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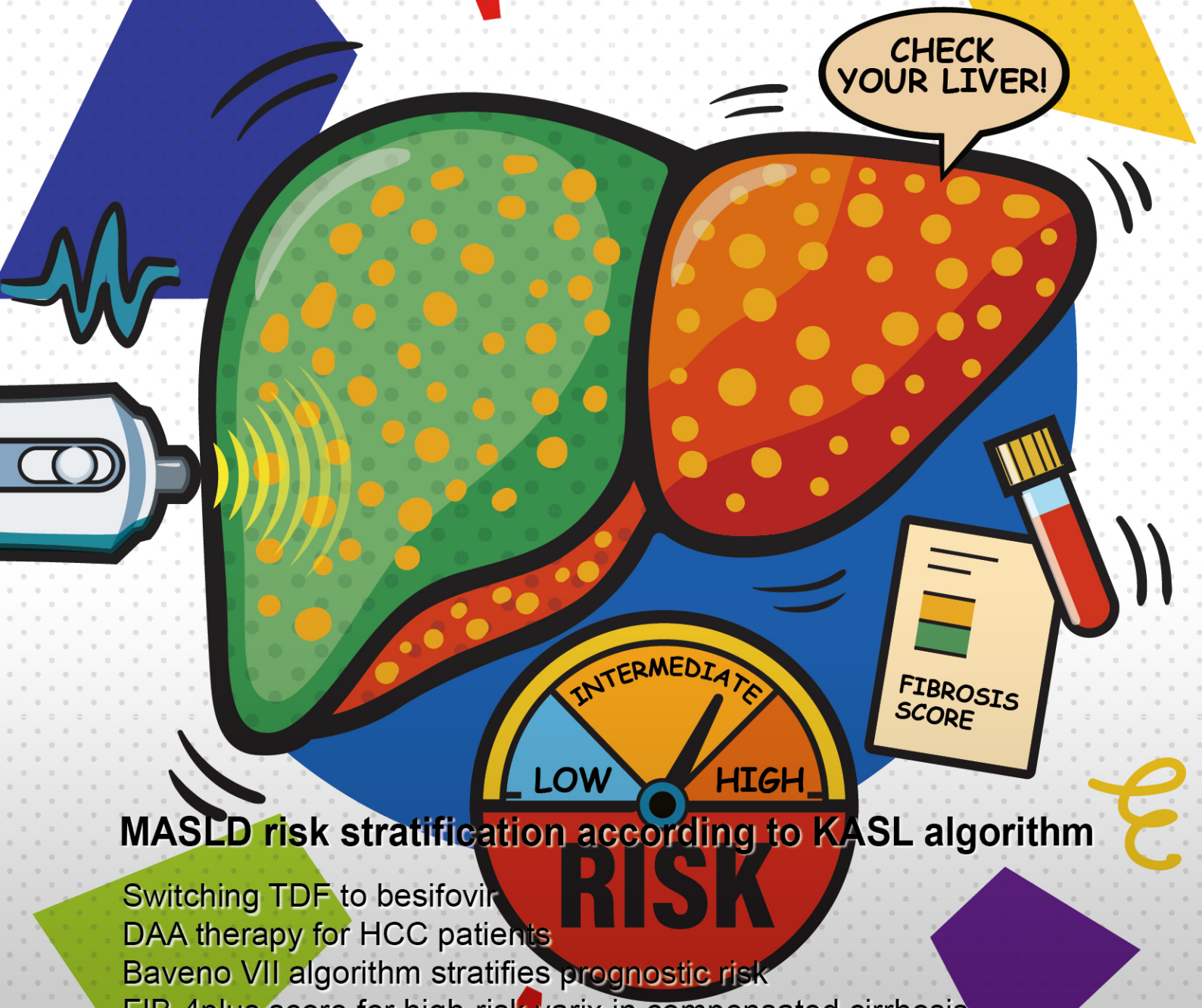

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Review

Deciphering adenosine signaling in hepatocellular carcinoma: Pathways, prognostic models, and therapeutic implications

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Hepatocellular carcinoma (HCC) is a highly lethal cancer due to its aggressive nature and poor prognosis. Adenosine, a key metabolic regulator in the tumor microenvironment (TME), plays a crucial role in cancer progression. In this review, we first described adenosine triphosphate adenosine metabolism in the TME and summarized its effects on tumor growth, immune suppression, angiogenesis, and metastasis in HCC. Given the limited number of clinical studies on adenosine signaling in HCC, we conducted LASSO-Cox analysis using the TCGA-LIHC cohort to develop a prognostic risk model composed of eight adenosine signaling-related genes. This model stratified the patients into low- and high-risk groups, with Kaplan-Meier survival analysis revealing poorer overall survival in the high-risk group. Additionally, differential gene expression analysis between the two groups identified 24 enriched signaling pathways for further investigation. Immune infiltration and single cell RNA-seq analyses revealed a correlation between adenosine and immunosuppressive activity in the TME, with a particularly strong association observed in macrophages, dendritic cells, and monocytes. Finally, we provided an overview of the advancements of antagonists that target adenosine receptors' progress in both preclinical research and clinical trials. In conclusion, this review aims to deepen our understanding of the biological role of adenosine and highlights emerging therapeutic strategies that may improve treatment outcomes for HCC. **(Clin Mol Hepatol 2025;31:706-729)**

Keywords: Hepatocellular carcinoma; Adenosine; Receptors, purinergic; Signal transduction; Prognosis

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most commonly diagnosed cancer and ranks as the fourth leading cause of cancer-related mortality globally, underscoring its aggressive nature and poor prognosis.[1] Approximately 90% of HCC cases arise from cirrhosis, which develops through a complex and multistep process. This progression is driven by the interplay of various etiological factors that initiate the early transformation of hepatocytes and lead to HCC development.[2] Currently, chronic infection with hepatitis B virus or hepatitis C virus remains the predominant cause of HCC, accounting for more than 50% of cases worldwide.[3] Additional risk factors, such as excessive alcohol con-

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sumption and nonalcoholic steatohepatitis (NASH), also play significant roles in the pathogenesis of HCC.[3] Moreover, mutational signature studies have implicated aristolochic acid and tobacco use as additional carcinogenic cofactors in HCC.[4] As reported by the World Health Organization, early-stage HCC diagnosis is associated with a 5-year survival rate exceeding 70%, whereas advanced-stage diagnosis drastically reduces this rate to less than 20%.[5] To improve survival outcomes for patients with advanced-stage HCC, systemic therapies, including targeted agents (e.g., sorafenib or lenvatinib), immunotherapies (e.g., immune checkpoint inhibitors), or a combination of both, are employed.[2] Unfortunately, a considerable proportion of patients experience limited long-term benefits from these treatments, largely due to the development of drug resistance, which continues to undermine therapeutic efficacy and contributes to the high fatality rate of HCC.[6] As a result, HCC remains a major public health challenge, and innovative therapeutic strategies are urgently needed. Research efforts focusing on the tumor microenvironment (TME), oncoimmunology, and cancer metabolism offer potential for discovering new treatment targets and enhancing the efficacy of current therapeutic interventions for advanced HCC.

The pivotal role of the metabolic landscape in HCC is widely acknowledged. Several metabolic molecules within the TME of HCC, such as lactate, glutamine, lipids, and adenosine, have been identified as key contributors to resistance to systemic therapies.[7,8] Notably, adenosine has been reported to exert an immunosuppressive effect in HCC, making it one of the main contributors to the poor efficacy of systemic therapies.[9] It is important to note that adenosine is distributed both intracellularly and extracellularly, where it contributes to various distinct aspects of cellular metabolism and signaling in HCC cells.[10] Intracellular adenosine primarily regulates cellular energy metabolism, thereby maintaining cell growth and supporting cellular functions under stress.[11] In contrast, substantial accumulation of extracellular adenosine (eADO) alters the TME, promoting immune evasion and creating an environment that supports tumor cell survival, proliferation, and migration.[12] In this context, research has preliminarily explored the mechanisms by which eADO regulates tumor cell survival, apoptosis, and its immunosuppressive effects within the TME in HCC.[13,14] Consequently, understanding the pathological mechanisms of adenosine in the TME is considered a critical strategy for therapeutic intervention in HCC, given its pivotal role in modulating the TME and influencing HCC progression.

This review provides a comprehensive overview of the role of the adenosine pathway in HCC, synthesizing insights from the literature and summarizing the processes of adenosine triphosphate (ATP)-adenosine metabolism within the TME of HCC. We discuss the pathological mechanisms by which adenosine influences tumor progression and review preclinical and clinical trials involving adenosine receptor antagonists. Furthermore, we utilize bulk and single-cell RNA sequencing (scRNA-seq) technologies to elucidate the impact of adenosine on HCC patient survival, its effects on immune infiltration, and alterations in the cellular composition and intercellular interactions within the TME. These data provide compelling clinical evidence supporting the significant role of adenosine in HCC progression. Therefore, the aim of this review is to deepen our understanding of the biological mechanisms underlying ATP-adenosine metabolism and the adenosine pathway in HCC, ultimately informing the development of more effective therapeutic strategies for treating HCC.

METHODS

Literature search

The search engines, including PubMed, Web of Science, PLoS, and Google scholar, were used for this review. The

Abbreviations:

ADA, adenosine deaminase; ADK, adenosine kinase; AK, adenylate kinase; AMP, adenosine monophosphate; ATP, adenosine triphosphate; AUC, area under the ROC curve; cAMP, cyclic AMP; CIs, confidence intervals; CNT, concentrative nucleoside transporter; DCs, dendritic cells; DEGs, differentially expressed genes; DPCPX, 1,3-Dipropyl-8-cyclopentylxanthine; eADO, extracellular adenosine; ENT, equilibrative nucleoside transporter; EL2, extracellular loop 2; GEO, Gene Expression Omnibus; GPCR, G protein-coupled receptor family; GSEA, gene set enrichment analysis; HCC, hepatocellular carcinoma; HRs, hazard ratios; ICGC, International Cancer Genome Consortium; IL, interleukin; KM, Kaplan-Meier; MDSCs, myeloid-derived suppressor cells; NASH, nonalcoholic steatohepatitis; NK, natural killer; NMF, nonnegative matrix factorization; OS, overall survival; ROC, receiver operating characteristic; SAH, S-adenosylhomocysteine; SAHH, SAH hydrolase; scRNA-seq, single-cell RNA sequencing; TCGA, The Cancer Genome Atlas; TME, tumor microenvironment; TMs, transmembrane domains; VEGF, vascular endothelial growth factor

keywords used for the search were “liver cancer”, “hepatocellular cancer”, “hepatocellular carcinoma”, “adenosine”, “adenosine metabolism, “adenosine pathway”, “bulk-RNA sequencing”, “clinical trials”, “preclinical study”, “single-cell RNA sequencing”, “adenosine receptors”, “adenosine receptor antagonists” and “prognostic effect”. All relevant literature was reviewed from November 2003 to January 2025.

Identification of adenosine signaling genes

A comprehensive set of adenosine signaling-related genes was generated on the basis of data from the STRING database and relevant literature.[15] A total of 134 adenosine signaling-related genes were included in the analysis in this study. The list of candidate genes is shown in Supplementary Table 1.

Database preprocessing

Transcriptome data and corresponding clinical metadata for HCC (TGCA_LIHC), pancreatic adenocarcinoma (TCGA_PAAD), esophageal cancer (TCGA_ESCA), and cholangiocarcinoma (TCGA_CHOL) patients were downloaded from The Cancer Genome Atlas (TCGA) through the UCSC Xena platform (https://xena.ucsc.edu/) for use as the training set. Two cohorts of HCC patients, including 22 patients from GSE14520 and 260 patients from Japan (ICGC_JP), were selected from the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) and International Cancer Genome Consortium (ICGC, https://dcc.icgc.org/) as the validation sets. Two cohort data of HCC patients treated by PD1 therapy (GSE202069) and targeted therapy (GSE109211) were downloaded from GEO. The Asia PD1-treated HCC dataset (PRJEB34724) was downloaded from European Nucleotide Archive (ENA, https://www.ebi.ac.uk/ena/browser/ home). An scRNA-seq cohort (GSE156625) was downloaded from the GEO database.

Construction and validation of the adenosine signaling signature prognostic risk model

The prognostic model was developed using the TCGA_LIHC cohort with completed survival data. Univariate Cox proportional regression analysis was initially conducted to evaluate the associations between the expression levels of genes in the adenosine signaling pathway and overall survival (OS) in HCC patients. Genes that were significantly associated with OS were selected for further analysis. Subsequently, LASSO-Cox regression analysis was employed to refine the gene signature by reducing the number of variables and generating a risk formula on the basis of a linear combination of gene expression levels and their respective coefficients. Using this risk formula, a risk score was calculated for each patient, and patients were then stratified into low- and high-risk groups according to the median risk score. Subsequently, Kaplan-Meier (KM) survival analysis and receiver operating characteristic (ROC) analysis were conducted to evaluate the survival differences between these groups and to assess the predictive accuracy of the risk score. To validate the robustness of the established risk formula, the independent GEO (GSE14520), ICGC_JP, TCGA_PAAD, TCGA_ESCA, and TCGA_CHOL cohorts were used. In this cohort, patients were similarly divided into low- and high-risk groups on the basis of the median risk score calculated using the gene signature. Similarly, KM survival and ROC analyses were performed.

Prognostic independence analysis

HCC patients from the TCGA_LIHC cohort with sufficient clinical information were included in the prognostic independence analysis. Both adenosine signaling-related signatures and clinicopathological features were selected as potential prognostic factors. To evaluate the prognostic value of these factors, multivariate Cox regression analyses for OS were performed to identify independent prognostic factors.

Nonnegative matrix factorization (NMF) analysis

To investigate the association between the prognostic risk model of adenosine signaling and the molecular subtype of HCC, we performed molecular subtype clustering analysis of HCC samples in TCGA_LIHC using the NMF R package (version: 0.28).[16] The analysis was based on 134 adenosine signaling-related genes, which allowed us to classify the HCC samples into distinct molecular subtypes. Subsequently, we plotted and compared the OS curves for

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patients in each subtype.

Differentially expressed genes (DEGs) and gene set enrichment analysis (GSEA)

DEGs were identified between the low- and high-risk groups via the DESeq2 (version: 1.40.2) R package. Genes with an adjusted *P*-value>0.05 and a $\log_2$ fold change (Log2FC)<1 were excluded. The filtered DEGs were subsequently used for GSEA to predict signaling pathways potentially enriched in the adenosine-enriched landscape in HCC patients. Patients from the TCGA cohort were divided into low- and high-risk groups on the basis of the median risk score. The C2.cp.kegg.v 7.4.symbols.gmt dataset served as the reference gene set, and pathways with *P*<0.05 and FDR <0.25 were considered significantly enriched.

Immune infiltration analysis

The abundance of infiltrating immune cells in HCC patients in TCGA cohort was assessed using the CIBERSORTx algorithm, a widely applied deconvolution method for analyzing immune cell composition on the basis of gene expression levels in solid tumors.[17] CIBERSORTx (version 0.1.0), with the LM22 signature, estimates the relative proportions of 22 immune cell types, including various B cells, T cells, natural killer (NK) cells, plasma cells, and distinct myeloid subsets. Comparisons of these 22 immune cell types were conducted between HCC patients in the low- and high-adenosine signature risk groups. Patients with *P*>0.05 were excluded from the analysis.

scRNA-seq and cell-cell interaction analyses

Transcriptomic analysis of single cells (GSE156625) was performed using the R package "Seurat" (version 4.3.0.1). For dimension reduction and visualization, uniform manifold approximation and projection was applied to the scRNA-seq data. Additionally, the risk score associated with the adenosine signature was calculated for each individual cell. The CellChat package was utilized to evaluate the interactions among different cell types within the TME of HCC patients. The median risk score of the adenosine signature was used to divide various cells into low- and high-risk groups according to the median risk score. Differential signaling pathways between these groups were identified on the basis of the criteria of *P*<0.05 and Log2FC>1.

Statistical analysis

All the statistical analyses were conducted via R software (version 4.3.1). A time-dependent ROC curve was generated to assess the performance of the risk score in predicting OS in HCC patients, and the area under the ROC curve (AUC) was calculated. Risk stratification of HCC patients was performed using the optimal cutoff value for risk scores, as determined by ROC analysis. The KM method was used to compare OS between the low- and high-risk groups, with statistical significance evaluated using the log-rank test. For prognostic analysis, the risk signature was treated as a dichotomous variable. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using the LASSO-Cox regression model. A random effects model was used to calculate pooled HRs and 95% CIs. *P*<0.05 was considered statistically significant.

OVERVIEW OF ATP-ADENOSINE METABOLISM IN THE TME

As mentioned in the Introduction section, adenosine is present in both the intracellular and the extracellular environments of cells. In the cytoplasm, adenosine is generated from endogenous ATP, which is produced primarily by mitochondria and is massively released during apoptosis or necrosis induced by mechanical or chemical stimuli.[9] Intracellularly, endogenous ATP is first converted to adenosine monophosphate (AMP) through the actions of nucleotide diphosphokinase and adenylate kinase (AK), and AMP is subsequently metabolized into adenosine by cytosolic nucleotidase.[18] Additionally, S-adenosylhomocysteine (SAH) can be hydrolyzed to adenosine and homocysteine by SAH hydrolase (SAHH), whereas S-adenosylmethionine serves as a methyl group donor in transmethylation reactions catalyzed by methyltransferases.[19,20] Given that mitochondria are the primary source of ATP production, mitochondrial bioenergetics are closely linked to the maintenance of intracellular adenosine homeostasis.[21,22] Intracellular adenosine is involved in energy metabolism, nucleic acid me-

tabolism, and the methionine cycle (Fig. 1).[23,24]

Extracellular ATP, the main precursor of eADO, originates primarily from two sources: the massive release of endogenous ATP during cellular damage and the nonlytic export of endogenous ATP into the extracellular space. Nonlytic endogenous ATP export occurs via connexin hemichannels, vesicular exocytosis, and other ion channels and transporters.[25] Once released, endogenous ATP is metabolized into eADO primarily by ectonucleoside triphosphate diphosphohydrolase-1 (CD39/ENTP1) and 5′-ectonucleotidase (CD73/NT5E), which constitute the main pathway for ATP-adenosine metabolism.[26] Additionally, eADO is generated from ATP and inorganic polyphosphates via tissue-nonspecific alkaline phosphatase, AMP hydrolysis through prostatic acid phosphatase, and NAD^+ through the CD38-ENPP1-CD73 axis.[27] Finally, eADO is transported into the intracellular space via equilibrative (ENT) and concentrative (CNT) nucleoside transporters, creating a dynamic transport cycle that modulates adenosine concentrations between the intracellular and extracellular environments (Fig. 1).[28] Consequently, adenosine concentrations are significantly elevated in the TME compared with normal conditions, fostering an immunosuppressive environment favorable for tumor cell growth.[14,29]

PATHOLOGIC MECHANISMS OF ADENOSINE IN THE TME

Adenosine shown in Figure 2A is a common molecule with a relatively simple structure, consisting of an adenine attached to a ribose via a β-N9-glycosidic bond.[30] Adenosine exerts its pathological effects in HCC primarily through the activation of adenosine receptors.[31] Four adenosine receptors have been identified, namely, A_1, A_{2A}, A_{2B}, and A_3, all of which belong to the G protein-coupled receptor family

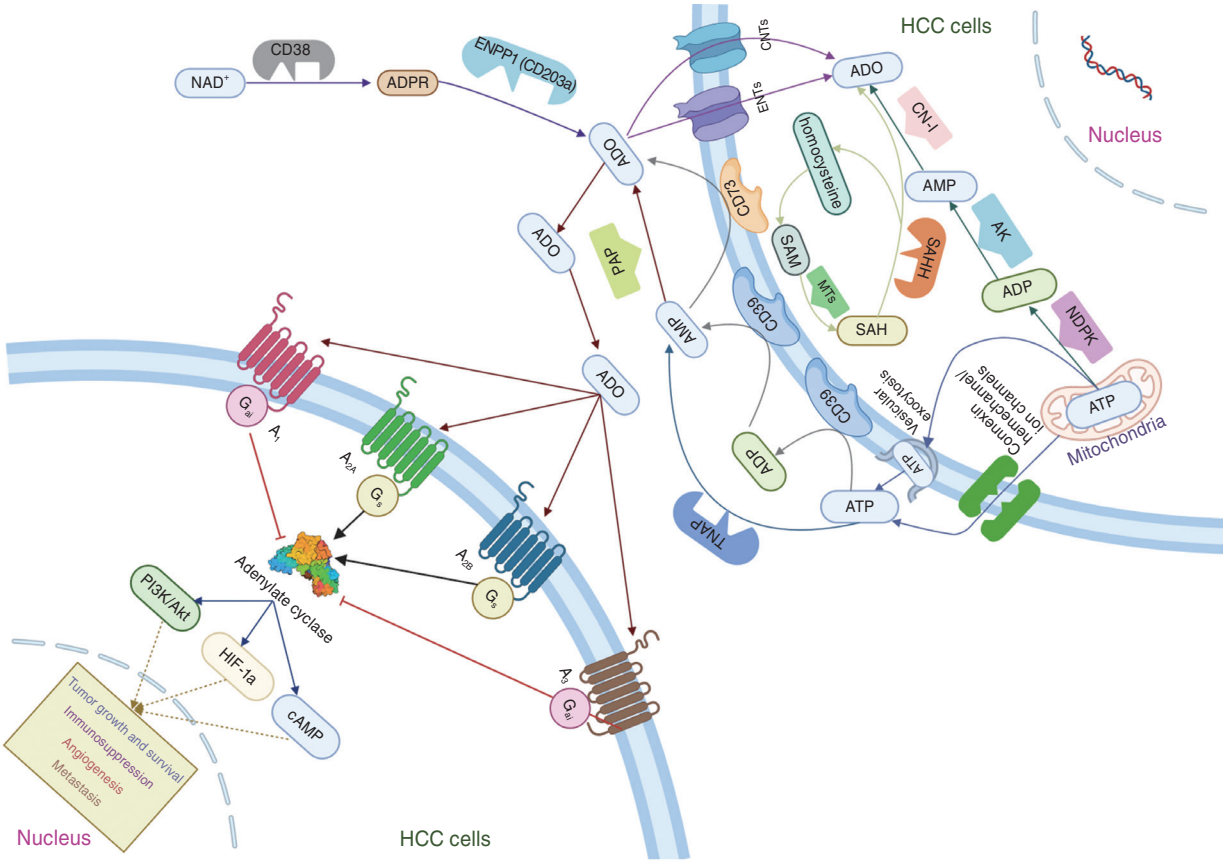

Figure 1. Schematic diagram of intracellular and extracellular adenosine metabolism and pathologic mechanisms of adenosine in the TME of HCC. HCC, hepatocellular carcinoma; TME, tumor microenvironment.

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(GPCR).[32] As shown in Figure 2B, the human A_1 receptor, composed of 326 amino acids and organized into seven transmembrane domains (TMs), exhibits highly conserved regions in TM3 and TM7 that are critical for ligand interactions. Additionally, the extracellular loop 2 (EL2) not only contributes to ligand binding affinity and signal transduction but also harbors an allosteric site. Recent structural analyses have revealed a distinct extracellular cavity, providing insights into selective ligand binding.[33] The human A_{2A} receptor (Fig. 2C) is composed of 412 amino acids, with slight variations ranging from 409 to 412 residues observed in other species. In contrast to other adenosine receptor subtypes, it possesses an extended carboxy-terminal region, contributing to its larger 45 kDa molecular weight. Structurally, it consists of seven TMs, each comprising 20-27 amino acids, with cysteine residues in TM3 and the EL2 forming a disulfide bond. Additionally, a short TM8 segment is located near the cytoplasmic surface.[34] Human adenosine receptor A_{2B} (Fig. 2D), consisting of 328 amino acids, follows the typical GPCR architecture with seven TMs, including three extracellular loops and three intracellular loops, with an extracellular N-terminus and an intracellular C-terminus. Notably, the EL2 domain of adenosine receptor A_{2B} is the longest among adenosine receptors and contains cysteine residues that form disulfide bonds, with the bond between C171 in EL2 and C78 in TM3 being crucial for ligand binding and receptor function. The extended EL2 in A_{2B} receptors may hinder ligand inter-

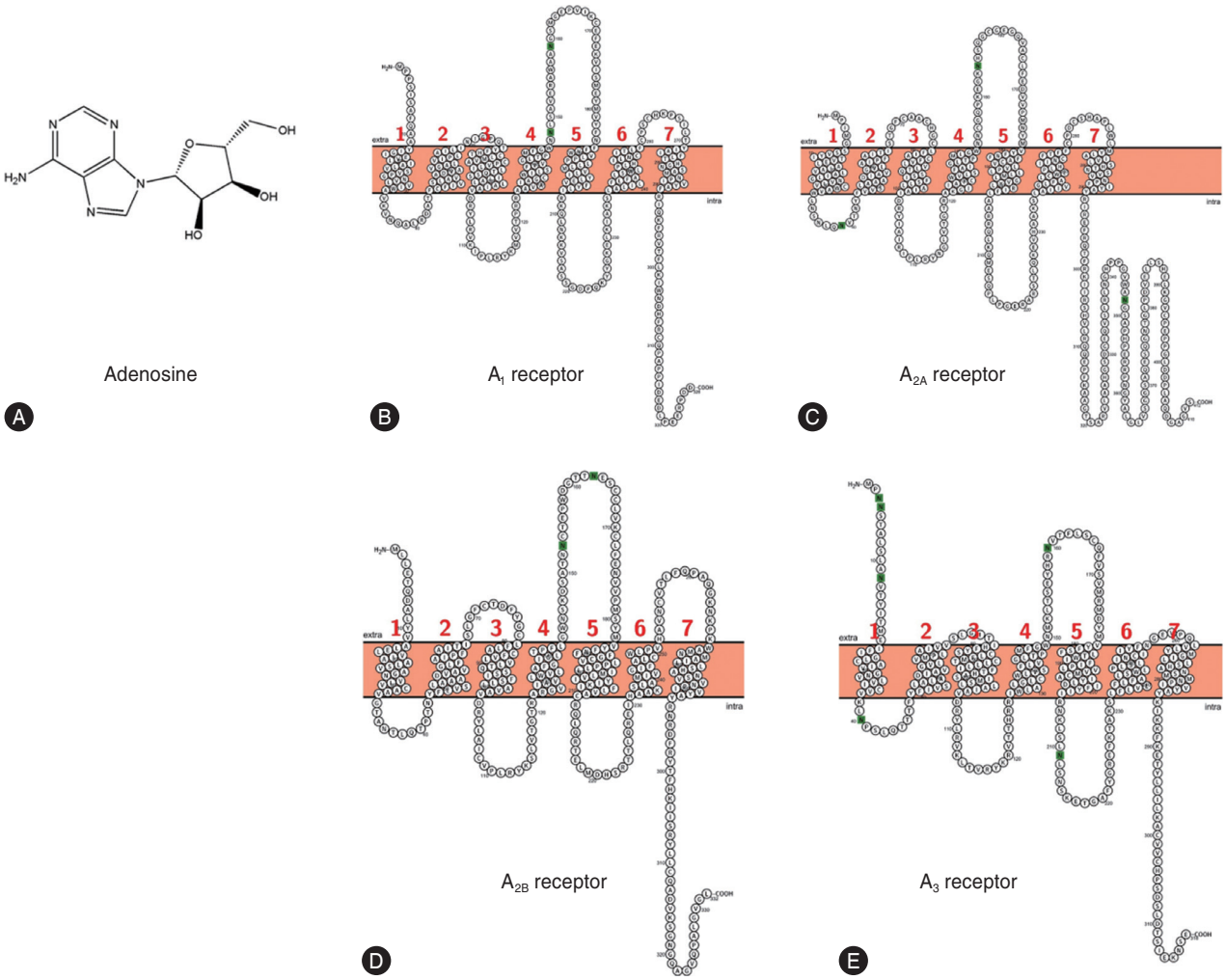

Figure 2. Chemical structures of adenosine and serpentine diagram of adenosine receptors. (A) Chemical structure of adenosine. (B) Human adenosine receptor A_1. (C) Human adenosine receptor A_{2A}. (D) Human adenosine receptor A_{2B}. (E) Human adenosine receptor A_3. Serpentine plots were generated by Protter (https://wlab.ethz.ch/protter/start/).

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action more than in A_{2A} receptors.[35] The human A_3 adenosine receptor (Fig. 2E), consisting of 318 amino acids, follows the typical GPCR structure with seven TMs and a C-terminal sequence containing Ser and Thr residues that undergo phosphorylation during rapid desensitization. Key to its function, the conserved Trp (W6.48) in TM6 is essential for signal transduction, β-arrestin2 interaction, and receptor internalization.[36] These receptors are expressed by various cell types within the TME of HCC, including tumor cells, stromal cells, endothelial cells, and immune cells.[35] Moreover, recent evidence indicates that these four receptors have varying affinities for adenosine, to which they are linked through G proteins.[37] For example, the activation of A_1 and A_3 receptors inhibits adenylate cyclase via $G_{i/o}$ protein interactions, leading to reduced cyclic AMP (cAMP) production.[38] Conversely, A_{2A} and A_{2B} receptors couple with the G_s protein family, activating adenylate cyclase and increasing intracellular cAMP levels.[39] As a result, these receptors play distinct roles in the pathological processes of HCC, including tumor growth and survival, immunosuppression, angiogenesis, and metastasis,[40] as discussed below.

Effects of adenosine on tumor growth and survival in the TME

As previously discussed, adenosine levels are elevated in the TME compared with normal physiological conditions. This can promote apoptosis in some cells, although certain tumor cells exhibit resistance to this apoptotic effect of adenosine.[41] In HCC, the overexpression of the A_1 adenosine receptor enhances cell proliferation and invasion, and promotes tumor growth by augmenting the activity of the PI3K/AKT oncogenic pathway in a subcutaneous xenograft mouse model.[42] Besides, knockdown of the A_1 receptor can enhance sensitivity to chemotherapy in the HCC mouse model.[42] Moreover, the activation of the A_{2B} adenosine receptor, regulated by HIF-1α, also promotes the proliferation of HepG2 liver cancer cells.[43] Hence, inhibitors targeting adenosine receptors may lead to the development of new therapeutic strategies on tumor suppression for HCC.

Effects of adenosine on immunosuppression in the TME

Adenosine is widely recognized as an immunosuppressive factor in the TME, where it can inhibit tumor antigen presentation and immune cell activation by binding to adenosine receptors on immune cells, thereby modulating tumor adaptive immunity.[44] Adenosine receptors are ubiquitously expressed across various immune cells, including T cells, NK cells, dendritic cells (DCs), myeloid-derived suppressor cells (MDSCs), and macrophages, within the TME of HCC.[45] Extensive preclinical studies have indicated that adenosine primarily exerts its immunosuppressive effects through the activation of A_{2A} and A_{2B} receptors on the cell surface of immune cells.[46] The A_{2A} receptor forms a complex with intracellular G_s and G_β–G_γ subunits on the surface of these cells, leading to the upregulation of immunosuppressive cytokines, such as interleukin (IL)-10 and forkhead box P3 (Foxp3), while suppressing the release of antitumor cytokines, including tumor necrosis factor-alpha (TNF-α), IL-1β, and IL-6, by activating the adenylyl cyclase/cAMP signaling cascade.[47,48] In contrast, the activation of A_1 and A_3 adenosine receptors on immune cells inhibits adenylyl cyclase activity, thereby preventing the activation of cAMP-dependent downstream signaling events.[49] Adenosine also inhibits T-cell activation and infiltration by binding to the A_{2A} receptor on T cells, thereby activating the cAMP-protein kinase A-Src kinase pathway.[50] The A_{2B} receptor, which is predominantly expressed by macrophages and DCs, inhibits their antigen-presenting function through adenosine binding.[35] Notably, the expression of adenosine receptors, especially A_{2A} and A_{2B}, is significantly increased in HCC.[51] Cheu et al. demonstrated that adenosine exerts an immunosuppressive effect on T cells and MDSCs via activation of the A_{2A} adenosine receptor in a $Tp53^{KO}$/c-Myc^{OE} HCC mouse model.[14] Moreover, pharmacological studies revealed that the activation of the A_{2A} receptor exacerbates immunosuppressive effects in a NASH-induced HCC mouse model.[52] Importantly, after immunotherapy, A_{2A} expression was elevated in an orthotopic HCC tumor mouse model, supporting the immunosuppressive role of adenosine in the TME of HCC.[53] Additionally, adenosine promotes macrophage infiltration into the tumor milieu, suggesting new avenues for immunotherapy in HCC.[29] These findings indicate that inhibitors targeting these adenosine receptors

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may enhance immunotherapeutic efficacy in HCC.

Effects of adenosine on angiogenesis in the TME

Owing to the insufficient oxygen supply in the TME, the release of adenosine exerts direct mitogenic effects on vascular cells, thereby enhancing angiogenesis and promoting tumor progression.[54] Adenosine stimulates endothelial cells to release various proangiogenic factors, such as IL-8, basic fibroblast growth factor, and vascular endothelial growth factor (VEGF), by activating A_{2B} adenosine receptors that are coupled to both G_s and G_q proteins.[32] In addition, adenosine stimulates endothelial cells' DNA synthesis and cell migration further amplifying its proangiogenic effects.[55] Simultaneously, adenosine inhibits the secretion of antiangiogenic factor (thrombospondin-1) through the G_s protein-coupled A_{2A} adenosine receptor.[56] Therefore, the main proangiogenic actions of adenosine can be attributed to its ability to regulate the production of proangiogenic and antiangiogenic substances from vascular cells and stromal cells within the TME. Moreover, both A_1 and A_{2A} adenosine receptors contribute to this process by increasing VEGF levels, thereby promoting the formation of the tumor microvascular network.[57] However, the specific effect of adenosine on angiogenesis in HCC remains unreported and requires further investigation.

Effects of adenosine on metastasis in the TME

Angiogenesis not only supplies nutrients to tumor cells but is also a critical element in the metastatic cascade.[58] Furthermore, the extracellular matrix, which is composed of essential components such as collagen, laminin, chondroitin sulfate proteoglycans, and hyaluronic acid, plays a pivotal role in tumor invasion and metastasis.[59] In HCC, pharmacological research indicates that metastasis is suppressed via inhibition of the A_{2A}/PI3K/AKT signaling pathway in NOD/SCID HCC mouse model.[60] Therefore, developing antagonists targeting A_{2A} and A_{2B} adenosine receptors holds significant therapeutic potential for managing HCC in metastatic stages, potentially improving the survival and quality of life of patients with advanced HCC.

THE CLINICAL SIGNIFICANCE OF ADENOSINE SIGNALING IN HCC

To date, limited meta-analysis on the role of adenosine signaling in HCC has been conducted,[61] but it excluded certain prognosis-related genes during the construction of the prognostic model. Consequently, the established model may not fully and accurately reflect the prognostic implications of adenosine signaling-related genes in HCC patients. Therefore, to gain a deeper understanding of the prognostic impact of adenosine signaling in HCC, our study systematically analyzed and developed a prognostic model based on a 134 gene adenosine signaling-related signature. The model was constructed and validated using data from the TCGA_LIHC, GEO, and ICGC databases, providing a more comprehensive evaluation of the prognostic role of adenosine signaling in HCC.

Construction of the prognostic signature of adenosine signaling in TCGA cohort

TCGA_LIHC cohort of 373 HCC patients with complete clinical information was selected as the training cohort of the prognostic signature. First, the univariate Cox proportional hazards regression analysis was performed to select adenosine signaling-related genes that are associated with the OS of HCC patients. The analytical results revealed that 38 genes were significantly associated with OS, including 32 high-risk genes with HRs greater than 1, and 6 low-risk genes with HRs less than 1 (Supplementary Table 2). Then, LASSO-Cox proportional hazards regression analysis was performed with these 38 genes, and the variables were further reduced. A total of eight genes were identified as prognostic genes. The following risk formula was constructed according to the expression of these genes and their regression coefficients:

Risk score=0.086265×*ENTPD2*-0.885792×*RAPGEF3*
+0.064217×*VEGFA*-0.333192×*VIPR1*+0.210808×*SLC6A3*+
0.076007×*RPIA*+0.193528×*CXCL8*+0.077439×*ADA*.

HCC patients were divided into low- and high-risk groups on the basis of the median risk score obtained using the risk formula. The expression of adenosine signature genes, the distribution of risk scores and the survival status of these

two groups are shown in Figure 3A and 3B. The KM survival curve revealed that patients in the high-risk group had significantly worse OS than those in the low-risk group did (Fig. 3C). The time-dependent ROC curves revealed that the AUCs for the risk score were 0.813, 0.753, and 0.682 for predicting 1-, 3-, and 5-year OS, respectively (Fig. 3D).

Validation of the adenosine signature in the GEO and ICGC cohorts

To further confirm the established adenosine signature model in HCC patients, the GEO and ICGC cohorts of HCC patients were employed. According to the risk formula, a risk score was calculated for each patient in the GSE14520 and ICGC_JP cohorts. The patients were then separately divided into low- and high-risk groups on the basis of the median risk score. The KM survival curves revealed that patients in the high-risk group had poorer OS than low-risk patients did (Fig. 3E, 3G). The time-dependent ROC curves revealed that the AUCs for the risk score were 0.578, 0.615, and 0.615 in GSE14520 cohort, and 0.759, 0.728, and 0.744 in ICGC_JP cohort, for predicting 1-, 3-, and 5-year OS, respectively (Fig. 3F, 3H).

Independence and specificity analysis of the prognostic signature

To evaluate the independent prognostic value of our signature in predicting OS in TCGA_LIHC cohort, multivariate Cox regression analysis demonstrated that a high-risk score in the prognostic model is independently associated with worse OS (Fig. 4A). Furthermore, to explore the specific effects of the prognostic model across various HCC molecular subtypes, the HCC samples in the TCGA_LIHC cohort were classified into five subtypes using the NMF method. The KM survival analysis exhibited no statistically significant differences in OS among these five molecular subtypes, suggesting our prognostic model is broadly applicable to HCC patients across various molecular subtypes in clinic (Fig. 4B). Finally, to further validate the specificity of the prognostic model for HCC, we assessed its performance in other cancers, including pancreatic adenocarcinoma (TCGA_PAAD), esophageal cancer (TCGA_ESCA), and cholangiocarcinoma (TCGA_CHOL). The KM survival curves showed that there were no statistically significant differences between low- and high-risk groups in the TCGA_PAAD, TCGA_ESCA, and TCGA_CHOL cohorts, indicating our model is specifically applicable to HCC patients (Fig. 4C-4E).

Exploration of adenosine signature-related biological pathways and immune infiltration in the TCGA_LIHC cohort

Given that adenosine-related biological pathways remain incompletely understood, GSEA was performed to predict enriched adenosine-associated pathways. A total of 2593 DEGs were identified within TCGA_LIHC cohort using DESeq2 method (Supplementary Table 3). KEGG pathway analysis revealed that 24 enriched pathways associated with the adenosine signature (Fig. 5A), including retinol metabolism, neuroactive ligand-receptor interaction, drug metabolism-cytochrome P450, metabolism of xenobiotics by cytochrome P450, and chemical carcinogenesis-receptor activation, presented the lowest *P*-values. CIBERSORTx analysis revealed that T-cell memory, Treg, M0 macrophage, and resting DC infiltration were significantly greater in the high-risk adenosine signature group than in the low-risk group. In contrast, the infiltration of memory B cells, resting NK cells, monocytes, and M2 macrophages was obviously greater in the low-risk groups than in the high-risk groups (Fig. 5B). These findings further support the immunosuppressive role of adenosine within the TME of HCC.

Deciphering the adenosine signaling signature at the single-cell level

To gain further insight into the effects of the adenosine pathway on each cell type in the TME of HCC, the scRNA-seq technique was used in this review. First, we evaluated the adenosine signature risk score of each cell in the HCC single-cell transcriptomic dataset (GSE156525). This single-cell dataset was subjected to quality control analysis and effectively annotated the cell types (Fig. 5C). Consequently, we calculated the risk score of the adenosine signature in each cell type (Fig. 5D), revealing relatively high risk scores in M1 and M2 macrophages, DCs, and monocytes. Moreover, we observed increased recruitment of immune cells and hepatocytes in the adenosine-enriched

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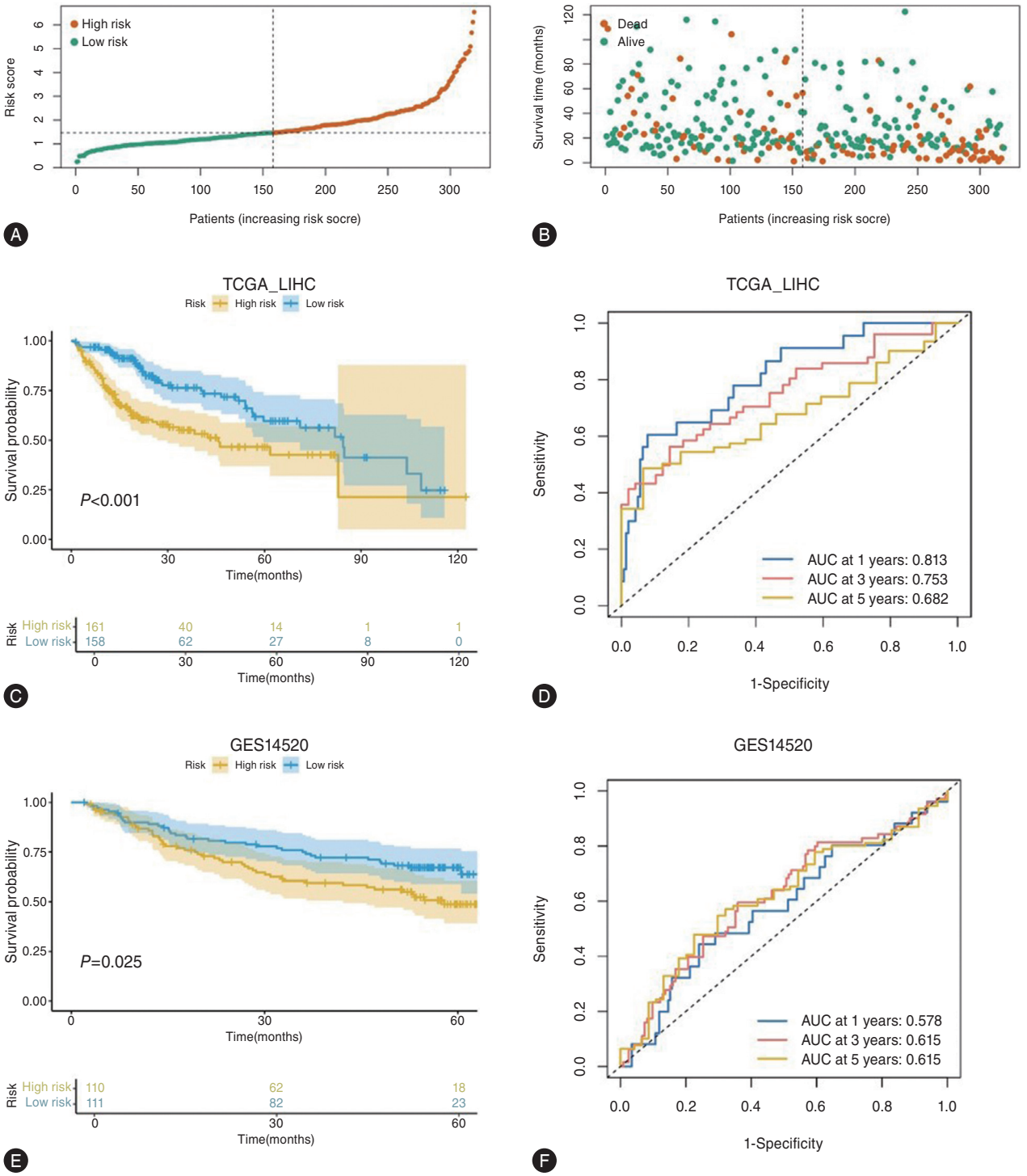
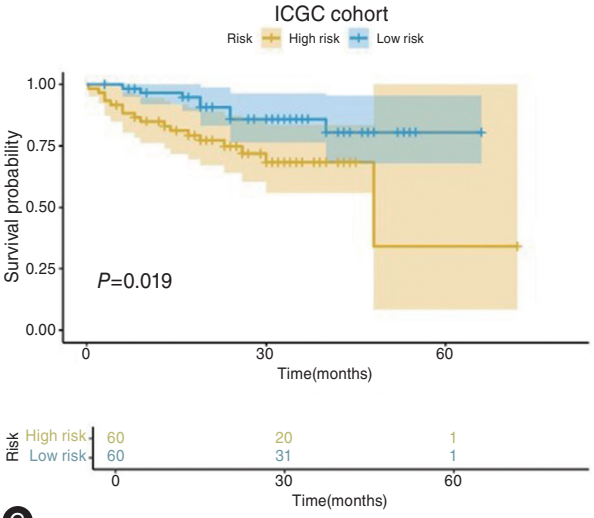


Figure 3. Construction and validation of prognostic model based on adenosine signaling signature in TCGA_LIHC and GEO cohorts. (A) Risk score distribution of the patients with low and high risk in TGCA_LIHC cohort. (B) Survival status of the patients with low and high risk in TGCA_LIHC cohort. (C) Kaplan–Meier curve of overall survival (OS) of TCGA_LIHC patients who are divided into low- and high-risk groups. (D) Time-dependent ROC curves for the prognostic model in TCGA_LIHC cohort. (E) Kaplan–Meier curve of OS of GSE14520 cohort. (F) Time-dependent ROC curves for the prognostic model in the GSE14520 cohort. (G) Kaplan-Meier curve of OS of ICGC cohort. (H) Time-dependent ROC curves for the prognostic model in the ICGC cohort. AUC, area under the ROC curve; GEO, Gene Expression Omnibus; ICGC, International Cancer Genome Consortium; ROC, receiver operating characteristic; TCGA, The Cancer Genome Atlas.



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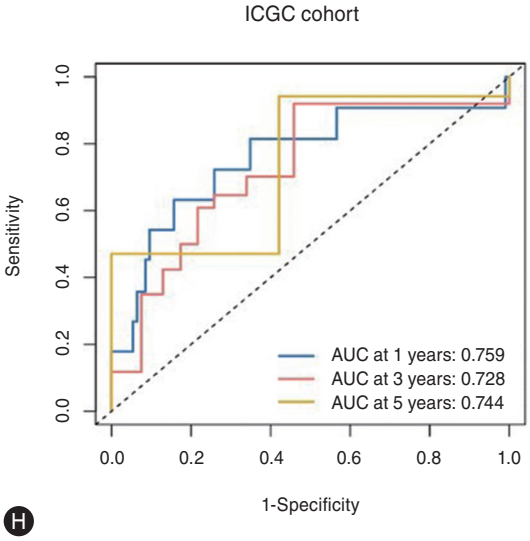

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Figure 3. Continued.

landscape, whereas the proportions of endothelial cells and M1 macrophages were relatively decreased (Fig. 5E, 5F). To further investigate the potential effects of adenosine on cell-cell communication, we predicted the ligand-receptor interaction strength between different cell types in low or high adenosine microenvironments (Fig. 5G, 5H) and found that the ligand-receptor interaction strength between immune cells and other cell types was decreased in the adenosine-enriched TME, which further supports the immunosuppressive effects of adenosine in the TME of HCC.

Effect of adenosine receptor expression on therapeutic response to immunotherapy or targeted therapy in HCC

To illustrate the association between adenosine receptors expression and therapeutic response in HCC, four HCC cohorts were analyzed including three PD-1 immunotherapy cohorts (GSE202069, GO30140, and PRJEB34724) and one sorafenib-treated cohort (GSE109211). In the PD-1-treated cohorts, high expression of adenosine receptors A_1 (Fig. 6A, 6E, 6I), A_{2B} (Fig. 6C, 6G, 6K), and A_3 (Fig. 6D, 6H, 6L) was associated with an increased response rate to PD-1 therapy. In contrast, elevated expression of adenosine receptor A_{2A} was linked to a reduced response rate (Fig. 6B, 6J), potentially due to its association with immunosuppressive effects in HCC. Meanwhile, in the sorafenib-treated cohorts, increased expression of all adenosine receptors was consistently correlated with an improved therapeutic response rate (Fig. 6M-6P). These results require further validation in HCC cohorts with a larger sample size. These findings suggest that increased expression of these adenosine receptors may enhance drug sensitivity, with the exception of adenosine receptor A_{2A}. This highlights the potential of adenosine receptor signaling as a therapeutic target in HCC.

DEVELOPMENT OF ADENOSINE RECEPTOR ANTAGONISTS

Many studies and clinical trials have focused primarily on inhibiting ATPase/ADPase activity via the use of anti-CD39 or anti-CD73 inhibitors.[62,63] However, these inhibitors reduce adenosine levels exclusively in the TME or blood circulation without directly targeting adenosine receptors to suppress their function. Consequently, recent studies have explored the development of both single and dual adenosine receptor antagonists for targeted cancer therapies, as discussed below (Table 1).

A_1 adenosine receptor antagonist

1,3-Dipropyl-8-cyclopentylxanthine (DPCPX, Fig. 7A) functions as an A_1 adenosine receptor antagonist, efficiently suppressing cancer cell proliferation in vitro and inhibit-

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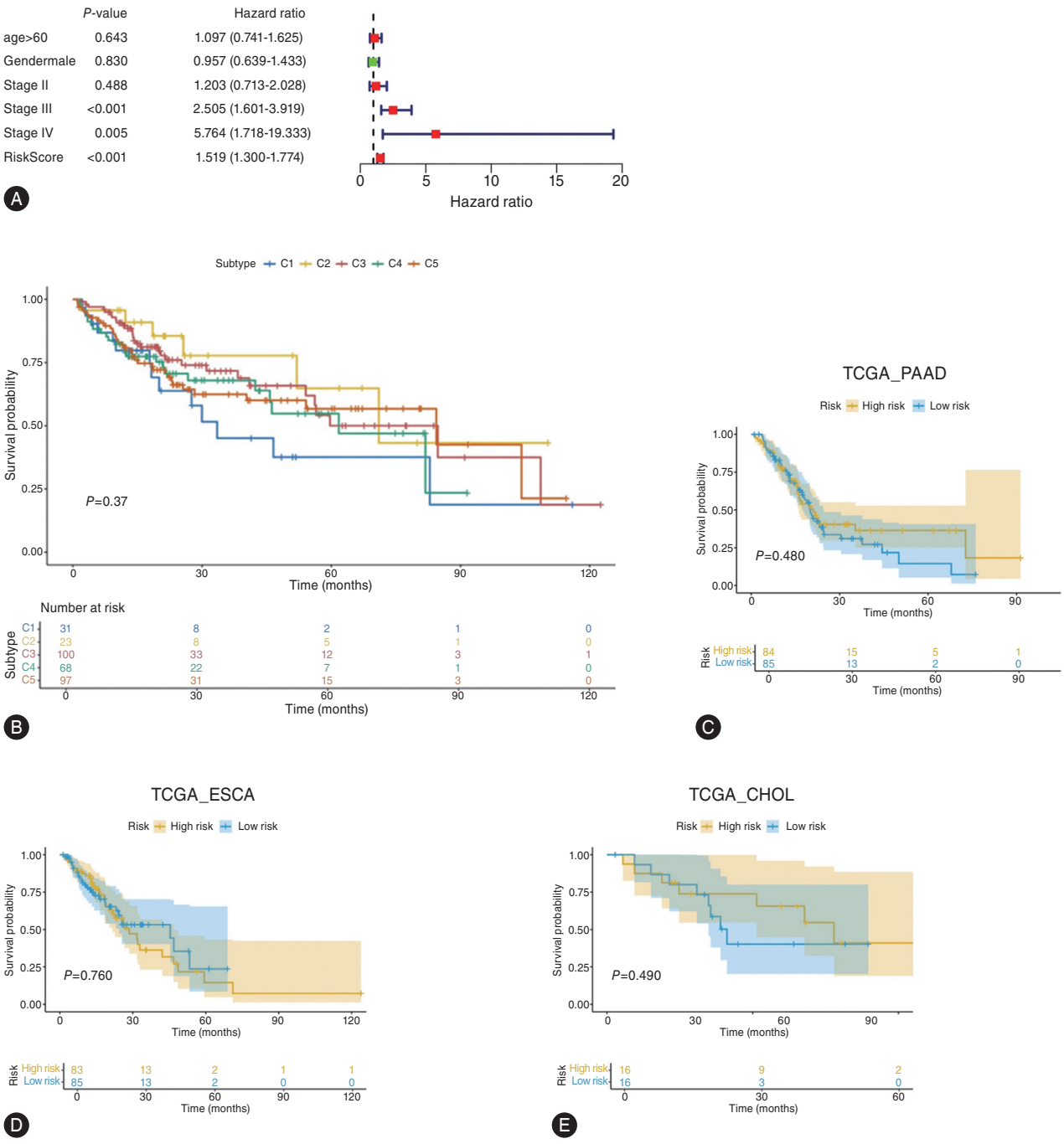

Figure 4. The independence and specificity analysis of the prognostic model. (A) Forest plot of OS multivariable Cox regression analysis from TCGA_LIHC cohort. (B) Survival curve of five molecular subtypes of TCGA_LIHC cohort OS. (C-E) Survival curve of the OS of TCGA_PAAD, TCGA_ESCA, and TCGA_CHOL, respectively. OS, overall survival; TCGA, The Cancer Genome Atlas.

ing tumor growth in renal cancer and HCC xenograft models.[42,64] Moreover, 10 μM DPCPX was found to counteract the immunosuppressive effects of adenosine in both in vitro and in vivo HCC models.[65] However, no clinical trials of DPCPX in HCC therapy have been reported to date. In future studies, DPCPX warrants clinical trials to further evaluate its potential for clinical application.

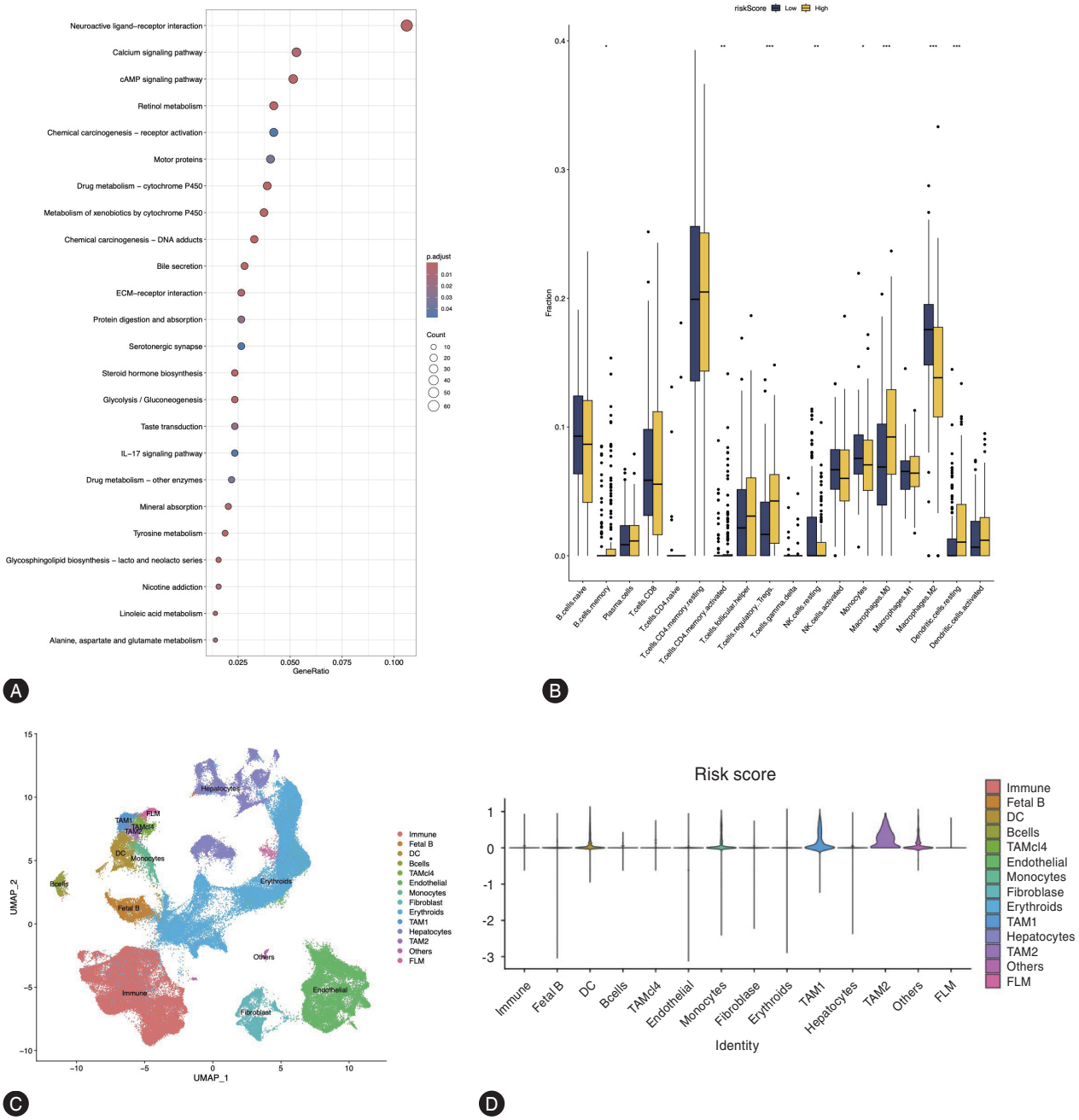

Figure 5. Functional association of the risk score of adenosine signaling signature in bulk-RNA and scRNA-seq analysis. (A) KEGG enriched pathways by GSEA of differentially expressed genes. (B) Immune infiltration analysis of the patients with low-and high-risk in TGCA_LIHC cohort. (C) UMAP plot of scRNA-seq profile from GSE156625. (D) Violin plot of risk score of adenosine signaling signature in each cell type of GSE156625 cohort. (E, F) Cell composition in low-and high-risk score groups. (G, H) Interaction strength of cell communication on each cell type in low-and high-risk score groups. GSEA, gene set enrichment analysis; scRNA-seq, single-cell RNA sequencing; TCGA, The Cancer Genome Atlas; UMAP, uniform manifold approximation and projection. $^{*}P< 0.05$, $^{**}P< 0.01$, $^{***}P< 0.001$.

A_{2A} adenosine receptor antagonists

Ciforadenant (CPI-444, Fig. 7B) is a selective A_{2A} adenosine receptor antagonist that is currently under investigation in the clinic. It has completed a phase I clinical trial for advanced cancers (ClinicalTrials ID: NCT02655822, NCT03454451)[66,67] and a phase Ib/II trial for lung cancer (ClinicalTrials ID: NCT03337698).[68] Imaradenant

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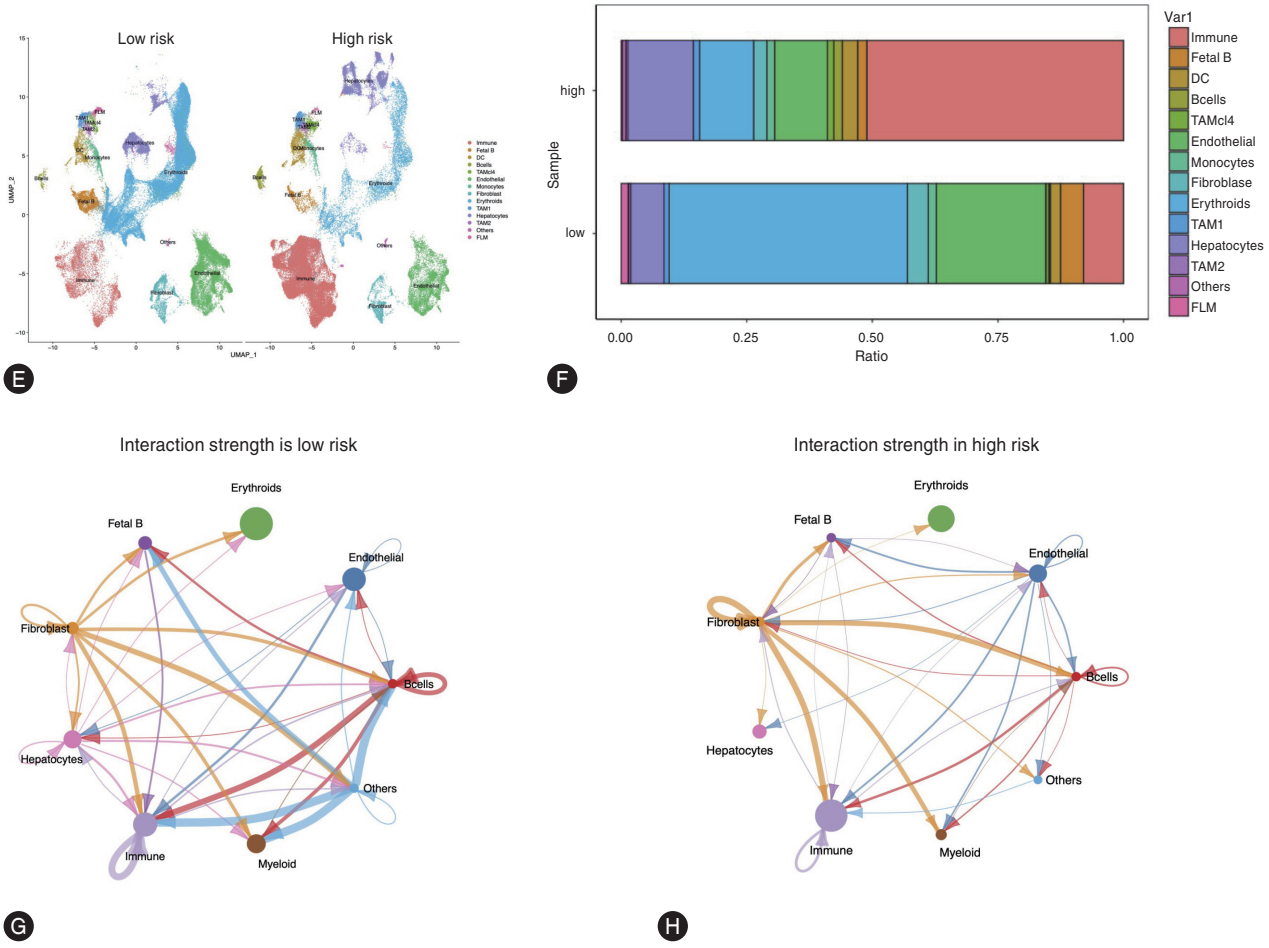

Figure 5. Continued.

(AZD4635, Fig. 7C) is an oral A_{2A} receptor antagonist that has completed a phase I clinical trial for advanced cancers (ClinicalTrials ID: NCT0398082).[69] Additionally, a phase II study combining AZD4635 with durvalumab (an anti-PD-L1 immunotherapy) or oleclumab (an anti-CD73 drug) in patients with metastatic prostate cancer revealed minimal antitumor activity but manageable safety (ClinicalTrials ID: NCT04089553).[70] Taminadenant (NIR178/PBF509, Fig. 7D), another selective A_{2A} receptor antagonist, has also completed a phase I trial for advanced cancers (ClinicalTrials ID: NCT03549000).[71] Furthermore, a phase I study evaluating taminadenant in combination with spartalizumab (an anti-PD1 therapy) in advanced lung cancer patients demonstrated clinical benefits beyond those of anti-PD1 monotherapy (ClinicalTrials ID: NCT02403193).[72] Etrumadenant (AB928, Fig. 7E) is a dual A_{2A}/A_{2B} receptor antagonist that has completed phase I and phase I/II clinical trials, both as monotherapy and in combination with anti-PD1 therapy (zimberelimab) in colorectal cancer (ClinicalTrials ID: NCT03720678, NCT04660812), esophagogastric cancer (ClinicalTrials ID: NCT03720678), breast cancer (ClinicalTrials ID: NCT03719326), prostate cancer (ClinicalTrials ID: NCT03629756, NCT04381832), lung cancer (ClinicalTrials ID: NCT04262856, NCT03846310), and head and neck cancers (ClinicalTrials ID: NCT04892875).[73] In addition, other high-affinity and selective A_{2A} receptor inhibitors have demonstrated clinical potential in preclinical studies. For example, ZM241385 (4-(2-((7-amino-2-(furan-2-yl)-(1,2,4)triazolo(1,5-a)(1,3,5)triazin-5-yl)amino)ethyl)-2-(125I)iodophenol, Fig. 7F) acts as an A_{2A} adenosine receptor antagonist and reduces fibrosis in CCl_4-induced and metabolic dysfunction-associated steatohepatitis mouse models.[74,75] Similarly, SCH58261 (2-(furan-2-yl)-7-phenethyl-7H-pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidin-5-amine, Fig. 7G),

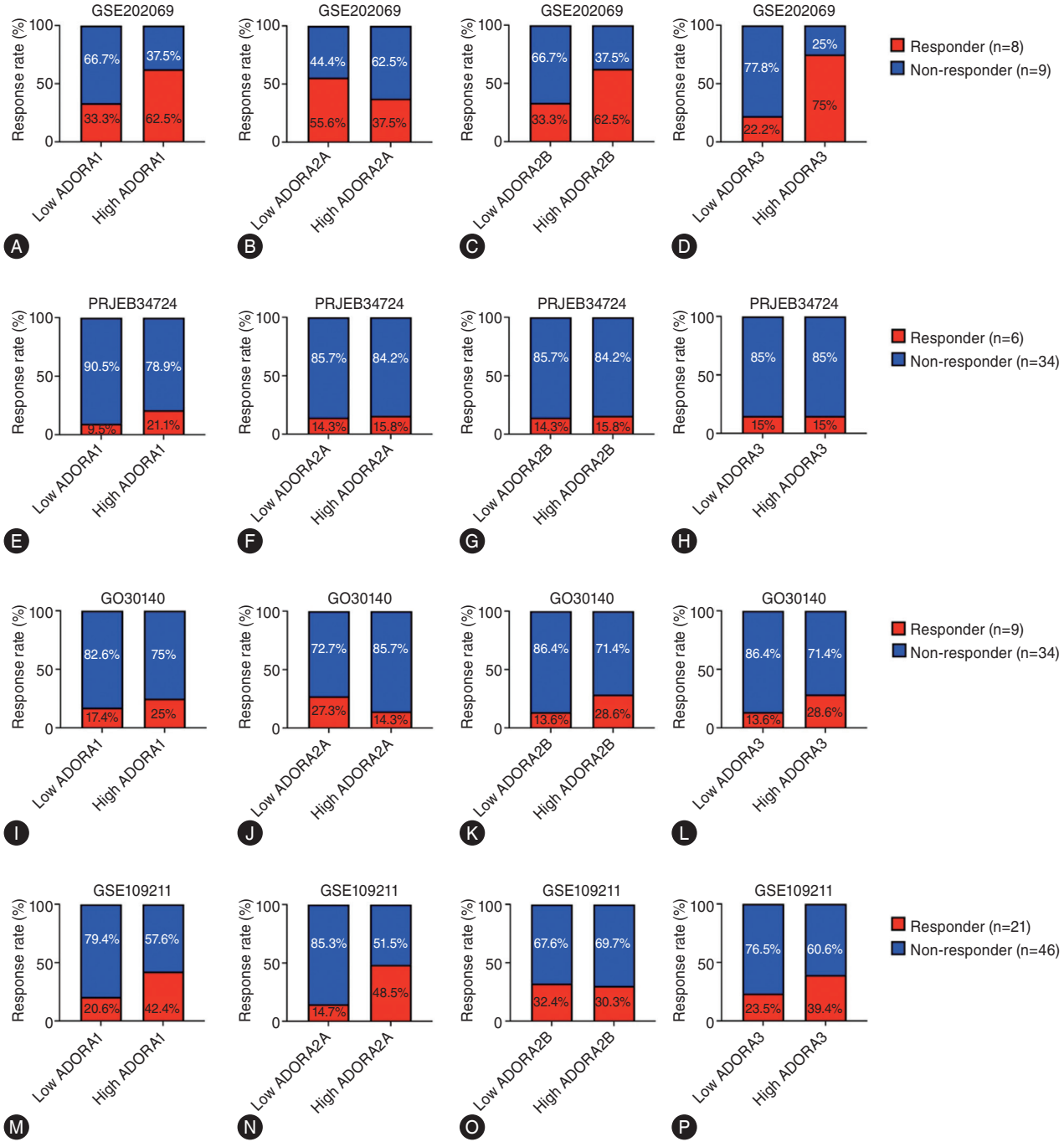

Figure 6. Association between adenosine receptor expression and therapeutic response towards immunotherapy and targeted therapy in hepatocellular carcinoma (HCC). (A–D) PD-1-treated HCC patients (GSE202069); (E–H) PD-1-treated HCC patients (PRJEB34724); (I–L) PD-1-treated HCC patients (GO30140); (M–P) Sorafenib-treated HCC patients (GSE109211).

another A_{2A} receptor antagonist, exhibited synergistic effects when combined with anti-PD1 treatment, activating T cells and reducing tumor size in orthotopic liver cancer models.[53] In future studies, a few early-stage clinical trials have explored the use of A_{2A} antagonists, either alone or in combination with immune checkpoint inhibitors (e.g., anti-PD-1 or anti-PD-L1 therapies), but further phase III and IV clinical trials with larger sample sizes are still needed to validate their efficacy and support their clinical application.

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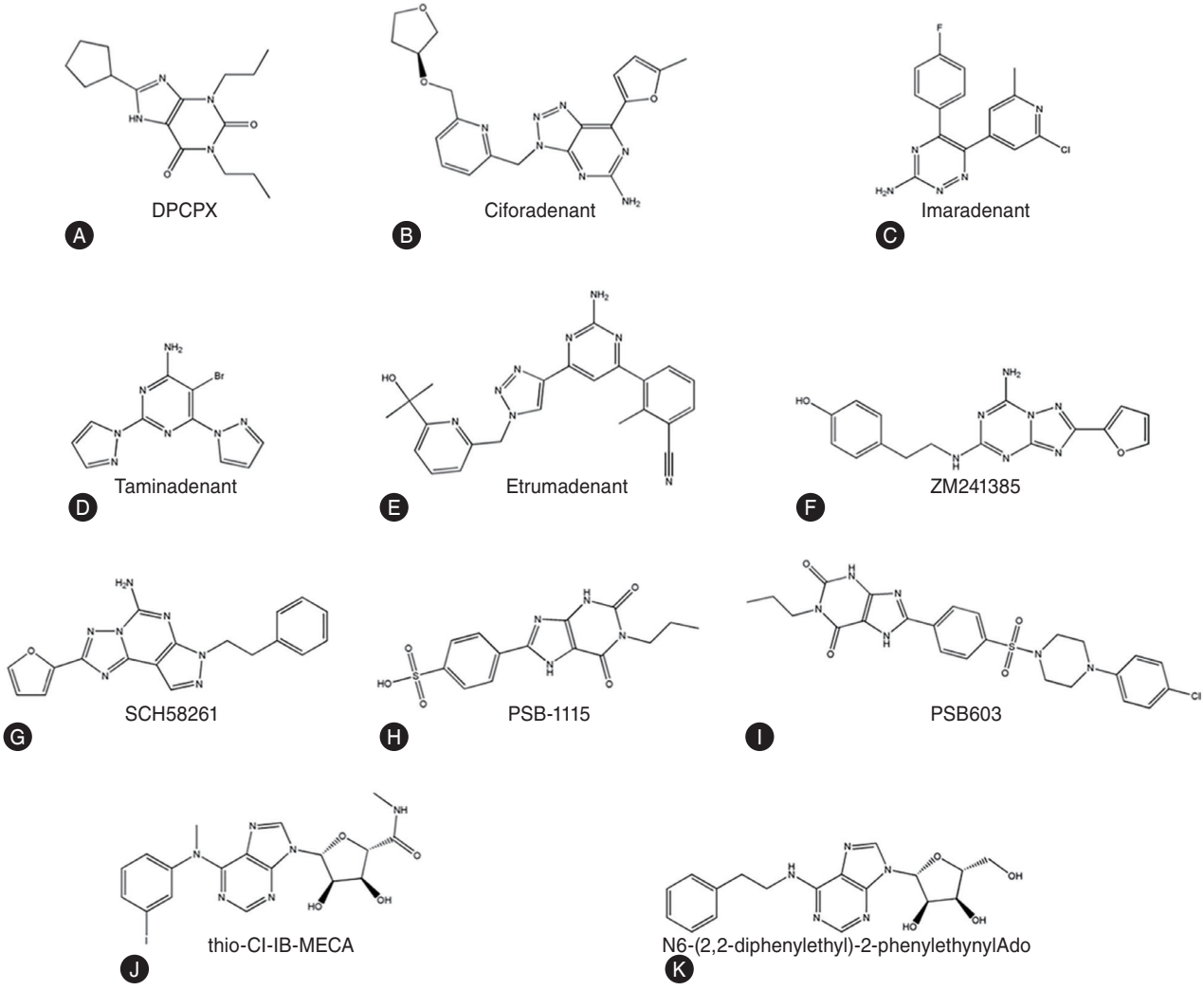

Figure 7. Chemical structures of adenosine receptor antagonists. (A) A_1 adenosine receptor antagonists. (B-G) A_{2A} adenosine receptor antagonists. (H, I) A_{2B} adenosine receptor antagonists. (J, K) A_3 adenosine receptor antagonists.

A_{2B} adenosine receptor antagonists

PBF-1129 (structure not reported) is a novel A_{2B} adenosine receptor antagonist that has been used in a phase I trial in patients with advanced non-small cell lung cancer (ClinicalTrials ID: NCT03274479), which demonstrated that PBF-1129 is safe and well tolerated in these patients.[76] Another phase I trial is currently evaluating PBF-1129 in combination with nivolumab (anti-PD1 therapy) in lung cancer patients (ClinicalTrials ID: NCT05234307). Similarly, several small molecules have exhibited high selectivity as A_{2B} receptor inhibitors; however, none have progressed to clinical trials. Examples include PSB-1115 (4-(2,6-dioxo-1-propyl-3,7-dihydropurin-8-yl) benzenesulfonic acid, Fig. 7H),[77] the xanthine derivative PSB603 (8-[4-[4-(4-chlorophenzyl) piperazide-1-sulfonyl)phenyl]]-1-propylxanthine, Fig. 7I),[78,79] and ATL801 (structure not reported).[80] In the future, similar to A_{2A} antagonists, phase III and IV clinical trials with larger sample sizes are required to further evaluate its potential for clinical application.

A_3 adenosine receptor antagonists

Currently, the A_3 receptor antagonist PBF-1650 (structure not reported) has completed a phase I clinical trial for psoriasis (ClinicalTrials ID: NCT03798236), whereas PBF-677 (structure not reported) has progressed through both phase I and II trials (ClinicalTrials ID: NCT02639975)

Table 1. Adenosine receptors and theirs' antagonists

Adenosine receptor	Mechanisms	Antagonists	Tumor types	Study type	Clinical trial IDs	Phases	Outcomes
A_1	Activation of A1 inhibits adenylate cyclase via Gi/o protein interactions leading to reduced cyclic AMP (cAMP) production	1,3-Dipropyl-8-cyclopentylxanthine (DPCPX)	Hepatocellular carcinoma	Preclinical study	Nil	Nil	Nil
A_{2A}	A2A receptor couple with the Gs and Gβ–Gγ protein family leading to activating adenylate cyclase, increasing intracellular cAMP levels	Ciforadenant (CPI-444)	Renal cell carcinoma; prostate cancer	Clinical trial	NCT02655822	I	Completed, prostate cancer may respond to A2AR blockade with ciforadenant.
			Advanced malignancies	Clinical trial	NCT03454451	I	Completed, the treatment has been well-tolerated and exhibited anti-tumor effect.
			Non-small cell lung cancer	Clinical trial	NCT03337698	Ib/II	On going
		Imaradenant (AZD4635)	Advanced solid malignancies	Clinical trial	NCT0398082	I	Completed, no new or unexpected safety concerns were identified.
			Prostate cancer	Clinical trial	NCT04089553	II	AZD4635 with durvalumab or oleclumab demonstrated minimal antitumor activity with a manageable safety profile.
	HIF-1α regulates the overexpression of A2A leading to promoting cell proliferation; activting PI3K/Akt oncogenic pathway	Taminadenant (NIR178/PBF509)	Advanced malignancies	Clinical trial	NCT03549000	I	Terminated
			Lung cancer	Clinical trial	NCT02403193	I	Completed, the treatment has been well-tolerated and exhibited some clinical benefit.
		Etrumadenant (AB928)	Gastrointestinal malignancies	Clinical trial	NCT03720678	I	Completed, the treatment has been well-tolerated without additive toxicity.
			Colorectal cancer	Clinical trial	NCT04660812	I/II	On going
			Breast cancer	Clinical trial	NCT03719326	I	Completed, the treatment has been well-tolerated.
			Advanced malignancies	Clinical trial	NCT03629756	I	Completed, no outcomes have been reported yet.
			Prostate cancer	Clinical trial	NCT04381832	Ib/II	Completed, no outcomes have been reported yet.
			Lung cancer	Clinical trial	NCT04262856	II	On going
			Lung cancer	Clinical trial	NCT03846310	I/Ib	Completed, no outcomes have been reported yet.

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Table 1. Continued.

Adenosine receptor	Mechanisms	Antagonists	Tumor types	Study type	Clinical trial IDs	Phases	Outcomes
		ZM241385	Liver cancer	Preclinical study	Nil	Nil	Nil
		SCH58261	Liver cancer	Preclinical study	Nil	Nil	Nil
A_{2B}	A_{2B} receptor couple with the $G_{s\ and}$ G_q protein family to active adenylate cyclase and increase intracellular cAMP levels	PBF-1129	Lung cancer	Clinical trial	NCT03274479	I	Completed, the treatment has been well-tolerated and safe.
			Lung cancer	Clinical trial	NCT05234307	I	On going
		PSB-1115	Breast cancer	Preclinical study	Nil	Nil	Nil
		PSB603	Breast cancer	Preclinical study	Nil	Nil	Nil
		ATL801	Bladder and breast tumors	Preclinical study	Nil	Nil	Nil
A_3	Activation of A3 inhibits adenylate cyclase via Gi/o protein interactions leading to reduced cAMP production	PBF-1650	Healthy volunteers	Clinical trial	NCT03798236	I	Completed, the compound exhibited well-tolerated and safe.
		PBF-677	Glaucoma	Clinical trial	NCT02639975	I	Completed, the compound is safe.
			Colorectal cancer	Clinical trial	NCT03773952	II	Completed, the compound exhibited well-tolerated and safe.
		thio-Cl-IB-MECA	Leukemia/lung cancers	Preclinical study	Nil	Nil	Nil

(ClinicalTrials ID: NCT03773952), underscoring the therapeutic potential of these A_3 receptor antagonists not only in inflammatory diseases but also potentially in cancers. Additionally, small molecules such as thio-Cl-IB-MECA (2-chloro-N^6-(3-iodobenzyl)-4′-thioadenosine-5′-N-methyluronamide, Fig. 7J) have been developed as selective A_3 receptor antagonists with demonstrated anti-proliferative effects in leukemia and lung cancers.[81] Another compound, N^6-(2,2-diphenylethyl)-2-phenylethynylAdo (Fig. 7K), has shown potential as an antitumor agent.[82] Although some A_3 receptor antagonists have undergone preclinical studies and early-phase clinical trials, clinical research on their application in HCC remains lacking. Therefore, future studies should focus on conducting late-phase clinical trials (phase III and IV) to provide more robust scientific evidence for the commercial application of A_3 receptor antagonists in clinic.

Taken together, a comprehensive review revealed that pyrimidine derivatives, triazine derivatives, coumarin derivatives, benzothiazole derivatives, adenine derivatives, pyrazine-based molecules, xanthine-based molecules, and thioxothiazole-based molecules hold significant potential for the development of adenosine receptor antagonists for clinical applications.[83] Thus, continued preclinical and clinical research into these molecules may facilitate the discovery of potent and selective adenosine receptor antagonists with therapeutic potential, providing a promising avenue for targeted HCC treatments.

CHALLENGES AND PERSPECTIVES

Undoubtedly, ATP-adenosine metabolism and adenosine signaling play critical roles in the development and progression of HCC.[84] As discussed, ATP-adenosine metabolism is primarily produced through ATP breakdown via two pathways: the canonical pathway, where ATP is hydrolyzed by CD39 and CD73 enzymes on the cell surface within the TME, and the noncanonical pathway, where NAD^+ serves as a substrate to generate eADO monophosphate (AMP) via CD38 (an NAD^+ ectohydrolase) and CD203a (ENPP1), followed by metabolism by CD73 enzyme.[85] Consequently, the increased accumulation of adenosine within the TME serves as a critical prerequisite for exploring the pathological mechanisms associated with adenosine in HCC. Ele-

vated adenosine levels are associated with poor prognosis and TME remodeling in HCC.[86] In other words, tumor cells benefit from eADO, as it plays a crucial role in the TME by promoting tumor progression and enabling immune evasion.[87] This review sought to summarize the potential mechanisms linking adenosine to the TME in different aspects of HCC, including tumor growth and survival, immunosuppression, angiogenesis, and metastasis. We found that current research has focused primarily on the immunosuppressive effects of eADO accumulation in the TME of HCC. However, studies on the impact of eADO accumulation on cancer stemness and drug resistance in HCC remain limited. Therefore, further investigation is needed to elucidate how elevated eADO in the TME influences cancer stemness and contributes to drug resistance in HCC. Understanding these mechanisms may reveal new therapeutic targets to counteract adenosine-mediated resistance and improve treatment outcomes for HCC patients.

Additionally, eADO-consuming pathways have been explored. In the TME, eADO has a short half-life, lasting only seconds, as it is swiftly metabolized to inosine by adenosine deaminase (ADA) or transported into the intracellular space of cells by both ENT and CNT.[88] Such a short half-life poses significant challenges for laboratory studies. Intracellular adenosine is metabolized by three different enzymes: ADA, SAHH and adenosine kinase (ADK). The primary intracellular adenosine metabolic pathway involves phosphorylation by ADK to generate AMP, which is subsequently converted to ATP by AK.[89] However, the prognostic implications of these adenosine-catabolizing genes in HCC remain largely unreported, with the exception that elevated expression of equilibrative nucleoside transporter 3 (ENT3) is associated with poor prognosis in HCC patients.[90] Hence, substantial research is warranted to elucidate the precise roles of these adenosine-metabolizing genes in HCC. Investigating these genes may reveal novel prognostic markers or therapeutic targets that may contribute to improving HCC management.

Currently, limited meta-analysis or prognostic risk model related to adenosine signaling in HCC research, indicates that the significance of ATP-adenosine metabolism in the development and progression of HCC has not been fully recognized in clinical practice. In this review, we developed a risk model composed of eight genes significantly associated with adenosine signaling via LASSO-Cox analysis. Most of these genes have been reported to be closely associated with the development and progression of HCC,[91-97] except for the RAPGEF3 gene, which encodes cAMP isoform 1 and is involved in the adenosine signaling pathway. This model was then applied to calculate risk scores for HCC patients in TCGA-LIHC, GSE14520, and ICGC_JP cohorts. Patients were divided into high- and low-risk groups on the basis of the median risk score, and an OS analysis was conducted. The risk model revealed that patients in the high-risk group had worse OS. Furthermore, GSEA of DEGs between the low- and high-risk groups revealed 24 enriched signaling pathways that warrant further investigation. Immune infiltration and single-cell analyses revealed that macrophages, DCs, and monocytes presented increased infiltration scores in the high-risk adenosine signature group. This finding suggests an increased abundance of these immune cells in the TME characterized by elevated adenosine in HCC. Macrophages contribute to immune evasion in HCC by expressing a variety of immunosuppressive molecules, including specific cytokines, chemokines, and enzymes.[98] As antigen-presenting cells, DCs also exert immunosuppressive effects in HCC, with a high density of plasmacytoid DCs linked to increased infiltration of Tregs and poorer prognosis.[99-101] Additionally, monocytes not only directly inhibit T-cell function but also promote the induction of Th17 cells in HCC, further highlighting their immunosuppressive role in HCC.[102,103] Thus, our prognostic risk model not only supports the immunosuppressive effects of adenosine in HCC patients and aids in predicting patient prognosis in a clinical context, but also provides valuable insights for further investigation into the precise biological mechanisms mediated by adenosine within the TME of HCC. However, the present risk model has several limitations, including an insufficient sample size. Larger HCC cohorts are needed to train the model adequately, which will improve its predictive accuracy. Additionally, the impact of adenosine signaling across different stages of HCC requires further investigation to fully elucidate its role in disease progression and potential as a therapeutic target.

Given the crucial roles of adenosine receptors in mediating various aspects of cancer progression, including tumor growth, angiogenesis, immune suppression, and metastasis, targeting these receptors represents a promising strategy for the treatment of HCC.[104] In this review, we compre-

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hensively summarize the functions of adenosine receptors across various cancer types and provide an overview of the designed adenosine receptor antagonists and their corresponding clinical trials, including combination therapies involving adenosine receptor antagonists and other immunotherapeutic agents (such as anti-PD-1 antibodies). However, these antagonists still encounter several limitations and challenges in the clinical application for HCC treatment. First, although a few antagonists have completed early-phase clinical trials in HCC patients, there is still a lack of late-phase clinical trial data to further validate their clinical efficacy and safety. Moreover, while a few clinical trials have investigated the efficacy of these antagonists in combination with immune checkpoint inhibitors, such as PD-1 and PD-L1, current research has mainly focused on the combination effects with only these two immune targets, which limits their broader applicability. Therefore, future studies should further evaluate the potential of these antagonists in combination with other immune targets, such as CTLA-4,VEGF, etc., to expand their application in HCC treatment and enhance their clinical application. This highlights the urgent need for further preclinical investigations and clinical trials to develop more effective and targeted adenosine receptor antagonists, providing novel strategies and options for HCC therapy.

In conclusion, this review not only provides a comprehensive overview of ATP-adenosine metabolism, the pathological mechanisms of adenosine within the TME, and the preclinical and clinical trials of adenosine receptor antagonists, but also develops a prognostic risk model associated with adenosine signaling to predict patient prognostic outcomes and offers valuable insights into the therapeutic potential of targeting adenosine signaling in HCC treatment. Undeniably, this model fills a critical gap in the clinical research on the adenosine signaling pathway in HCC and offers a new perspective for investigating the pathological mechanisms of adenosine signaling in HCC. Although our understanding of ATP-adenosine metabolism and adenosine signaling in HCC remains incomplete, the development of new therapeutic strategies targeting ATP-adenosine metabolism and adenosine signaling holds great promise for HCC treatment.

Authors' contribution

Huihai Yang: Conceptualization, Formal analysis, Investigation, Methodology, Writing-original draft, Martina Mang Leng Lei: Formal analysis, Investigation, Longfei Xie: Methodology, Resources, Yinuo Shou: Investigation, Writing-original draft, Terence Kin Wah Lee: Funding acquisition, Supervision, Writing-review & editing.

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Conflicts of Interest

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIAL

Supplementary material is available at Clinical and Molecular Hepatology website (http://www.e-cmh.org).

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