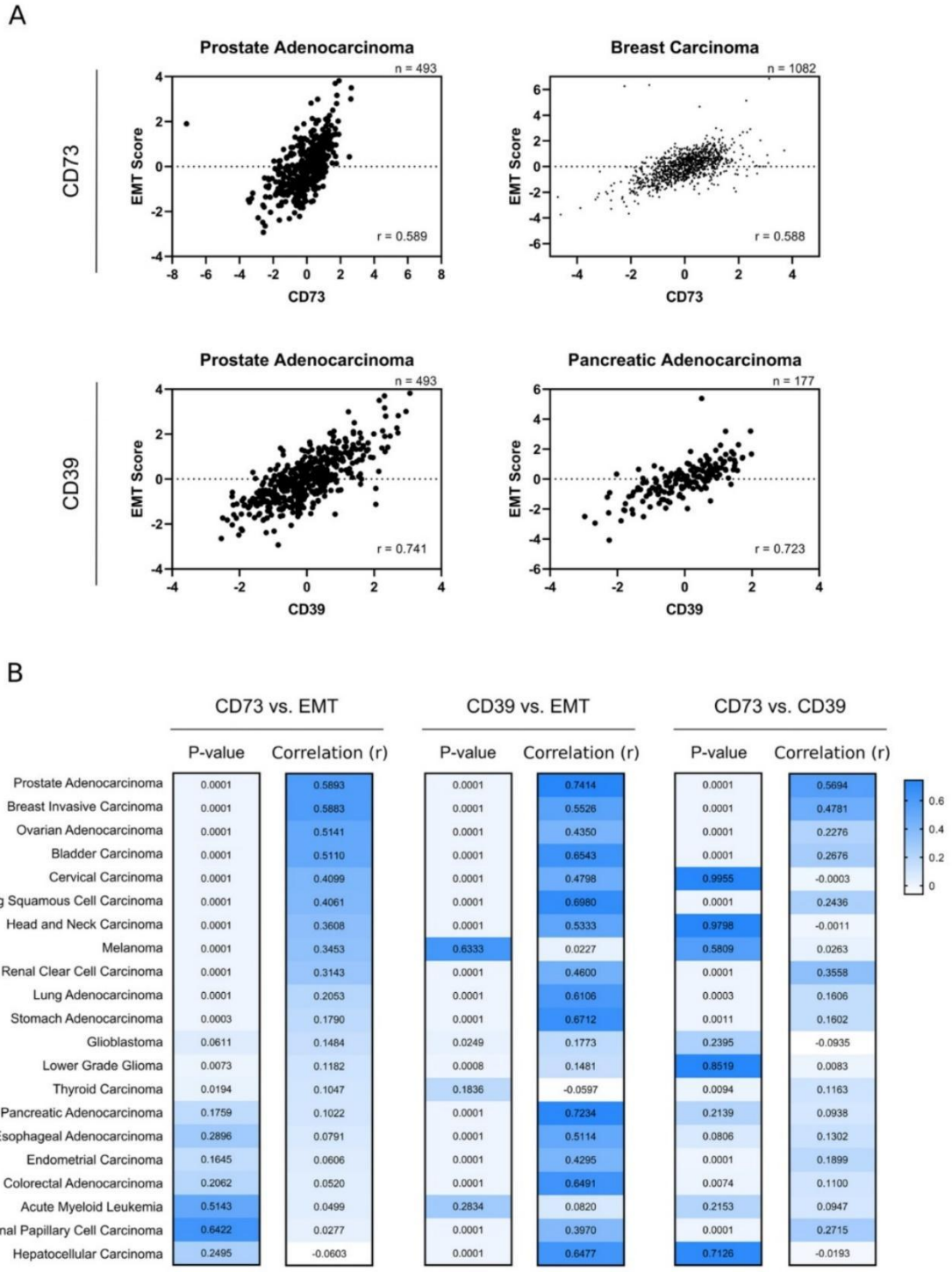


**Fig. 2.** Correlation between *NT5E* (CD73) and *ENTPD1* (CD39) expression with EMT score in tumor samples. The EMT score was calculated for each patient sample as the mean z-score expression of mesenchymal markers subtracted by the mean z-score expression of the epithelial markers, as previously described by Mak et al. [147]. Pearson correlation analysis was performed using RNA-seq expression data from The Cancer Genome Atlas (TCGA) PanCancer Atlas dataset obtained through the cBioPortal for Cancer Genomics [148,149]. (A) Scatter plots of the two tumors with the strongest correlations between EMT score and *NT5E* and *ENTPD1* expression. Sample size (n) and Pearson's correlation coefficient (r) are shown for each cancer type. (B) Heatmap of the p-values and correlation coefficients between CD73 with EMT, CD39 with EMT, and CD73 with CD39 for the 21 tumor types analyzed. Statistical analyses were carried out using GraphPad Prism 8.0.2 Software. Two-sided p-values $< 0.05$ were considered statistically significant.

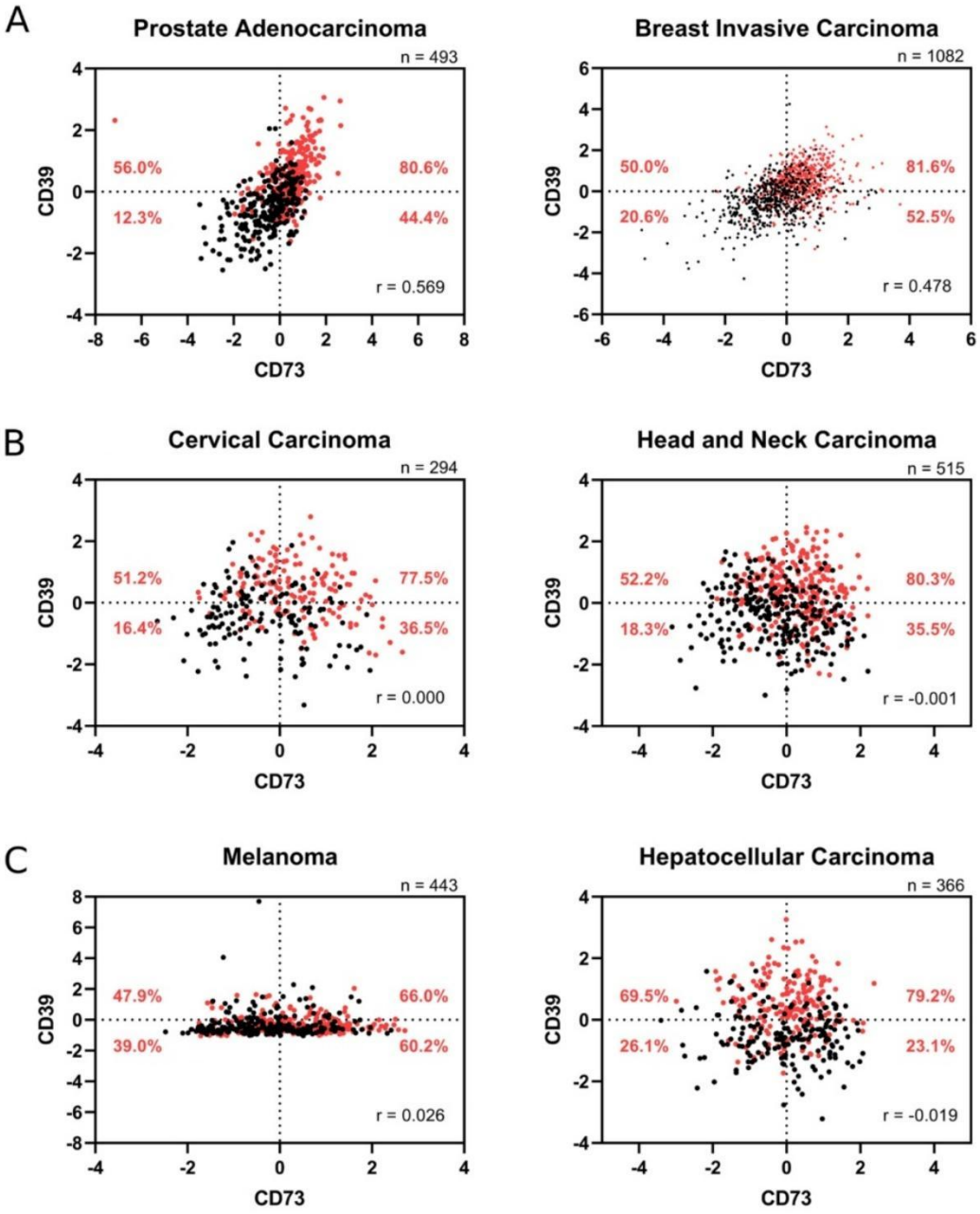


**Fig. 3.** The gene expression of both CD39 and CD73 contributes to the correlations observed with the EMT score. (A-C) Scatter plots of the correlations between *NT5E* (CD73) and *ENTPD1* (CD39) expressions using RNA-seq expression data from the TCGA PanCancer Atlas dataset. Patient samples with an EMT score greater than the median EMT score for that tumor type are colored red, and the percentages of samples colored red in each quadrant of the plot are indicated. Sample size (n) and Pearson's correlation coefficient (r) are shown for each cancer type. (A) Scatter plots of prostate adenocarcinoma and breast carcinoma are shown to represent tumor types with strong correlations between EMT score and both CD73 and CD39 expression (Fig. 2), as well as between their expressions. (B) Scatter plots of cervical and head and neck carcinomas are displayed to represent tumors with significant correlations between the EMT score and both genes (Fig. 2) but not between their expressions. (C) Scatter plots of melanoma and hepatocellular carcinoma are shown as examples of tumors with strong correlations with the EMT score for only one of the genes (CD73 in melanoma and CD39 in hepatocellular carcinoma) (Fig. 2) and that did not present a significant correlation between their expressions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

gallbladder cancer cells and pancreatic ductal adenocarcinoma, promoting cell migration and invasion via the up-regulation of Vimentin and down-regulation of E-cadherin [164,174]. In order to summarize the main roles of CD73 in tumor EMT, comprehending the more recurrent alterations in EMT markers and tumor outcome, a schematic representation is elaborated in Fig. 4.

**Table 1**
Summary of studies endorsing CD73 influence in EMT.

| Cancer type | Study design | Intervention | Overall effect | Relation to EMT | Reference |
|---|---|---|---|---|---|
| Breast | *In vivo and in vitro* | CD73 shRNA or APCP | ↑ E-cadherin, ↓ vimentin | CD73 is important for mesenchymal phenotype | [150] |
| Breast | *In vivo and in vitro* | TGFβ | ↑ CD73 | EMT accompanied by ↑ CD73 | [155, 156] |
| Breast | *In vitro* | | | | |
| Cervical | *In vitro* | APCP | ↓ CD73 | ↑ CD73 and TGFβ levels favors tumor progression | [157] |
| Cervical | *In vitro* | Anti-hTGF-β neutralizing antibodies | ↓ CD73 | Interaction of MSC with tumor cells increase TGFβ, inducing CD73 expression | [158] |
| Cervical | *In vitro* | ADO; Blockade of $A_2AR$ and $A_2BR$ | ↑ TGFβ and CD73; ↓ TGFβ and CD73 | TGF-β1 was necessary to maintain CD73 expression; ADO treatment was necessary to ↑ the production and expression of TGF-β1 | [159] |
| Cervical | *In situ* | Comparison between normal donors and precursor lesion patients | ↑ CD73 in precursor lesion patients | Production of TGFβ in precursor lesions promotes the expression of CD73 generating an immunosuppressive microenvironment and allowing the progression to Cervical Cancer | [160] |
| Cervical | *In vitro* | ADO | ↑ E-cadherin; ↓ N-cadherin and fibronectin | ADO repressed EMT progress | [161] |
| Cholangiocarcinoma | *In vitro* | TGFβ-induced EMT | ↑ CD73, ↑ mesenchymal markers and ↓ epithelial markers | ↑ CD73 was related to poor prognosis | [162] |
| Endometrial | *In vivo and in vitro* | TGFβ; CD73 siRNA, APCP | ↓ CD73 = TGFβ goes from tumor suppressor to promoter | ↓ CD73 was related to ↑ migration, invasion and poor prognosis | [163] |
| Gallbladder | *In vitro* | TGFβ; CD73 siRNA | ↑ CD73; ↑ E-cadherin, ↓ vimentin | ↑ CD73 was negatively associated with survival period and positively associated with mortality after surgery | [164] |
| Gastric | *In vivo and in vitro* | CD73 siRNA and APCP | ↓ Metastasis | CD73 was related to poor prognosis | [165] |
| Glioblastoma | *In vitro* | ADO | ↑ Snail, ZEB1 and vimentin; ↓ E-cadherin | ADO supported cell proliferation and invasiveness | [166] |
| Glioma | *In vitro* | CD73 shRNA and ZEB1 shRNA | ↓ SNAIL1, MMP2 and CD73 | ↓ CD73 caused suppression of clonogenicity, cell invasion and proliferation | [70] |
| Head and neck squamous cell carcinoma | *In vivo and in vitro* | CD73 siRNA | ↓ N-cadherin, Slug and Vimentin, ↑ E-cadherin | CD73 and ADO signaling promote tumor progression and metastasis | [167] |
| Hepatocellular | *In vivo and in vitro* | CD73 shRNA and overexpression | ↓ Migration and invasion; ↑ N-cadherin in cells CD73-high | CD73 promotes proliferation, migration, invasion, and EMT | [168] |
| Hepatocellular | *In situ* | Gene expression databases analysis | ↓ CD73 in normal hepatocytes | CD73 is a predictor of poor overall survival and recurrence-free survival | [169] |
| Hepatocellular | *In vitro* | TNF-α | ↑ CD73; ↓ CD73 upon reversal of EMT | TNF-α treatment upregulates CD73 and EMT genes | [170] |
| Hepatobilio- pancreatic | *In situ* | Immunohistochemistry of surgical specimens quantifying CD73 and E-cadherin | ↑ CD73 was inversely correlated with differentiation and was helpful to identify isolated discohesive tumor cells; ↓ E-cadherin | ↑ CD73 was associated with reduced overall survival | [171] |
| Lung | *In vitro* | Databases analysis; CD73 overexpression | In silico: ↑ CD73 was correlated with poor overall survival; *In vitro*: ↓ E-cadherin and ↑ N-cadherin and Vimentin | CD73 promoted migration and EMT | [172] |
| Lung | *In vivo, in situ and in vitro* | APCP | CD73 enrichment in tumor and peritumoral stroma | Tumor TGFβ stimulated CD73 expression, inhibited antitumor activity, and protected tumor cells from the cytotoxic effect of chemotherapy | [143] |
| Melanoma | *In vitro* | CD73 siRNA | Enzymatic activity of CD73 is involved in invasion, whereas the non-enzymatic action contributes to cell adhesion and migration | CD73 is involved with cell adhesion, migration and invasion | [69] |
| Ovarian | *In vivo and in vitro* | CD73 shRNA or APCP | ↓ Snail, Twist and Zeb1 | CD73 is important for mesenchymal phenotype | [173] |
| Ovarian | *In vitro* | ADO | ↓ Migration, ↑ E-Cadherin | ADO induced epithelial phenotype | [102] |
| Pancreatic | *In vitro* | Overexpression and Knockdown of CD73 | ↑ CD73 and ZEB-1 in radioresistant cells; CD73-mediated radioresistance is independent of the enzymatic activity of CD73 | CD73 is required for maintaining radioresistant cells in a mesenchymal state | [174] |

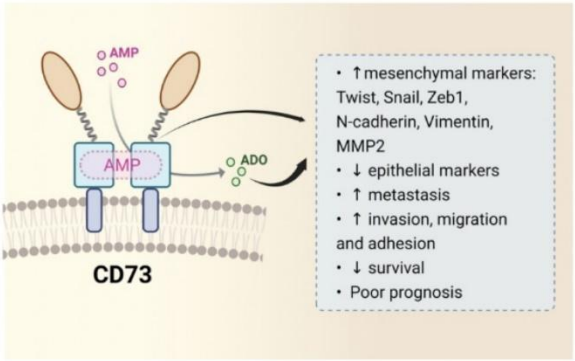


**Fig. 4.** Schematic representation of CD73 roles in EMT in cancer.

## 4. Conclusions

Comprehending how intricately connected the adenosinergic system and the EMT are is a powerful resource when it comes to understanding cancer growth and response to therapy. The possibility to apply this knowledge to clinical approaches is of utmost relevance since it comprises many types of tumors. Here we reviewed the interaction between these two processes and their effect on metastasis and tumor invasion, discussing the latest existing body of knowledge regarding the matter. We hope the presented overview contributes to the development of strategies aiming for better management of metastasis risk and greater survival expectancy.

## Declaration of Competing Interest

None.

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## References

[1] H. Zimmermann, M. Zebisch, N. Sträter, Cellular function and molecular structure of ecto-nucleotidases, Purinergic Signal 8 (2012) 437–502.
[2] G. Burnstock, Purinergic signalling: therapeutic developments, Front. Pharmacol. 8 (2017) 661.
[3] S.C. Robson, J. Sévigny, H. Zimmermann, The E-NTPDase family of ectonucleotidases: structure function relationships and pathophysiological significance, Purinergic Signal 2 (2006) 409–430.
[4] G. Burnstock, Purine and pyrimidine receptors, Cell. Mol. Life Sci. 64 (2007) 1471–1483.
[5] J.D. Young, S.Y.M. Yao, J.M. Baldwin, C.E. Cass, S.A. Baldwin, The human concentrative and equilibrative nucleoside transporter families, SLC28 and SLC29, Mol. Asp. Med. 34 (2013) 529–547.
[6] B.B. Fredholm, Adenosine receptors as drug targets, Exp. Cell Res. 316 (2010) 1284–1288.
[7] A.L. Horenstein, et al., A CD38/CD203a/CD73 ectoenzymatic pathway independent of CD39 drives a novel adenosinergic loop in human T lymphocytes, Oncoimmunology 2 (2013), e26246.
[8] B. Allard, D. Allard, L. Buisseret, J. Stagg, The adenosine pathway in immuno-oncology, Nat. Rev. Clin. Oncol. 17 (2020) 611–629.
[9] H. Zimmermann, Extracellular metabolism of ATP and other nucleotides, Naunyn. Schmiede Arch. Pharmacol. 362 (2000) 299–309.
[10] L.I.S. Naasani, et al., Extracellular nucleotide hydrolysis in dermal and limbal mesenchymal stem cells: a source of adenosine production, J. Cell. Biochem. 118 (2017) 2430–2442.
[11] M.R. Wink, et al., Altered extracellular ATP, ADP and AMP catabolism in glioma cell lines, Cancer Lett. 198 (2003) 211–218.
[12] I.C. Iser, et al., Mesenchymal stem cells from different murine tissues have differential capacity to metabolize extracellular nucleotides, J. Cell. Biochem. 115 (2014) 1673–1682.
[13] A. Clayton, S. Al-Taei, J. Webber, M.D. Mason, Z. Tabi, Cancer exosomes express CD39 and CD73, which suppress T cells through adenosine production, J. Immunol. 187 (2011) 676–683.
[14] G. Burnstock, Di Virgilio, F, Purinergic signalling and cancer, Purinergic Signal 9 (2013) 491–540.
[15] M.M. Faas, T. Sáez, P. de Vos, Extracellular ATP and adenosine: The Yin and Yang in immune responses? Mol. Asp. Med. 55 (2017) 9–19.
[16] C. Cekic, J. Linden, Purinergic regulation of the immune system, Nat. Rev. Immunol. 16 (2016) 177–192.
[17] F.D. Virgilio, F. Di Virgilio, E. Adinolfi, Extracellular purines, purinergic receptors and tumor growth, Oncogene vol. 36 (2017) 293–303.
[18] F. Di Virgilio, A.C. Sarti, S. Falzoni, E. De Marchi, E. Adinolfi, Extracellular ATP and P2 purinergic signalling in the tumour microenvironment, Nat. Rev. Cancer 18 (2018) 601–618.
[19] O. Kepp, F. Loos, P. Liu, G. Kroemer, Extracellular nucleosides and nucleotides as immunomodulators, Immunol. Rev. 280 (2017) 83–92.
[20] X.-Y. Li, et al., Targeting CD39 in cancer reveals an extracellular ATP- and inflammasome-driven tumor immunity, Cancer Disco 9 (2019) 1754–1773.
[21] L. Antonioli, C. Blandizzi, P. Pacher, G. Haskó, Immunity, inflammation and cancer: a leading role for adenosine, Nat. Rev. Cancer 13 (2013) 842–857.
[22] S. Sheth, R. Brito, D. Mukherjea, L.P. Rybak, V. Ramkumar, Adenosine receptors: expression, function and regulation, Int. J. Mol. Sci. 15 (2014) 2024–2052.
[23] P.A. Beavis, et al., Adenosine receptor 2A blockade increases the efficacy of anti-PD-1 through enhanced antitumor T-cell responses, Cancer Immunol. Res. 3 (2015) 506–517.
[24] E.Y. Tan, C.L. Richard, H. Zhang, D.W. Hoskin, J. Blay, Adenosine downregulates DPPIV on HT-29 colon cancer cells by stimulating protein tyrosine phosphatase(s) and reducing ERK1/2 activity via a novel pathway, Am. J. Physiol. Cell Physiol. 291 (2006) C433–C444.
[25] W.N. Dinjens, et al., Adenosine deaminase complexing protein (ADCP) expression and metastatic potential in prostatic adenocarcinomas, J. Pathol. 160 (1990) 195–201.
[26] H. Kajiyama, et al., Prolonged survival and decreased invasive activity attributable to dipeptidyl peptidase IV overexpression in ovarian carcinoma, Cancer Res 62 (2002) 2753–2757.
[27] J. Kotulová, M. Hajdúch, P. Džubák, Current adenosinergic therapies: what do cancer cells stand to gain and lose, Int. J. Mol. Sci. 22 (2021).
[28] M. Roh, D.A. Wainwright, J.D. Wu, Y. Wan, B. Zhang, Targeting CD73 to augment cancer immunotherapy, Curr. Opin. Pharmacol. 53 (2020) 66–76.
[29] L.-L. Feng, Y.-Q. Cai, M.-C. Zhu, L.-J. Xing, X. Wang, The yin and yang functions of extracellular ATP and adenosine in tumor immunity, Cancer Cell Int. 20 (2020) 110.
[30] L. Li, et al., Metformin-induced reduction of CD39 and CD73 blocks myeloid-derived suppressor cell activity in patients with ovarian Cancer, Cancer Res 78 (2018) 1779–1791.
[31] I. Perrot, et al., Blocking antibodies targeting the CD39/CD73 immunosuppressive pathway unleash immune responses in combination cancer therapies, Cell Rep. 27 (2411–2425) (2019), e9.
[32] S. Guo, F. Han, W. Zhu, CD39 - a bright target for cancer immunotherapy, Biomed. Pharmacother. 151 (2022), 113066.
[33] D. Allard, P. Chrobak, B. Allard, N. Messaoudi, J. Stagg, Targeting the CD73-adenosine axis in immuno-oncology, Immunol. Lett. 205 (2019) 31–39.
[34] L. Buisseret, et al., Clinical significance of CD73 in triple-negative breast cancer: multiplex analysis of a phase III clinical trial, Ann. Oncol. 29 (2018) 1056–1062.
[35] A.M. Chambers, et al., Functional expression of CD73 on human natural killer cells, Cancer Immunol. Immunother. (2022), https://doi.org/10.1007/s00262-022-03219-z.
[36] F. Yan, et al., Human dental pulp stem cells regulate allogeneic NK cells' function via induction of anti-inflammatory purinergic signalling in activated NK cells, Cell Prolif. 52 (2019), e12595.
[37] D. Allard, M. Turcotte, J. Stagg, Targeting A2 adenosine receptors in cancer, Immunol. Cell Biol. 95 (2017) 333–339.
[38] B. Allard, P.A. Beavis, P.K. Darcy, J. Stagg, Immunosuppressive activities of adenosine in cancer, Curr. Opin. Pharmacol. 29 (2016) 7–16.
[39] A. Ohta, et al., A2A adenosine receptor protects tumors from antitumor T cells, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 13132–13137.
[40] D. Jin, et al., CD73 on tumor cells impairs antitumor T-cell responses: a novel mechanism of tumor-induced immune suppression, Cancer Res 70 (2010) 2245–2255.
[41] D. Mittal, et al., Adenosine 2B receptor expression on cancer cells promotes metastasis, Cancer Res 76 (2016) 4372–4382.
[42] R. Iannone, L. Miele, P. Maiolino, A. Pinto, S. Morello, Blockade of A2b adenosine receptor reduces tumor growth and immune suppression mediated by myeloid-derived suppressor cells in a mouse model of melanoma, Neoplasia 15 (2013) 1400–1409.

[43] A. Young, et al., Co-inhibition of CD73 and A2AR adenosine signaling improves anti-tumor immune responses, Cancer Cell 30 (2016) 391–403.
[44] F. Morandi, et al., A non-canonical adenosinergic pathway led by CD38 in human melanoma cells induces suppression of T cell proliferation, Oncotarget 6 (2015) 25602–25618.
[45] N.W.C.J. van de Donk, Immunomodulatory effects of CD38-targeting antibodies, Immunol. Lett. 199 (2018) 16–22.
[46] J. Krejcik, et al., Daratumumab depletes CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma, Blood 128 (2016) 384–394.
[47] L. Antonioli, P. Pacher, E.S. Vizi, G. Haskó, CD39 and CD73 in immunity and inflammation, Trends Mol. Med. 19 (2013) 355–367.
[48] J. Kiss, et al., IFN-beta protects from vascular leakage via up-regulation of CD73, Eur. J. Immunol. 37 (2007) 3334–3338.
[49] K.P. Alcedo, J.L. Bowser, N.T. Snider, The elegant complexity of mammalian ecto-5′-nucleotidase (CD73), Trends Cell Biol. 31 (2021) 829–842.
[50] Z.-W. Gao, et al., CD73 promotes proliferation and migration of human cervical cancer cells independent of its enzyme activity, BMC Cancer 17 (2017) 135.
[51] N. Bonnin, et al., MiR-422a promotes loco-regional recurrence by targeting NT5E/CD73 in head and neck squamous cell carcinoma, Oncotarget 7 (2016) 44023–44038.
[52] M. Xie, et al., MicroRNA-30a regulates cell proliferation and tumor growth of colorectal cancer by targeting CD73, BMC Cancer 17 (2017) 305.
[53] J. Zhu, et al., CD73/NT5E is a target of miR-30a-5p and plays an important role in the pathogenesis of non-small cell lung cancer, Mol. Cancer 16 (2017) 34.
[54] F. Zhang, et al., MicroRNA-187, a downstream effector of TGFβ pathway, suppresses Smad-mediated epithelial-mesenchymal transition in colorectal cancer, Cancer Lett. 373 (2016) 203–213.
[55] R. Wang, et al., CircNT5E acts as a sponge of miR-422a to promote glioblastoma tumorigenesis, Cancer Res 78 (2018) 4812–4825.
[56] J. Yang, et al., CircNT5E promotes the proliferation and migration of bladder cancer via sponging miR-502-5p, J. Cancer 12 (2021) 2430–2439.
[57] C. Lo Nigro, et al., NT5E CpG island methylation is a favourable breast cancer biomarker, Br. J. Cancer 107 (2012) 75–83.
[58] H. Wang, et al., NT5E (CD73) is epigenetically regulated in malignant melanoma and associated with metastatic site specificity, Br. J. Cancer 106 (2012) 1446–1452.
[59] W. Xu, et al., Integrative analysis of DNA methylation and gene expression identified cervical cancer-specific diagnostic biomarkers, Signal Transduct. Target Ther. 4 (2019) 55.
[60] N.T. Snider, et al., Alternative splicing of human NT5E in cirrhosis and hepatocellular carcinoma produces a negative regulator of ecto-5′-nucleotidase (CD73), Mol. Biol. Cell 25 (2014) 4024–4033.
[61] X. Zhi, et al., RNAi-mediated CD73 suppression induces apoptosis and cell-cycle arrest in human breast cancer cells, Cancer Sci. 101 (2010) 2561–2569.
[62] L. Bavaresco, et al., The role of ecto-5′-nucleotidase/CD73 in glioma cell line proliferation, Mol. Cell. Biochem. 319 (2008) 61–68.
[63] J.H. Azambuja, et al., CD73 Downregulation Decreases In Vitro and In Vivo Glioblastoma Growth, Mol. Neurobiol. 56 (2019) 3260–3279.
[64] J.H. Azambuja, et al., Nasal Administration of Cationic Nanoemulsions as CD73-siRNA Delivery System for Glioblastoma Treatment: a New Therapeutical Approach, Mol. Neurobiol. vol. 57 (2020) 635–649.
[65] H.K. Oh, et al., Overexpression of CD73 in epithelial ovarian carcinoma is associated with better prognosis, lower stage, better differentiation and lower regulatory T cell infiltration, J. Gynecol. Oncol. 23 (2012) 274–281.
[66] P.-O. Gaudreau, B. Allard, M. Turcotte, J. Stagg, CD73-adenosine reduces immune responses and survival in ovarian cancer patients, Oncoimmunology 5 (2016), e1127496.
[67] A.R. Cappellari, G.J. Vasques, L. Bavaresco, E. Braganhol, A.M.O. Battastini, Involvement of ecto-5′-nucleotidase/CD73 in U138MG glioma cell adhesion, Mol. Cell. Biochem. 359 (2012) 315–322.
[68] Z.-W. Gao, K. Dong, H.-Z. Zhang, The roles of CD73 in cancer, Biomed. Res. Int 2014 (460654) (2014).
[69] R. Sadej, A.C. Skladanowski, Dual, enzymatic and non-enzymatic, function of ecto-5′-nucleotidase (eN, CD73) in migration and invasion of A375 melanoma cells, Acta Biochim. Pol. 59 (2012) 647–652.
[70] J. Tsiampali, et al., Enzymatic Activity of CD73 Modulates Invasion of Gliomas via Epithelial-Mesenchymal Transition-Like Reprogramming, Pharmaceuticals 13 (2020).
[71] R. Turiello, et al., Exosomal CD73 from serum of patients with melanoma suppresses lymphocyte functions and is associated with therapy resistance to anti-PD-1 agents, J. Immunother. Cancer 10 (2022).
[72] B. Zhang, et al., The expression and clinical significance of CD73 molecule in human rectal adenocarcinoma, Tumour Biol. 36 (2015) 5459–5466.
[73] J. Wang, S. Matosevic, /CD73 as correlative factor of patient survival and natural killer cell infiltration in glioblastoma, J. Clin. Med. Res. 8 (2019).
[74] A.P.S. Bertoni, et al., Activity of ecto-5′-nucleotidase (NT5E/CD73) is increased in papillary thyroid carcinoma and its expression is associated with metastatic lymph nodes, Mol. Cell. Endocrinol. 479 (2019) 54–60.
[75] J. Stella, et al., Differential ectonucleotidase expression in human bladder cancer cell lines, Urol. Oncol. 28 (2010) 260–267.
[76] X.-X. Lu, et al., Expression and clinical significance of CD73 and hypoxia-inducible factor-1α in gastric carcinoma, World J. Gastroenterol. 19 (2013) 1912–1918.
[77] Y.I. Yu, et al., Ecto-5′-nucleotidase expression is associated with the progression of renal cell carcinoma, Oncol. Lett. 9 (2015) 2485–2494.
[78] B.G. Leclerc, et al., CD73 Expression is an independent prognostic factor in prostate cancer, Clin. Cancer Res 22 (2016) 158–166.
[79] Q. Chen, et al., CD73 acts as a prognostic biomarker and promotes progression and immune escape in pancreatic cancer, J. Cell. Mol. Med. 24 (2020) 8674–8686.
[80] J. Zhao, et al., Overexpression of CD73 in pancreatic ductal adenocarcinoma is associated with immunosuppressive tumor microenvironment and poor survival, Pancreatology 21 (2021) 942–949.
[81] R. Sadej, J. Spychala, A.C. Skladanowski, Expression of ecto-5′-nucleotidase (eN, CD73) in cell lines from various stages of human melanoma, Melanoma Res 16 (2006) 213–222.
[82] J. Spychala, et al., Role of estrogen receptor in the regulation of ecto-5′-nucleotidase and adenosine in breast cancer, Clin. Cancer Res 10 (2004) 708–717.
[83] S. Serra, et al., CD73-generated extracellular adenosine in chronic lymphocytic leukemia creates local conditions counteracting drug-induced cell death, Blood 118 (2011) 6141–6152.
[84] Y. Inoue, et al., Prognostic impact of CD73 and A2A adenosine receptor expression in non-small-cell lung cancer, Oncotarget 8 (2017) 8738–8751.
[85] P.A. Borea, S. Gessi, S. Merighi, K. Varani, Adenosine as a Multi-Signalling Guardian Angel in Human Diseases: When, Where and How Does it Exert its Protective Effects? Trends Pharmacol. Sci. 37 (2016) 419–434.
[86] A.R. Cappellari, et al., Ecto-5′-Nucleotidase Overexpression Reduces Tumor Growth in a Xenograph Medulloblastoma Model, PLoS One 10 (2015), e0140996.
[87] L. Madi, et al., A3 adenosine receptor activation in melanoma cells: association between receptor fate and tumor growth inhibition, J. Biol. Chem. 278 (2003) 42121–42130.
[88] S. Bar-Yehuda, et al., CF101, an agonist to the A3 adenosine receptor, enhances the chemotherapeutic effect of 5-fluorouracil in a colon carcinoma murine model, Neoplasia 7 (2005) 85–90.
[89] G. Ohana, et al., Inhibition of primary colon carcinoma growth and liver metastasis by the A3 adenosine receptor agonist CF101, Br. J. Cancer 89 (2003) 1552–1558.
[90] P. Fishman, et al., Targeting the A3 adenosine receptor for cancer therapy: inhibition of prostate carcinoma cell growth by A3AR agonist, Anticancer Res 23 (2003) 2077–2083.
[91] S. Bar-Yehuda, et al., The A3 adenosine receptor agonist CF102 induces apoptosis of hepatocellular carcinoma via de-regulation of the Wnt and NF-kappaB signal transduction pathways, Int. J. Oncol. 33 (2008) 287–295.
[92] S. Merighi, et al., Adenosine receptors as mediators of both cell proliferation and cell death of cultured human melanoma cells, J. Invest. Dermatol. 119 (2002) 923–933.
[93] F. Dietrich, et al., Ecto-5′-nucleotidase/CD73 contributes to the radiosensitivity of T24 human bladder cancer cell line, J. Cancer Res. Clin. Oncol. 144 (2018) 469–482.
[94] H. Hosseinzadeh, M.R. Jaafari, J. Shamsara, Selective inhibitory effect of adenosine A1 receptor agonists on the proliferation of human tumor cell lines, Iran. Biomed. J. 12 (2008) 203–208.
[95] P. Koszałka, et al., Specific Activation of A3, A2A and A1 Adenosine Receptors in CD73-Knockout Mice Affects B16F10 Melanoma Growth, Neovascularization, Angiogenesis and Macrophage Infiltration, PLoS One 11 (2016), e0151420.
[96] S. Daniele, E. Zappelli, L. Natali, C. Martini, M.L. Trincavelli, Modulation of A1 and A2B adenosine receptor activity: a new strategy to sensitise glioblastoma stem cells to chemotherapy, Cell Death Dis. 5 (2014), e1539.
[97] E. Polycarpou, et al., Resveratrol 3-O-D-glucuronide and resveratrol 4′-O-D-glucuronide inhibit colon cancer cell growth: evidence for a role of A3 adenosine receptors, cyclin D1 depletion, and G1 cell cycle arrest, Mol. Nutr. Food Res. 57 (2013) 1708–1717.
[98] S. Gessi, et al., Modulation of metalloproteinase-9 in U87MG glioblastoma cells by A3 adenosine receptors, Biochem. Pharmacol. 79 (2010) 1483–1495.
[99] S. Jajoo, D. Mukherjea, K. Watabe, V. Ramkumar, Adenosine A(3) receptor suppresses prostate cancer metastasis by inhibiting NADPH oxidase activity, Neoplasia 11 (2009) 1132–1145.
[100] J.L. Bowser, et al., Loss of CD73-mediated actin polymerization promotes endometrial tumor progression, J. Clin. Invest 126 (2016) 220–238.
[101] A. Supernat, et al., CD73 expression as a potential marker of good prognosis in breast carcinoma, Appl. Immunohistochem. Mol. Morphol. 20 (2012) 103–107.
[102] A.S. Martínez-Ramírez, et al., Cellular Migration Ability Is Modulated by Extracellular Purines in Ovarian Carcinoma SKOV-3 Cells, J. Cell. Biochem. 118 (2017) 4468–4478.
[103] S. Hajiahmadi, M. Panjehpour, M. Aghaei, M. Shabani, Activation of A2b adenosine receptor regulates ovarian cancer cell growth: involvement of Bax/Bcl-2 and caspase-3, Biochem. Cell Biol. 93 (2015) 321–329.
[104] P. Sureechatchaiyan, A. Hamacher, N. Brockmann, B. Stork, M.U. Kassack, Adenosine enhances cisplatin sensitivity in human ovarian cancer cells, Purinergic Signal 14 (2018) 395–408.
[105] J.H. Azambuja, N. Ludwig, E. Braganhol, T.L. Whiteside, Inhibition of the Adenosinergic Pathway in Cancer Rejuvenates Innate and Adaptive Immunity, Int. J. Mol. Sci. 20 (2019).
[106] S. Chen, et al., CD73: an emerging checkpoint for cancer immunotherapy, Immunotherapy 11 (2019) 983–997.
[107] C.M. Hay, et al., Targeting CD73 in the tumor microenvironment with MEDI9447, Oncoimmunology 5 (2016), e1208875.
[108] S.M. Stemmer, et al., CF102 for the treatment of hepatocellular carcinoma: a phase I/II, open-label, dose-escalation study, Oncologist 18 (2013) 25–26.

[109] S.B. Willingham, et al., A2AR Antagonism with CPI-444 Induces Antitumor Responses and Augments Efficacy to Anti-PD-(L)1 and Anti-CTLA-4 in Preclinical Models, Cancer Immunol. Res 6 (2018) 1136–1149.
[110] R. Hermann, et al., Cladribine as a potential object of nucleoside transporter-based drug interactions, Clin. Pharmacokinet. 61 (2022) 167–187.
[111] D. Hanahan, Hallmarks of Cancer: New Dimensions, Cancer Disco 12 (2022) 31–46.
[112] G. Lenz, et al., The Origins of Phenotypic Heterogeneity in Cancer, Cancer Res 82 (2022) 3–11.
[113] L.S. Lenz, et al., Cancer Cell Fitness Is Dynamic, Cancer Res 81 (2021) 1040–1051.
[114] E.D. Hay, A. Zuk, Transformations between epithelium and mesenchyme: normal, pathological, and experimentally induced, Am. J. Kidney Dis. 26 (1995) 678–690.
[115] M.A. Nieto, R.Y.-J. Huang, R.A. Jackson, J.P. Thiery, EMT: 2016, Cell 166 (2016) 21–45.
[116] A. Dongre, R.A. Weinberg, New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer, Nat. Rev. Mol. Cell Biol. 20 (2019) 69–84.
[117] S. Xu, M. Zhan, J. Wang, Epithelial-to-mesenchymal transition in gallbladder cancer: from clinical evidence to cellular regulatory networks, Cell Death Disco 3 (2017) 17069.
[118] A. Dongre, et al., Epithelial-to-Mesenchymal Transition Contributes to Immunosuppression in Breast Carcinomas, Cancer Res 77 (2017) 3982–3989.
[119] K. Gravdal, O.J. Halvorsen, S.A. Haukaas, L.A. Akslen, A switch from E-cadherin to N-cadherin expression indicates epithelial to mesenchymal transition and is of strong and independent importance for the progress of prostate cancer, Clin. Cancer Res 13 (2007) 7003–7011.
[120] A.M. Krebs, et al., The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer, Nat. Cell Biol. 19 (2017) 518–529.
[121] N. Aruga, et al., Epithelial-mesenchymal Transition (EMT) is Correlated with Patient's Prognosis of Lung Squamous Cell Carcinoma. *Tokai*, J. Exp. Clin. Med. 43 (2018) 5–13.
[122] L. Prudkin, et al., Epithelial-to-mesenchymal transition in the development and progression of adenocarcinoma and squamous cell carcinoma of the lung, Mod. Pathol. vol. 22 (2009) 668–678.
[123] M. Shioiri, et al., Slug expression is an independent prognostic parameter for poor survival in colorectal carcinoma patients, Br. J. Cancer 94 (2006) 1816–1822.
[124.] T.K. Lee, et al., Twist overexpression correlates with hepatocellular carcinoma metastasis through induction of epithelial-mesenchymal transition, Clin. Cancer Res 12 (2006) 5369–5376.
[125] J. Chen, et al., Aquaporin 3 promotes epithelial-mesenchymal transition in gastric cancer, J. Exp. Clin. Cancer Res. 33 (2014) 38.
[126] B. Roth, et al., Employing an orthotopic model to study the role of epithelial-mesenchymal transition in bladder cancer metastasis, Oncotarget 8 (2017) 34205–34222.
[127] I.C. Iser, M.B. Pereira, G. Lenz, M.R. Wink, The Epithelial-to-Mesenchymal Transition-Like Process in Glioblastoma: An Updated Systematic Review and In Silico Investigation, Med. Res. Rev. 37 (2017) 271–313.
[128] Y.-T. Zeng, X.-F. Liu, W.-T. Yang, P.-S. Zheng, REX1 promotes EMT-induced cell metastasis by activating the JAK2/STAT3-signaling pathway by targeting SOCS1 in cervical cancer, Oncogene 38 (2019) 6940–6957.
[129] S. Brabletz, H. Schuhwerk, T. Brabletz, M.P. Stemmler, Dynamic EMT: a multi-tool for tumor progression, EMBO J. 40 (2021), e108647.
[130] S. Bhatia, P. Wang, A. Toh, E.W. Thompson, New Insights Into the Role of Phenotypic Plasticity and EMT in Driving Cancer Progression, Front Mol. Biosci. 7 (2020) 71.
[131] K.T. Yeung, J. Yang, Epithelial-mesenchymal transition in tumor metastasis, Mol. Oncol. 11 (2017) 28–39.
[132] S. Bhatia, et al., Interrogation of Phenotypic Plasticity between Epithelial and Mesenchymal States in Breast Cancer, J. Clin. Med. Res. 8 (2019).
[133] S. Zhang, et al., Mesenchymal phenotype of circulating tumor cells is associated with distant metastasis in breast cancer patients, Cancer Manag. Res. 9 (2017) 691–700.
[134] M. Yu, et al., Circulating breast tumor cells exhibit dynamic changes in epithelial and mesenchymal composition, Science 339 (2013) 580–584.
[135] X. Zhang, et al., Epithelial-mesenchymal transition phenotype of circulating tumor cells is associated with distant metastasis in patients with NSCLC, Mol. Med. Rep. 19 (2019) 601–608.
[136] L. Alonso-Alconada, et al., Molecular profiling of circulating tumor cells links plasticity to the metastatic process in endometrial cancer, Mol. Cancer 13 (2014) 223.
[137] R. Negishi, et al., Transcriptomic profiling of single circulating tumor cells provides insight into human metastatic gastric cancer, Commun. Biol. 5 (2022) 20.
[138] M. Lim, et al., Circulating tumor cell clusters are cloaked with platelets and correlate with poor prognosis in unresectable pancreatic cancer, Cancers 13 (2021).
[139] C.R. Lindsay, et al., Vimentin and Ki67 expression in circulating tumour cells derived from castrate-resistant prostate cancer, BMC Cancer 16 (2016) 168.
[140] M.K. Jolly, et al., Implications of the hybrid epithelial/mesenchymal phenotype in metastasis, Front. Oncol. 5 (2015) 155.
[141] A.J. Armstrong, et al., Circulating tumor cells from patients with advanced prostate and breast cancer display both epithelial and mesenchymal markers, Mol. Cancer Res 9 (2011) 997–1007.
[142] M.K. Koivisto, et al., Cell-type-specific CD73 expression is an independent prognostic factor in bladder cancer, Carcinogenesis 40 (2019) 84–92.
[143] J. Li, et al., CD39/CD73 upregulation on myeloid-derived suppressor cells via TGF-β-mTOR-HIF-1 signaling in patients with non-small cell lung cancer, Oncoimmunology 6 (2017), e1320011.
[144] Z. Zeng, et al., Significantly different immunoscores in lung adenocarcinoma and squamous cell carcinoma and a proposal for a new immune staging system, Oncoimmunology 9 (2020) 1828538.
[145] P. Koszałka, et al., CD73 on B16F10 melanoma cells in CD73-deficient mice promotes tumor growth, angiogenesis, neovascularization, macrophage infiltration and metastasis, Int. J. Biochem. Cell Biol. 69 (2015) 1–10.
[146] L. Song, et al., Ecto-5′-nucleotidase (CD73) is a biomarker for clear cell renal carcinoma stem-like cells, Oncotarget 8 (2017) 31977–31992.
[147] M.P. Mak, et al., A Patient-Derived, Pan-Cancer EMT Signature Identifies Global Molecular Alterations and Immune Target Enrichment Following Epithelial-to-Mesenchymal Transition, Clin. Cancer Res 22 (2016) 609–620.
[148] E. Cerami, et al., The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data, Cancer Disco 2 (2012) 401–404.
[149] J. Gao, et al., Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal, Sci. Signal. 6 (2013) l1.
[150] N. Petruk, et al., CD73 facilitates EMT progression and promotes lung metastases in triple-negative breast cancer, Sci. Rep. 11 (2021) 6035.
[151] B. Allard, et al., Anti-CD73 therapy impairs tumor angiogenesis, Int. J. Cancer 134 (2014) 1466–1473.
[152] C. Giacomelli, et al., The A Adenosine Receptor Modulates the Epithelial-Mesenchymal Transition through the Balance of cAMP/PKA and MAPK/ERK Pathway Activation in Human Epithelial Lung Cells, Front. Pharmacol. 9 (2018) 54.
[153] L. Weng, et al., The effect of cAMP-PKA activation on TGF-β1-induced profibrotic signaling, Cell. Physiol. Biochem. 36 (2015) 1911–1927.
[154] H. Yang, et al., Protein kinase A modulates transforming growth factor-β signaling through a direct interaction with Smad4 protein, J. Biol. Chem. 288 (2013) 8737–8749.
[155] M. Turcotte, et al., CD73 Promotes Resistance to HER2/ErbB2 Antibody Therapy, Cancer Res 77 (2017) 5652–5663.
[156] J. Yu, et al., A preliminary study of the role of extracellular -5′- nucleotidase in breast cancer stem cells and epithelial-mesenchymal transition, Vitr. Cell. Dev. Biol. Anim. 53 (2017) 132–140.
[157] R. Muñóz-Godínez, et al., Detection of CD39 and a Highly Glycosylated Isoform of Soluble CD73 in the Plasma of Patients with Cervical Cancer: Correlation with Disease Progression, Mediat. Inflamm. 2020 (2020) 1678780.
[15] L.R. Ávila-Ibarra, et al., Mesenchymal Stromal Cells Derived from Normal Cervix and Cervical Cancer Tumors Increase CD73 Expression in Cervical Cancer Cells Through TGF-β1 Production, Stem Cells Dev. 28 (2019) 477–488.
[159] R. García-Rocha, et al., Cervical cancer cells produce TGF-β1 through the CD73-adenosine pathway and maintain CD73 expression through the autocrine activity of TGF-β1, Cytokine 118 (2019) 71–79.
[160] M. de Lourdes Mora-García, et al., HPV-16 Infection Is Associated with a High Content of CD39 and CD73 Ectonucleotidases in Cervical Samples from Patients with CIN-1, Mediat. Inflamm. 2019 (2019) 4651627.
[161] Z.W. Gao, et al., Adenosine inhibits migration, invasion and induces apoptosis of human cervical cancer cells, Neoplasma 63 (2016) 201–207.
[162] L. Cao, et al., Prognostic Role of Immune Checkpoint Regulators in Cholangiocarcinoma: A Pilot Study, J. Clin. Med. Res. 10 (2021).
[163] K.C. Kurnit, et al., Loss of CD73 shifts transforming growth factor-β1 (TGF-β1) from tumor suppressor to promoter in endometrial cancer, Cancer Lett. 505 (2021) 75–86.
[164] L. Xiong, Y. Wen, X. Miao, Z. Yang, NT5E and FcGBP as key regulators of TGF-1-induced epithelial-mesenchymal transition (EMT) are associated with tumor progression and survival of patients with gallbladder cancer, Cell Tissue Res 355 (2014) 365–374.
[165] Z. Xu, et al., CD73 promotes tumor metastasis by modulating RICS/RhoA signaling and EMT in gastric cancer, Cell Death Dis. 11 (2020) 202.
[166] D. Pietrobono, C. Giacomelli, L. Marchetti, C. Martini, M.L. Trincavelli, High Adenosine Extracellular Levels Induce Glioblastoma Aggressive Traits Modulating the Mesenchymal Stromal Cell Secretome, Int. J. Mol. Sci. 21 (2020).
[167] Z.-H. Ren, et al., CD73 is associated with poor prognosis in HNSCC, Oncotarget 7 (2016) 61690–61702.
[168] X.-L. Ma, et al., CD73 promotes hepatocellular carcinoma progression and metastasis via activating PI3K/AKT signaling by inducing Rap1-mediated membrane localization of P110β and predicts poor prognosis, J. Hematol. Oncol. 12 (2019) 37.
[169] R. Shrestha, et al., Monitoring Immune Checkpoint Regulators as Predictive Biomarkers in Hepatocellular Carcinoma, Front. Oncol. 8 (2018) 269.
[170] R. Shrestha, K.R. Bridle, D.H.G. Crawford, A. Jayachandran, TNF-α-mediated epithelial-to-mesenchymal transition regulates expression of immune checkpoint molecules in hepatocellular carcinoma, Mol. Med. Rep. 21 (2020) 1849–1860.
[171] A. Sciarra, et al., CD73 expression in normal and pathological human hepatobiliopancreatic tissues, Cancer Immunol. Immunother. 68 (2019) 467–478.
[172] Z.-W. Gao, et al., CD73 severed as a potential prognostic marker and promote lung cancer cells migration enhancing EMT progression, Front. Genet. 12 (2021), 728200.
[173] M. Lupia, et al., CD73 regulates stemness and epithelial-mesenchymal transition in ovarian cancer-initiating cells, Stem Cell Rep. 10 (2018) 1412–1425.
[174] A.M. Nguyen, J. Zhou, B. Sicairos, S. Sonney, Y. Du, Upregulation of CD73 confers acquired radioresistance and is required for maintaining irradiation-selected pancreatic cancer cells in a Mesenchymal State, Mol. Cell. Proteom. 19 (2020) 375–389.

[175] T.J. Vogt, et al., Detailed analysis of adenosine A2a receptor and CD73 (5′-nucleotidase, ecto,) methylation and gene expression in head and neck squamous cell carcinoma patients, Oncoimmunology 7 (2018), e1452579.

[176] Q.-F. Yang, et al., Development and validation of an individualized immune prognostic model in stage I-III lung squamous cell carcinoma, Sci. Rep. 11 (2021) 12727.

[177] K. Torres-Poveda, et al., Role of IL-10 and TGF-β1 in local immunosuppression in HPV-associated cervical neoplasia, World J. Clin. Oncol. 5 (2014) 753–763.

# CHAPTER II

## Interconnections between CD73 and ZEB1 in papillary thyroid carcinoma

Article to be submitted to Endocrine-Related Cancer

The second chapter aims to investigate the CD73-ZEB1 relationship, two important players in adenosinergic signaling and epithelial-mesenchymal plasticity, specifically on cell lines derived from papillary thyroid carcinoma. My contributions to the article went from theme suggestion and article organization to experiments conduction and literature review.

**OBS: Suprimido da tese até publicação**

# DISCUSSION

Papillary thyroid carcinoma (PTC) is the most prevalent form of malignant lesions affecting the thyroid gland, accounting for the majority of cases (ALI; CIBAS, 2017). Although this variety of carcinoma has a favorable prognosis, it has an estimated recurrence rate of 30% (SCHREINEMAKERS et al., 2012). The heterogeneous nature of this tumor, as demonstrated by disparities in histopathological characteristics, genetic factors, and prognosis, creates diverse variables to take into consideration when studying PTC. This variation influences treatment selection, which results in divergent protocols and contradictory data collection. In that setting, a better understanding of possible mechanisms contributing to PTC progression is of utmost importance and, since the epithelial-mesenchymal transition and the adenosinergic signaling were already demonstrated to be involved with PTC development, they are interesting targets of investigation (BERTONI *et al.*, 2019; JEONG *et al.*, 2020).

The process represented by epithelial-mesenchymal transition provides a diverse set of tumor aggressiveness markers, with the possibility of epithelial and mesenchymal markers making combinations associated with more resistant and migratory phenotypes (PASTUSHENKO *et al.*, 2018; PASTUSHENKO; BLANPAIN, 2019). Similarly, players of the adenosinergic pathway are involved with aggressive phenotypes in cancer, which can contribute to poor prognosis. CD73, an ectonucleotidase that converts AMP into adenosine, is an important participant of the adenosinergic contribution to malign traits, being associated with tumor

progression in various types of cancer (ALLARD *et al.*, 2014, 2020; LI *et al.*, 2018; WANG *et al.*, 2012; YU *et al.*, 2015). Similarly, ZEB1, one of the EMT markers, is a crucial transcription factor correlated with cancerous cell progression and poor survival (PEREZ-OQUENDO; GIBBONS, 2022). From this perspective, we investigated potential associations between CD73 and ZEB1 expression, partially connecting the adenosinergic pathway and the epithelial-mesenchymal transition.

Cancer cell heterogeneity involves genetic and non-genetic variations that are manifested as a complex gathering of genotypic and phenotypically different cells in the tumor microenvironment (LENZ *et al.*, 2021). Considering these heterogeneities, different approaches were taken to study PTC cells individually, enabling us to observe cancer cell diversity in cell lines derived from PTC. Beyond the assessment of the post-transcriptional regulation of ZEB1 via GFP lentiviral transduction, cell polarity index and cell migration speed were also quantified, via cell axis measurements and manual tracking of individual cells. An additional layer of analysis was presented by the comparison of these cell variables between WT cells and cells in which CD73 was inactivated via CRISPR-Cas9.

Through the evaluation of the post-transcriptional regulation of ZEB1 via GFP fluorescence in WT and CD73 negative cell lines derived from papillary thyroid carcinoma, we were able to explore latent links between EMT and the adenosinergic pathway. Notably, ZEB1 expression was altered in BCPAP cells lacking CD73 but was not modified in TPC cells. As so, it could be hypothesized that the differences between TPC and BCPAP cell lines may play a role in ZEB1 expression dissimilarities, since BCPAP is $BRAF^{V600E}$-mutated and this mutation was previously associated with high levels of ZEB1 (RICHARD *et al.*, 2016).

Interestingly, dabrafenib and trametinib treatment for $BRAF^{V600E}$-mutated melanomas caused profound CD73 downregulation in tumor cells (YOUNG *et al.*, 2017). In addition, ZEB1 knockdown was previously associated with a reduction of CD73 expression on the protein and mRNA level (TSIAMPALI *et al.*, 2020). Hence, CD73 and ZEB1 expressions association needs further investigation, because despite the results seen in BCPAP, TPC cell did not present significant differences, and, in order to understand this divergence, the mechanisms behind CD73 and ZEB1 connection would have to be elucidated.

The reduction of ZEB1 expression by the deletion of CD73 suggests an influence of CD73 on ZEB1, which is not clearly explained by the literature yet. Considering signaling pathways connection points between CD73 and ZEB1 regulation, we can hypothesize that miR-200c can play a role in the construction of a CD73-ZEB1 bridge, since it controls CD73 expression via SMAD2, the last functioning as a transcriptional activator of CD73, and it also represses ZEB1 mRNA via the 3'-untranslated region (3'-UTR) (GREGORY *et al.*, 2008; KORDAS; OSEN; EICHMÜLLER, 2018; KORPAL *et al.*, 2008). Besides, when trying to identify miRNAs that affect the invasive potential of anaplastic thyroid carcinoma, it was found that miR-200c and miR-30a–e target SMAD2, which contributes to our conjecture (BRAUN *et al.*, 2010).

As so, in addition to the hypothesis of an interconnection between the ZEB1/miR200 feedback loop and the regulation of CD73 by miR200c via SMAD2, another hypothesis that can be discussed here is the participation of the hypoxia inducible factor-1a (HIF-1a) in the CD73-ZEB1 relationship. Recently it was

demonstrated that TGFβ, a known EMT inducer, influences CD73 expression positively via the mTOR-HIF-1a pathway in a trophoblast cell line (ZHU *et al.*, 2022). The authors observed simultaneously increased levels of pmTOR, pSMAD2 and pSMAD3 as well as CD73 after TGFβ administration, indicating a link between SMAD signaling and mTOR activation. Moreover, after addition of a SMAD2/3 inhibitor these increases were diminished, suggesting a TGFβ-mTOR signaling pathway in the trophoblast cell line. TGFβ-induced phosphorylation of mTOR was also inhibited, suggesting that this phosphorylation was SMAD2/3-dependent (ZHU *et al.*, 2022).

Considering that CD73/*NT5E* is a direct target of HIF-1a, the last acting as a *NT5E* transcription factor, Zhu et al. (2022) also analyzed HIF-1a activation, observing that TGFβ enhanced HIF-1a expression in the presence of hypoxia mimetic $CoCl_2$, but it was reduced by rapamycin (ROZEN-ZVI *et al.*, 2013; SYNNESTVEDT *et al.*, 2002). $CoCl_2$ increased CD73 expression, but this effect was annihilated by a HIF-1a inhibitor. Furthermore, when rapamycin suppressed the mTOR pathway, TGFβ-mediated expression of CD73 also decreased. Altogether, the study indicates that TGFβ may induce CD73 expression via the mTOR-HIF-1a pathway (ZHU *et al.*, 2022). Brought to the context of our data and considering that TGFβ activates ZEB1 transcription by increasing pSMAD2, it can be suggested that SMAD2 is a central contributor to associations between CD73 and ZEB1 expressions, thus requiring future exploration.

With the intent to use this tool to study potential modulation mechanisms connecting EMT and adenosinergic signaling and aiming to examine the impacts on cell polarity index, we measured the cells axis from WT and CD73 negative

cells. Cell polarity can be apico-basal, as in epithelial cells, or front-rear, as in a migratory cell state (NELSON, 2009). The transition from apico-basal to front-rear polarity is considered a hallmark of cancer progression, turning the cells into more invasive and metastatic phenotypes (GANDALOVIČOVÁ *et al.*, 2016; ROYER; LU, 2011). When evaluating cell polarity index in cell lines derived from PTC, it was revealed that BCPAP CD73 negative cells were significantly less polar than WT cells, suggesting the importance of the ectonucleotidase for the maintenance of a fusiform phenotype. Apart from what was already discussed on polarity in the manuscript, it is important to notice that the absence of CD73 seemed to homogenize cell polarity, as seen in Figure 3. Cells from the WT group presented higher polarity index heterogeneity, which also contributed to the suggestion that CD73 may play a relevant role in cell polarity configuration.

ZEB1 can affect the expression of cell polarity complexes, repressing Crumbs, PATJ and Lgl, as well as tight junctions and adherens junctions proteins via their promoter regions (AIGNER *et al.*, 2007). Due to ZEB1's importance as an EMT transcription factor contributing to cell polarity, we evaluated its expression in cell lines derived from PTC, both WT and CD73 edited by CRISPR-Cas9. Concurrently, we analyzed polarity index and cell speed for the same cells individually. Contrastingly, the expected correlation between ZEB1 expression and cell polarity index and between ZEB1 expression and cell speed observed in WT cells were lost in CD73 negative cells, also indicating the importance of this gene to the maintenance of the link between these processes. Since SMAD2 may contribute to the connection between CD73 and ZEB1 expressions association, it

can also be related with the regulatory mechanism affecting the correlations between their expressions and the cellular parameters studied.

Interestingly, cell speed was significantly higher in TPC WT cells in comparison to CD73- cells, while BCPAP WT cells did not differ from CD73- cells in speed, but in ZEB1 expression and polarity index. Again, lineage differences as mutations can be responsible for the contrasting results, but further investigation is needed. As for differences caused by CD73 absence, they also suggest its importance to ZEB1 expression and consequently for the fusiform phenotype, as well as, for a greater extent, to cell migration potential.

To discriminate EMT transition states in PTC and explore its relation to BRAF mutation and CD73/*NT5E* and ZEB1 expressions, RNAseq data from PTC samples from a TCGA cohort were analyzed. PTC samples were divided based on EMT profile (epithelial, hybrid and mesenchymal), and it was possible to observe an increase in the hybrid signature expression in BRAF-mutated samples (Fig. 6A). The patients in the hybrid state and BRAF mutated were 84%, against 75% and 36% of epithelial and mesenchymal states, respectively (Fig. 6B). Interestingly, in response to NRAS/BRAF activation, EMT transcription factors alter their co-regulation to support TWIST1 and ZEB1, collaborating with BRAF and contributing to dedifferentiation and neoplastic transformation of melanocytes (CARAMEL *et al.*, 2013). Additionally, there are also suggestions that, at the tumor front, TGFβ could exhibit synergistic effects with $BRAF^{V600E}$, stimulating EMT (ELOY *et al.*, 2012). Intriguingly, both *ZEB1* and *NT5E* expressions are increased in the hybrid state, which encourages the hypothesis of the transition state being of interest for further study on the *ZEB1* and *NT5E* association.

Unfortunately, several limitations can be pointed out in our study. First of all, it would be ideal to have a normal thyroid cell line for comparison, enabling us to discuss the differences between the normal cell and the tumor cell lines when it comes to ZEB1 and CD73 expressions. Secondly, it would enrich our study if other EMT markers were analyzed. E-cadherin, for example, would give us an informative image to trace the opposite phenotype of ZEB1. Other markers would also be interesting, because we could quantify different co-expressions to make a parallel with cell variables such as speed and polarity index, as well as associations between them. Another subject that would be interesting to be addressed is the participation or influence of other players of the adenosinergic pathway, using inhibitors of CD73, as APCP, or introducing ways to evaluate adenosinergic receptors.

Despite the appointed limitations, we were able to shed light over potential links between CD73 and ZEB1, using cellular parameters as polarity and speed to better evaluate their interrelation. We showed that CD73 impacts ZEB1 expression negatively in PTC, as well as has negative repercussions on cell morphology and migration in vitro. Furthermore, we were also able to distinguish the hybrid EMT transition state as a relevant condition to BRAF mutation presence and ZEB1 and CD73 expressions. Altogether, we were able to superficially touch the dark matter of EMT-adenosinergic signaling relationship, contributing to further research with the aim to advance cancer therapy in the near future.

# PERSPECTIVES

- To add other EMT markers to the analysis, contemplating epithelial and mesenchymal phenotypes;
- To study how different players of the adenosinergic pathway would affect ZEB1 expression.

# CONCLUSIONS

Understanding the interdependence of the adenosinergic system and the EMT is a valuable asset in comprehending cancer progression and therapeutic response. The potential to utilize this knowledge in clinical settings is of significant importance as it encompasses a wide range of neoplastic conditions. In this study, we conducted a comprehensive analysis of the interplay between these two biological processes and their impact on the progression of metastasis and tumor invasion. The influence of CD73 on the post-transcriptional regulation of ZEB1 via 3'UTR in cell lines derived from PTC presented an insightful observation for future exploration of the EMT-Adenosinergic signaling relationship. The correlation differences between cell polarity index, cell migration speed and ZEB1 expression in CD73 negative cells and WT cells contributed to the comprehension of possible mechanisms associating EMT and the adenosinergic pathway. Our emphasis on the potential interplay between CD73 and ZEB1 may contribute to a better understanding of the contribution of these processes to papillary thyroid carcinoma progression. The overview presented herein is anticipated to make a valuable contribution to the formulation of strategies that are geared towards enhancing the management of metastasis risk and increasing the overall survival rate.

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5. 1. STEFANI, GIUSEPPE POTRICK; CAPALONGA, LUCAS; DA SILVA, LUCAS RIBEIRO; HECK, THIAGO GOMES; FRIZZO, MATIAS NUNES; SULZBACHER, LUCAS MACHADO; SULZBACHER, MAICON MACHADO; DE BATISTA, DIOVANA; VEDOVATTO, SAMLAI; BERTONI, ANA PAULA SANTIN; WINK, MÁRCIA ROSÂNGELA; DAL LAGO, PEDRO Effects of aerobic and

resistance exercise training associated with carnosine precursor supplementation on maximal strength and $O^2$max in rats with heart failure. LIFE SCIENCES. v.282, p.119816 -, 2021.

5. 2. VEDOVATTO, SAMLAI; FACCHINI, JORDANO C.; BATISTA, RAQUEL K.; PAIM, THAÍS C.; LIONZO, MARIA ISMENIA Z.; WINK, MÁRCIA R. Development of chitosan, gelatin and liposome film and analysis of its biocompatibility in vitro. INTERNATIONAL JOURNAL OF BIOLOGICAL MACROMOLECULES. v.160, p.750 - 757, 2020.

5. 3. SOUS NAASANI, LILIANA IVET; DAMO SOUZA, ALINE FRANCIELLE; RODRIGUES, CRISTIANO; VEDOVATTO, SAMLAI; AZEVEDO, JÉSSICA GONÇALVES; SANTIN BERTONI, ANA PAULA; DA CRUZ FERNANDES, MARILDA; BUCHNER, SILVIO; WINK, MÁRCIA ROSÂNGELA. Decellularized human amniotic membrane associated with adipose derived mesenchymal stromal cells as a bioscaffold: Physical, histological and molecular analysis. BIOCHEMICAL ENGINEERING JOURNAL. v.152, p.107366 -, 2019.

5.4. ISER, ISABELE CRISTIANA; VEDOVATO, SAMLAI; DITTRICH OLIVEIRA, FERNANDA; BECKENKAMP, LIZIANE RAQUEL; LENZ, GUIDO; WINK, MÁRCIA R. The crossroads of adenosinergic pathway and epithelial-mesenchymal plasticity in cancer. SEMINARS IN CANCER BIOLOGY. v.86(Pt 2):202-213, 2022.

## 6. RESUMOS E TRABALHOS APRESENTADOS EM CONGRESSO:

6. 1. VEDOVATTO, S.; PAIM, T. C.; BATISTA, R. K.; LIONZO, M. I. Z.; WINK, M. R. IN VITRO EVALUATION OF CHITOSAN AND COLLAGEN COMPOSITE MATRIX ASSOCIATED WITH EPIDERMAL GROWTH FACTOR, 2019.

6. 2. VEDOVATTO, S.; BERTONI, A. P. S.; SOUZA, A. F. D.; WINK, M. R. Influence of amniotic membrane scaffold on the expression of adhesion molecules in stromal stem cells, 2018.

6. 3. VEDOVATTO, S.; SOUZA, A. F. D.; WINK, M. R.; BERTONI, A. P. S. Evaluation of amniotic membrane potential as scaffold for adipose-derived stem cells, 2017. (Outra,Apresentação de Trabalho) 4. VEDOVATTO, S.; BERTONI, A. P. S.; PREZZI, F.; WINK, M. R. Análise da Atividade das Ecto-Nucleotídeo Pirofosfatase-Fosfodiesterase (E-Npps) em Gliomas, 2016.

# REFERENCES


ABOELNAGA, Engy M.; AHMED, Rehab Allah. Difference between papillary and follicular thyroid carcinoma outcomes: an experience from Egyptian institution. **Cancer biology & medicine**, [*s. l.*], v. 12, n. 1, p. 53–59, 2015.

AIGNER, K. *et al.* The transcription factor ZEB1 (deltaEF1) promotes tumour cell dedifferentiation by repressing master regulators of epithelial polarity. **Oncogene**, [*s. l.*], v. 26, n. 49, p. 6979–6988, 2007.

AL-BRAHIM, Nabeel; ASA, Sylvia L. Papillary thyroid carcinoma: an overview. **Archives of pathology & laboratory medicine**, [*s. l.*], v. 130, n. 7, p. 1057–1062, 2006.

ALI, Syed Z.; CIBAS, Edmund S. **The Bethesda System for Reporting Thyroid Cytopathology: Definitions, Criteria, and Explanatory Notes**. [*S. l.*]: Springer, 2017.

ALLARD, Bertrand *et al.* Anti-CD73 therapy impairs tumor angiogenesis. **International journal of cancer. Journal international du cancer**, [*s. l.*], v. 134, n. 6, p. 1466–1473, 2014.

ALLARD, David *et al.* CD73-adenosine: a next-generation target in immuno-oncology. **Immunotherapy**, [*s. l.*], v. 8, n. 2, p. 145–163, 2016.

ALLARD, Bertrand *et al.* The adenosine pathway in immuno-oncology. **Nature reviews. Clinical oncology**, [*s. l.*], v. 17, n. 10, p. 611–629, 2020.

AL-RASHIDA, Mariya *et al.* Ectonucleotidase inhibitors: a patent review (2011-2016). **Expert opinion on therapeutic patents**, [*s. l.*], v. 27, n. 12, p. 1291–1304, 2017.

ALZAMELY, Khalid Odamizil *et al.* Combined inhibition of CD73 and ZEB1 by Arg-Gly-Asp (RGD)-targeted nanoparticles inhibits tumor growth. **Colloids and surfaces. B, Biointerfaces**, [*s. l.*], v. 197, p. 111421, 2021.

ANTONIOLI, Luca *et al.* CD39 and CD73 in immunity and inflammation. **Trends in molecular medicine**, [*s. l.*], v. 19, n. 6, p. 355–367, 2013.

BAE, Gab-Yong *et al.* Loss of E-cadherin activates EGFR-MEK/ERK signaling, which promotes invasion via the ZEB1/MMP2 axis in non-small cell lung cancer. **Oncotarget**, [*s. l.*], v. 4, n. 12, p. 2512–2522, 2013.

BARNES, Leon. **Surgical Pathology of the Head and Neck**. [*S. l.*]: CRC Press, 2019.

BEAVIS, Paul A. *et al.* Targeting the adenosine 2A receptor enhances chimeric antigen receptor T cell efficacy. **The Journal of clinical investigation**, [*s. l.*], v. 127, n. 3, p. 929–941, 2017.

BERGDORF, Kensey *et al.* Papillary thyroid carcinoma behavior: clues in the tumor microenvironment. **Endocrine-related cancer**, [*s. l.*], v. 26, n. 6, p. 601–614, 2019.

BERTONI, Ana Paula Santin *et al.* Activity of ecto-5'-nucleotidase (NT5E/CD73) is

increased in papillary thyroid carcinoma and its expression is associated with metastatic lymph nodes. **Molecular and cellular endocrinology**, [*s. l.*], v. 479, p. 54–60, 2019.

BERTONI, Ana Paula Santin *et al.* Extracellular ATP is Differentially Metabolized on Papillary Thyroid Carcinoma Cells Surface in Comparison to Normal Cells. **Cancer microenvironment: official journal of the International Cancer Microenvironment Society**, [*s. l.*], v. 11, n. 1, p. 61–70, 2018.

BOISON, Detlev; YEGUTKIN, Gennady G. Adenosine Metabolism: Emerging Concepts for Cancer Therapy. **Cancer cell**, [*s. l.*], v. 36, n. 6, p. 582–596, 2019.

BONO, María Rosa *et al.* CD73 and CD39 ectonucleotidases in T cell differentiation: Beyond immunosuppression. **FEBS letters**, [*s. l.*], v. 589, n. 22, p. 3454–3460, 2015.

BOREA, Pier Andrea *et al.* Pharmacology of Adenosine Receptors: The State of the Art. **Physiological reviews**, [*s. l.*], v. 98, n. 3, p. 1591–1625, 2018.

BOWSER, Jessica L. *et al.* Loss of CD73-mediated actin polymerization promotes endometrial tumor progression. **The Journal of clinical investigation**, [*s. l.*], v. 126, n. 1, p. 220–238, 2016.

BRABLETZ, Thomas *et al.* EMT in cancer. **Nature reviews. Cancer**, [*s. l.*], v. 18, n. 2, p. 128–134, 2018.

BRAUN, J. *et al.* Downregulation of microRNAs directs the EMT and invasive potential of anaplastic thyroid carcinomas. **Oncogene**, [*s. l.*], v. 29, n. 29, p. 4237–4244, 2010.

BUISSERET, L. *et al.* Clinical significance of CD73 in triple-negative breast cancer: multiplex analysis of a phase III clinical trial. **Annals of oncology: official journal of the European Society for Medical Oncology / ESMO**, [*s. l.*], v. 29, n. 4, p. 1056–1062, 2018.

BURGHOFF, Sandra *et al.* Growth and metastasis of B16-F10 melanoma cells is not critically dependent on host CD73 expression in mice. **BMC cancer**, [*s. l.*], v. 14, p. 898, 2014.

CAMPOS-CONTRERAS, Anaí Del Rocío; DÍAZ-MUÑOZ, Mauricio; VÁZQUEZ-CUEVAS, Francisco G. Purinergic Signaling in the Hallmarks of Cancer. **Cells** , [*s. l.*], v. 9, n. 7, 2020. Disponível em: http://dx.doi.org/10.3390/cells9071612.

CAO, Lu *et al.* Prognostic Role of Immune Checkpoint Regulators in Cholangiocarcinoma: A Pilot Study. **Journal of clinical medicine research**, [*s. l.*], v. 10, n. 10, 2021. Disponível em: http://dx.doi.org/10.3390/jcm10102191.

CARAMEL, Julie *et al.* A switch in the expression of embryonic EMT-inducers drives the development of malignant melanoma. **Cancer cell**, [*s. l.*], v. 24, n. 4, p. 466–480, 2013.

CEKIC, Caglar *et al.* Adenosine A2B receptor blockade slows growth of bladder and breast tumors. **Journal of immunology** , [*s. l.*], v. 188, n. 1, p. 198–205, 2012.

CHAN, Wendy W. L.; KWONG, Dora L. W. Radioactive Iodine for Papillary Thyroid Carcinoma. **Methods in molecular biology** , [*s. l.*], v. 2534, p. 225–241, 2022.

CHEN, Xin; PLEBANSKI, Magdalena. **The Role of TNF-TNFR2 Signal in**

**Immunosuppressive Cells and its Therapeutic Implications**. [*S. l.*]: Frontiers Media SA, 2020.

CHUA, H. L. *et al.* NF-kappaB represses E-cadherin expression and enhances epithelial to mesenchymal transition of mammary epithelial cells: potential involvement of ZEB-1 and ZEB-2. **Oncogene**, [*s. l.*], v. 26, n. 5, p. 711–724, 2007.

DE LOURDES MORA-GARCÍA, María *et al.* HPV-16 Infection Is Associated with a High Content of CD39 and CD73 Ectonucleotidases in Cervical Samples from Patients with CIN-1. **Mediators of inflammation**, [*s. l.*], v. 2019, p. 4651627, 2019.

DIAS LOPES, Natália Medeiros *et al.* Thyroid cancer and thyroid autoimmune disease: A review of molecular aspects and clinical outcomes. **Pathology, research and practice**, [*s. l.*], v. 216, n. 9, p. 153098, 2020.

DONGRE, Anushka; WEINBERG, Robert A. New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. **Nature reviews. Molecular cell biology**, [*s. l.*], v. 20, n. 2, p. 69–84, 2019.

ELOY, C. *et al.* TGF-beta/Smad pathway and BRAF mutation play different roles in circumscribed and infiltrative papillary thyroid carcinoma. **Virchows Archiv: an international journal of pathology**, [*s. l.*], v. 460, n. 6, p. 587–600, 2012.

FISHMAN, Pnina *et al.* An agonist to the A3 adenosine receptor inhibits colon carcinoma growth in mice via modulation of GSK-3 beta and NF-kappa B. **Oncogene**, [*s. l.*], v. 23, n. 14, p. 2465–2471, 2004.

FLAMENT. Crosstalk between adenosine receptor (A2A isoform) and ERα mediates ethanol action in MCF-7 breast cancer cells. **Oncology reports**, [*s. l.*], v. 21, n. 4, 2009. Disponível em: http://www.spandidos-publications.com/or/21/4/977.

GANDALOVIČOVÁ, Aneta *et al.* Cell polarity signaling in the plasticity of cancer cell invasiveness. **Oncotarget**, [*s. l.*], v. 7, n. 18, p. 25022–25049, 2016.

GAO, Z. W. *et al.* Adenosine inhibits migration, invasion and induces apoptosis of human cervical cancer cells. **Neoplasma**, [*s. l.*], v. 63, n. 2, p. 201–207, 2016.

GARCÍA-ROCHA, Rosario *et al.* Cervical cancer cells produce TGF-β1 through the CD73-adenosine pathway and maintain CD73 expression through the autocrine activity of TGF-β1. **Cytokine**, [*s. l.*], v. 118, p. 71–79, 2019.

GESSI, Stefania *et al.* Adenosine receptors and cancer. **Biochimica et biophysica acta**, [*s. l.*], v. 1808, n. 5, p. 1400–1412, 2011.

GESSI, Stefania *et al.* Elevated expression of A3 adenosine receptors in human colorectal cancer is reflected in peripheral blood cells. **Clinical cancer research: an official journal of the American Association for Cancer Research**, [*s. l.*], v. 10, n. 17, p. 5895–5901, 2004.

GHARIB, Hossein *et al.* American Association of Clinical Endocrinologists, American College of Endocrinology, and Associazione Medici Endocrinologi medical guidelines for clinical practice for the diagnosis and management of thyroid nodules--2016 update. **Endocrine practice: official journal of the American College of Endocrinology and**

**the American Association of Clinical Endocrinologists**, [*s. l.*], v. 22, n. 5, p. 622–639, 2016.

GORAIN, Bapi *et al.* Adenosine Receptors as Novel Targets for the Treatment of Various Cancers. **Current pharmaceutical design**, [*s. l.*], v. 25, n. 26, p. 2828–2841, 2019.

GREENBURG, G.; HAY, E. D. Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. **The Journal of cell biology**, [*s. l.*], v. 95, n. 1, p. 333–339, 1982.

GREGORY, Philip A. *et al.* An autocrine TGF-beta/ZEB/miR-200 signaling network regulates establishment and maintenance of epithelial-mesenchymal transition. **Molecular biology of the cell**, [*s. l.*], v. 22, n. 10, p. 1686–1698, 2011.

GREGORY, Philip A. *et al.* The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. **Nature cell biology**, [*s. l.*], v. 10, n. 5, p. 593–601, 2008.

GUENTHER, Matthew G. *et al.* A chromatin landmark and transcription initiation at most promoters in human cells. **Cell**, [*s. l.*], v. 130, n. 1, p. 77–88, 2007.

HAUGEN, Bryan R. *et al.* 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. **Thyroid: official journal of the American Thyroid Association**, [*s. l.*], v. 26, n. 1, p. 1–133, 2016.

HÄUSLER, Sebastian F. M. *et al.* Ectonucleotidases CD39 and CD73 on OvCA cells are potent adenosine-generating enzymes responsible for adenosine receptor 2A-dependent suppression of T cell function and NK cell cytotoxicity. **Cancer immunology, immunotherapy: CII**, [*s. l.*], v. 60, n. 10, p. 1405–1418, 2011.

HUMPHRIES, Brock *et al.* MicroRNA-200b targets protein kinase Cα and suppresses triple-negative breast cancer metastasis. **Carcinogenesis**, [*s. l.*], v. 35, n. 10, p. 2254–2263, 2014.

HUMPHRIES, Brock; YANG, Chengfeng. The microRNA-200 family: small molecules with novel roles in cancer development, progression and therapy. **Oncotarget**, [*s. l.*], v. 6, n. 9, p. 6472–6498, 2015.

ISER, Isabele Cristiana *et al.* The crossroads of adenosinergic pathway and epithelial-mesenchymal plasticity in cancer. **Seminars in cancer biology**, [*s. l.*], v. 86, n. Pt 2, p. 202–213, 2022.

JARZAB, Barbara; HANDKIEWICZ-JUNAK, Daria. Differentiated thyroid cancer in children and adults: same or distinct disease?. **Hormones** , [*s. l.*], v. 6, n. 3, p. 200–209, 2007.

JEONG, Young Mun *et al.* CD73 Overexpression Promotes Progression and Recurrence of Papillary Thyroid Carcinoma. **Cancers**, [*s. l.*], v. 12, n. 10, 2020. Disponível em: http://dx.doi.org/10.3390/cancers12103042.

KALLURI, Raghu. EMT: when epithelial cells decide to become mesenchymal-like cells. **The Journal of clinical investigation**, [*s. l.*], v. 119, n. 6, p. 1417–1419, 2009.

KASAMA, Hiroki *et al.* Adenosine A2b receptor promotes progression of human oral cancer. **BMC cancer**, [*s. l.*], v. 15, p. 563, 2015.

KHAN, Abdul Aziz; LEE, Andrew Jeoungun; ROH, Tae-Young. Polycomb group protein-mediated histone modifications during cell differentiation. **Epigenomics**, [*s. l.*], v. 7, n. 1, p. 75–84, 2015.

KONDO, T. *et al.* Expression of CD73 and its ecto-5'-nucleotidase activity are elevated in papillary thyroid carcinomas. **Histopathology**, [*s. l.*], v. 48, n. 5, p. 612–614, 2006.

KORDAS, Theresa; OSEN, Wolfram; EICHMÜLLER, Stefan B. Controlling the Immune Suppressor: Transcription Factors and MicroRNAs Regulating CD73/NT5E. **Frontiers in immunology**, [*s. l.*], v. 9, p. 813, 2018.

KORPAL, Manav *et al.* Direct targeting of Sec23a by miR-200s influences cancer cell secretome and promotes metastatic colonization. **Nature medicine**, [*s. l.*], v. 17, n. 9, p. 1101–1108, 2011.

KORPAL, Manav *et al.* The miR-200 family inhibits epithelial-mesenchymal transition and cancer cell migration by direct targeting of E-cadherin transcriptional repressors ZEB1 and ZEB2. **The Journal of biological chemistry**, [*s. l.*], v. 283, n. 22, p. 14910–14914, 2008.

KREBS, Angela M. *et al.* The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. **Nature cell biology**, [*s. l.*], v. 19, n. 5, p. 518–529, 2017.

KURNIT, Katherine C. *et al.* Loss of CD73 shifts transforming growth factor-β1 (TGF-β1) from tumor suppressor to promoter in endometrial cancer. **Cancer letters**, [*s. l.*], v. 505, p. 75–86, 2021.

LAM, Alfred K. Histopathological Assessment for Papillary Thyroid Carcinoma. **Methods in molecular biology** , [*s. l.*], v. 2534, p. 93–108, 2022.

LECLERC, Bruno G. *et al.* CD73 Expression Is an Independent Prognostic Factor in Prostate Cancer. **Clinical cancer research: an official journal of the American Association for Cancer Research**, [*s. l.*], v. 22, n. 1, p. 158–166, 2016.

LENZ, Luana S. *et al.* Cancer Cell Fitness Is Dynamic. **Cancer research**, [*s. l.*], v. 81, n. 4, p. 1040–1051, 2021.

LEONE, Robert D.; EMENS, Leisha A. Targeting adenosine for cancer immunotherapy. **Journal for immunotherapy of cancer**, [*s. l.*], v. 6, n. 1, p. 57, 2018.

LI, Jieyao *et al.* CD39/CD73 upregulation on myeloid-derived suppressor cells via TGF-β-mTOR-HIF-1 signaling in patients with non-small cell lung cancer. **Oncoimmunology**, [*s. l.*], v. 6, n. 6, p. e1320011, 2017.

LI, Lifeng *et al.* Metformin-Induced Reduction of CD39 and CD73 Blocks Myeloid-Derived Suppressor Cell Activity in Patients with Ovarian Cancer. **Cancer research**, [*s. l.*], v. 78, n. 7, p. 1779–1791, 2018.

LIN, Z. *et al.* Adenosine A1 receptor, a target and regulator of estrogen receptoralpha action, mediates the proliferative effects of estradiol in breast cancer. **Oncogene**, [*s. l.*], v.

29, n. 8, p. 1114–1122, 2010.

LIVOLSI, Virginia A. Papillary thyroid carcinoma: an update. **Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc**, [*s. l.*], v. 24 Suppl 2, p. S1–S9, 2011.

LOPEZ-CAMPISTROUS, Ana *et al.* Gene expression profile of epithelial-mesenchymal transition mediators in papillary thyroid cancer. **Endocrine**, [*s. l.*], v. 72, n. 2, p. 452–461, 2021.

LUPIA, Michela *et al.* CD73 Regulates Stemness and Epithelial-Mesenchymal Transition in Ovarian Cancer-Initiating Cells. **Stem cell reports**, [*s. l.*], v. 10, n. 4, p. 1412–1425, 2018.

MA, Xiao-Lu *et al.* CD73 promotes hepatocellular carcinoma progression and metastasis via activating PI3K/AKT signaling by inducing Rap1-mediated membrane localization of P110β and predicts poor prognosis. **Journal of hematology & oncology**, [*s. l.*], v. 12, n. 1, p. 37, 2019.

MAO, Jingxin *et al.* Risk Factors for Lymph Node Metastasis in Papillary Thyroid Carcinoma: A Systematic Review and Meta-Analysis. **Frontiers in endocrinology**, [*s. l.*], v. 11, p. 265, 2020.

MARTÍNEZ-RAMÍREZ, A. S. *et al.* Cellular Migration Ability Is Modulated by Extracellular Purines in Ovarian Carcinoma SKOV-3 Cells. **Journal of cellular biochemistry**, [*s. l.*], v. 118, n. 12, p. 4468–4478, 2017.

MERIGHI, Stefania *et al.* Adenosine receptors as mediators of both cell proliferation and cell death of cultured human melanoma cells. **The Journal of investigative dermatology**, [*s. l.*], v. 119, n. 4, p. 923–933, 2002.

MIRZA, Asra *et al.* RNA interference targeting of A1 receptor-overexpressing breast carcinoma cells leads to diminished rates of cell proliferation and induction of apoptosis. **Cancer biology & therapy**, [*s. l.*], v. 4, n. 12, p. 1355–1360, 2005.

MONTEIRO, Inês *et al.* CD73 expression in normal, hyperplastic, and neoplastic thyroid: a systematic evaluation revealing CD73 overexpression as a feature of papillary carcinomas. **Virchows Archiv: an international journal of pathology**, [*s. l.*], v. 479, n. 1, p. 209–214, 2021.

MOUSAVI, Samira *et al.* Expression of adenosine receptor subclasses in malignant and adjacent normal human prostate tissues. **The Prostate**, [*s. l.*], v. 75, n. 7, p. 735–747, 2015.

MUELLER, Dorothee *et al.* A role for the MLL fusion partner ENL in transcriptional elongation and chromatin modification. **Blood**, [*s. l.*], v. 110, n. 13, p. 4445–4454, 2007.

MUÑÓZ-GODÍNEZ, Ricardo *et al.* Detection of CD39 and a Highly Glycosylated Isoform of Soluble CD73 in the Plasma of Patients with Cervical Cancer: Correlation with Disease Progression. **Mediators of inflammation**, [*s. l.*], v. 2020, p. 1678780, 2020.

NELSON, W. James. Remodeling epithelial cell organization: transitions between front-rear and apical-basal polarity. **Cold Spring Harbor perspectives in biology**, [*s. l.*], v. 1, n. 1, p. a000513, 2009.

NGUYEN, Anna M. *et al.* Upregulation of CD73 Confers Acquired Radioresistance and is Required for Maintaining Irradiation-selected Pancreatic Cancer Cells in a Mesenchymal State. **Molecular & cellular proteomics: MCP**, [*s. l.*], v. 19, n. 2, p. 375–389, 2020.

NIETO, M. A. *et al.* Control of cell behavior during vertebrate development by Slug, a zinc finger gene. **Science**, [*s. l.*], v. 264, n. 5160, p. 835–839, 1994.

NTANTIE, Elizabeth *et al.* An adenosine-mediated signaling pathway suppresses prenylation of the GTPase Rap1B and promotes cell scattering. **Science signaling**, [*s. l.*], v. 6, n. 277, p. ra39, 2013.

PARK, Lee Chun *et al.* Immunologic and clinical implications of CD73 expression in non-small cell lung cancer (NSCLC). **Journal of clinical oncology: official journal of the American Society of Clinical Oncology**, [*s. l.*], v. 36, n. 15_suppl, p. 12050–12050, 2018.

PARK, Sun-Mi *et al.* The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. **Genes & development**, [*s. l.*], v. 22, n. 7, p. 894–907, 2008.

PASTUSHENKO, Ievgenia *et al.* Identification of the tumour transition states occurring during EMT. **Nature**, [*s. l.*], v. 556, n. 7702, p. 463–468, 2018.

PASTUSHENKO, Ievgenia; BLANPAIN, Cédric. EMT Transition States during Tumor Progression and Metastasis. **Trends in cell biology**, [*s. l.*], v. 29, n. 3, p. 212–226, 2019.

PEREZ-OQUENDO, Mabel; GIBBONS, Don L. Regulation of ZEB1 Function and Molecular Associations in Tumor Progression and Metastasis. **Cancers**, [*s. l.*], v. 14, n. 8, 2022. Disponível em: http://dx.doi.org/10.3390/cancers14081864.

PETRUK, Nataliia *et al.* CD73 facilitates EMT progression and promotes lung metastases in triple-negative breast cancer. **Scientific reports**, [*s. l.*], v. 11, n. 1, p. 6035, 2021.

PIETROBONO, Deborah *et al.* High Adenosine Extracellular Levels Induce Glioblastoma Aggressive Traits Modulating the Mesenchymal Stromal Cell Secretome. **International journal of molecular sciences**, [*s. l.*], v. 21, n. 20, 2020. Disponível em: http://dx.doi.org/10.3390/ijms21207706.

PUISIEUX, Alain; BRABLETZ, Thomas; CARAMEL, Julie. Oncogenic roles of EMT-inducing transcription factors. **Nature cell biology**, [*s. l.*], v. 16, n. 6, p. 488–494, 2014.

REN, Zhen-Hu *et al.* CD73 is associated with poor prognosis in HNSCC. **Oncotarget**, [*s. l.*], v. 7, n. 38, p. 61690–61702, 2016a.

REN, Zhen-Hu *et al.* CD73 is associated with poor prognosis in HNSCC. **Oncotarget**, [*s. l.*], v. 7, n. 38, p. 61690–61702, 2016b.

RICHARD, Geoffrey *et al.* ZEB1-mediated melanoma cell plasticity enhances resistance to MAPK inhibitors. **EMBO molecular medicine**, [*s. l.*], v. 8, n. 10, p. 1143–1161, 2016.

ROYER, C.; LU, X. Epithelial cell polarity: a major gatekeeper against cancer?. **Cell death and differentiation**, [*s. l.*], v. 18, n. 9, p. 1470–1477, 2011.

ROZEN-ZVI, Benaya *et al.* TGF-β/Smad3 activates mammalian target of rapamycin complex-1 to promote collagen production by increasing HIF-1α expression. **American journal of physiology. Renal physiology**, [*s. l.*], v. 305, n. 4, p. F485–F494, 2013.

SADEJ, Rafal *et al.* Tenascin C interacts with ecto-5'-nucleotidase (eN) and regulates adenosine generation in cancer cells. **Biochimica et biophysica acta**, [*s. l.*], v. 1782, n. 1, p. 35–40, 2008.

SADEJ, R.; SPYCHALA, J.; SKLADANOWSKI, A. C. Ecto-5'-nucleotidase (eN, CD73) is coexpressed with metastasis promoting antigens in human melanoma cells. **Nucleosides, nucleotides & nucleic acids**, [*s. l.*], v. 25, n. 9-11, p. 1119–1123, 2006.

SCHLUMBERGER, M.; BAUDIN, E.; TRAVAGLI, J. P. [Papillary and follicular cancers of the thyroid]. **Presse medicale** , [*s. l.*], v. 27, n. 29, p. 1479–1481, 1998.

SCIARRA, Amedeo *et al.* CD73 expression in normal and pathological human hepatobiliopancreatic tissues. **Cancer immunology, immunotherapy: CII**, [*s. l.*], v. 68, n. 3, p. 467–478, 2019.

SHAKIB, Heewa *et al.* Epithelial-to-mesenchymal transition in thyroid cancer: a comprehensive review. **Endocrine**, [*s. l.*], v. 66, n. 3, p. 435–455, 2019.

SHRESTHA, Ritu *et al.* Monitoring Immune Checkpoint Regulators as Predictive Biomarkers in Hepatocellular Carcinoma. **Frontiers in oncology**, [*s. l.*], v. 8, p. 269, 2018.

SITKOVSKY, Michail V. *et al.* Hostile, hypoxia-A2-adenosinergic tumor biology as the next barrier to overcome for tumor immunologists. **Cancer immunology research**, [*s. l.*], v. 2, n. 7, p. 598–605, 2014.

SMITA, Shuchi *et al.* Importance of EMT Factor ZEB1 in cDC1 "MutuDC Line" Mediated Induction of Th1 Immune Response. **Frontiers in immunology**, [*s. l.*], v. 9, p. 2604, 2018.

SPADERNA, Simone *et al.* The transcriptional repressor ZEB1 promotes metastasis and loss of cell polarity in cancer. **Cancer research**, [*s. l.*], v. 68, n. 2, p. 537–544, 2008.

STAGG, John *et al.* Anti-CD73 antibody therapy inhibits breast tumor growth and metastasis. **Proceedings of the National Academy of Sciences of the United States of America**, [*s. l.*], v. 107, n. 4, p. 1547–1552, 2010.

STEFANIA, De Domenico; VERGARA, Daniele. The Many-Faced Program of Epithelial-Mesenchymal Transition: A System Biology-Based View. **Frontiers in oncology**, [*s. l.*], v. 7, p. 274, 2017.

SYNNESTVEDT, Kristin *et al.* Ecto-5'-nucleotidase (CD73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal epithelia. **The Journal of clinical investigation**, [*s. l.*], v. 110, n. 7, p. 993–1002, 2002.

TIAN, Yin *et al.* MicroRNA-200 (miR-200) cluster regulation by achaete scute-like 2 (Ascl2): impact on the epithelial-mesenchymal transition in colon cancer cells. **The Journal of biological chemistry**, [*s. l.*], v. 289, n. 52, p. 36101–36115, 2014.

TSIAMPALI, Julia *et al.* Enzymatic Activity of CD73 Modulates Invasion of Gliomas via Epithelial-Mesenchymal Transition-Like Reprogramming. **Pharmaceuticals** , [*s. l.*], v. 13,

n. 11, 2020. Disponível em: http://dx.doi.org/10.3390/ph13110378.

TURCOTTE, Martin *et al.* CD73 is associated with poor prognosis in high-grade serous ovarian cancer. **Cancer research**, [*s. l.*], v. 75, n. 21, p. 4494–4503, 2015.

ULISSE, Salvatore *et al.* Papillary Thyroid Cancer Prognosis: An Evolving Field. **Cancers**, [*s. l.*], v. 13, n. 21, 2021. Disponível em: http://dx.doi.org/10.3390/cancers13215567.

VAN CALKER, Dietrich *et al.* The role of adenosine receptors in mood and anxiety disorders. **Journal of neurochemistry**, [*s. l.*], v. 151, n. 1, p. 11–27, 2019.

VIJAYAN, Dipti *et al.* Targeting immunosuppressive adenosine in cancer. **Nature reviews. Cancer**, [*s. l.*], v. 17, n. 12, p. 709–724, 2017.

VOGT, Timo J. *et al.* Detailed analysis of adenosine A2a receptor () and CD73 (5'-nucleotidase, ecto, ) methylation and gene expression in head and neck squamous cell carcinoma patients. **Oncoimmunology**, [*s. l.*], v. 7, n. 8, p. e1452579, 2018.

WANG, Li *et al.* Ecto-5'-nucleotidase (CD73) promotes tumor angiogenesis. **Clinical & experimental metastasis**, [*s. l.*], v. 30, n. 5, p. 671–680, 2013.

WANG, Li *et al.* Ecto-5'-nucleotidase promotes invasion, migration and adhesion of human breast cancer cells. **Journal of cancer research and clinical oncology**, [*s. l.*], v. 134, n. 3, p. 365–372, 2008.

WANG, Zhiyi *et al.* Novel crosstalk between KLF4 and ZEB1 regulates gemcitabine resistance in pancreatic ductal adenocarcinoma. **International journal of oncology**, [*s. l.*], v. 51, n. 4, p. 1239–1248, 2017.

WANG, H. *et al.* NT5E (CD73) is epigenetically regulated in malignant melanoma and associated with metastatic site specificity. **British journal of cancer**, [*s. l.*], v. 106, n. 8, p. 1446–1452, 2012.

XIONG, Li *et al.* NT5E and FcGBP as key regulators of TGF-1-induced epithelial-mesenchymal transition (EMT) are associated with tumor progression and survival of patients with gallbladder cancer. **Cell and tissue research**, [*s. l.*], v. 355, n. 2, p. 365–374, 2014.

XU, Zhijun *et al.* CD73 promotes tumor metastasis by modulating RICS/RhoA signaling and EMT in gastric cancer. **Cell death & disease**, [*s. l.*], v. 11, n. 3, p. 202, 2020.

YANG, Qi-Fan *et al.* Development and validation of an individualized immune prognostic model in stage I-III lung squamous cell carcinoma. **Scientific reports**, [*s. l.*], v. 11, n. 1, p. 12727, 2021.

YEGUTKIN, Gennady G.; BOISON, Detlev. ATP and adenosine metabolism in cancer: Exploitation for therapeutic gain. **Pharmacological reviews**, [*s. l.*], v. 74, n. 3, p. 797–822, 2022.

YOUNG, Arabella *et al.* Co-inhibition of CD73 and A2AR Adenosine Signaling Improves Anti-tumor Immune Responses. **Cancer cell**, [*s. l.*], v. 30, n. 3, p. 391–403, 2016.

YOUNG, Arabella *et al.* Targeting Adenosine in BRAF-Mutant Melanoma Reduces Tumor

Growth and Metastasis. **Cancer research**, [*s. l.*], v. 77, n. 17, p. 4684–4696, 2017.

YU, Y. I. *et al.* Ecto-5'-nucleotidase expression is associated with the progression of renal cell carcinoma. **Oncology letters**, [*s. l.*], v. 9, n. 6, p. 2485–2494, 2015.

ZHANG, Yu *et al.* The roles of ZEB1 in tumorigenic progression and epigenetic modifications. **Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie**, [*s. l.*], v. 110, p. 400–408, 2019.

ZHOU, Xuerui *et al.* Effects of ecto-5'-nucleotidase on human breast cancer cell growth in vitro and in vivo. **Oncology reports**, [*s. l.*], 2007. Disponível em: http://www.spandidos-publications.com/10.3892/or.17.6.1341.

ZHU, Jianan *et al.* CD39/CD73 Dysregulation of Adenosine Metabolism Increases Decidual Natural Killer Cell Cytotoxicity: Implications in Unexplained Recurrent Spontaneous Abortion. **Frontiers in immunology**, [*s. l.*], v. 13, p. 813218, 2022.