Research article

CD38 symmetric dimethyl site R58 promotes malignant tumor cell immune escape by regulating the cAMP-GSK3β-PD-L1 axis

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A B S T R A C T

In recent years, immunotherapy has emerged as an effective approach for treating tumors, with programmed cell death ligand 1 (PD-L1)/programmed cell death protein-1 (PD-1) immune checkpoint blockade (ICB) being a promising strategy. However, suboptimal therapeutic efficacy limits its clinical benefit. Understanding the regulation mechanism of PD-L1 expression is crucial for improving anti-PD-L1/PD-1 therapy and developing more effective tumor immunotherapy. Previous studies have revealed that resistance to PD-L1/PD-1 blockade therapy arises from the upregulation of CD38 on tumor cells induced by ATRA and IFN-β, which mediates the inhibition of CD8$^+$ T cell function through adenosine receptor signaling, thereby promoting immune evasion.Yet, the precise role of CD38 in regulating PD-L1 on malignant tumor cells and its impact on CD8$^+$ T cells through PD-L1 remain unclear. Here, we demonstrate that CD38 is highly expressed in malignant tumors (lung cancer, nasopharyngeal carcinoma, cervical cancer) and upregulates PD-L1 protein expression, impairing CD8$^+$ T cell function. Mechanistically, CD38 phosphorylates GSK3β via the adenosine-activated cAMP-PKA signaling pathway, leading to GSK3β inactivation and enhanced PD-L1 stability and expression, facilitating tumor immune escape. Furthermore, we identify PRMT5 as a novel CD38-interacting molecule that symmetrically dimethylates CD38 arginine position 58, augmenting PD-L1 stability and expression through the ADO-cAMP-GSK3β signaling axis. This inhibits CD8$^+$ T cell-mediated tumor cell killing, enabling tumor cells to evade immune surveillance. Our findings suggest that targeting the CD38 R58 site offers a new avenue for enhancing anti-PD-L1/PD-1 therapy efficacy in tumor treatment.

1. Introduction

In recent years, immune checkpoint blockade has emerged as a promising approach for the treatment of various malignant tumors,

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including nasopharyngeal carcinoma (NPC) and non-small cell lung cancer (NSCLC) [1–3]. Among these, the blockade of programmed cell death ligand 1 (PD-L1/PD-1) immune checkpoints has demonstrated efficacy in tumor immunotherapy [4,5]. PD-L1 is typically expressed on tumor cells as well as immune cells such as macrophages and dendritic cells. Its interaction with the PD-1 receptor on T cells suppresses T cell-mediated tumor killing activity. Tumor cells exploit upregulation of PD-L1 to evade immune surveillance and evade attacks by T cells [6–9]. Consequently, blocking the PD-L1/PD-1 axis has emerged as a promising therapeutic strategy. Currently, antibodies targeting PD-1 and PD-L1 proteins have been rapidly developed and utilized in treating various tumor types, yielding some therapeutic benefits. However, only a subset of patients derive clinical benefits from anti-PD-1/PD-L1 treatment, with the majority displaying primary or acquired resistance to PD-1 therapy [6,10–13]. Hence, elucidating the regulatory mechanisms underlying PD-L1 expression in tumor cells is crucial for improving anti-PD-L1/PD-1 therapy and enhancing its clinical efficacy. It has been observed that resistance to PD-L1/PD-1 blockade therapy arises from the upregulation of CD38 on tumor cells induced by ATRA and IFN-β, which mediates the inhibition of $CD8^+$ T cell function through adenosine receptor signaling, thereby promoting immune evasion [14]. Consequently, blockade of CD38 holds promise as an effective strategy to overcome resistance to anti-PD-1/PD-L1 therapy. However, the precise mechanism by which CD38 regulates PD-L1 expression in solid tumors remains largely unexplored.

CD38, a single-stranded transmembrane glycoprotein situated on the cell membrane, is recognized as a multifunctional membrane molecule [15]. It exhibits widespread expression across immune cells, including activated B-cells, T-cells, NK-cells, and macrophages [16–18]. Our initial investigations have revealed that CD38 exerts significant effects on the proliferation and metabolic pathways of NPC and cervical cance (CC) cells [19–25]. Specifically, CD38 promotes cell proliferation, facilitates the transition of NPC cells from G0-G1 to S phase, and influences intracellular concentrations of key metabolites such as ATP, lactate, and cyclic adenosine mono-phosphate (cAMP) in NPC cells. Similarly, CD38 also plays a role in promoting CC cell proliferation and modulating cell metabolism. These findings underscore the pivotal role of CD38 in the development of malignant tumors such as NPC and cervical cancer. As an extracellular enzyme, CD38 catalyzes the hydrolysis of NAD^+ to ADP-ribose (ADPR) and cADPR, which are essential for regulating extracellular metabolites, intracellular calcium levels, cell adhesion, and signal transduction pathways [26]. Emerging evidence suggests that CD38 is intricately involved in modulating the tumor immune microenvironment. For instance, the non-classical adenosine pathway guided by CD38/CD203a facilitates the production of immunosuppressive adenosine, which is critical for main-taining tissue homeostasis and preventing excessive immune responses [27,28]. Moreover, CD38 expressed on T cells regulates NAD^+ levels to convert cells into mixed effector Th1/17 cells, thereby enhancing adoptive T cell therapy [26]. Notably, in the context of NSCLC, resistance to anti-PD-1/PD-L1 therapy has been attributed to the upregulation of CD38 in tumor cells, which suppresses $CD8^+$ T cell function via adenosine receptor signaling [14]. Consequently, the role and mechanisms underlying CD38-mediated immune escape and immunotherapy in solid tumors, including NSCLC, CC, and NPC, warrant further investigation. It is crucial to determine whether CD38 participates in immune escape by regulating the expression of PD-L1 on tumor cells, as this may have significant implications for the development of novel therapeutic strategies targeting CD38 in cancer immunotherapy.

In our investigation, we observed a positive association between the expression levels of CD38 and PD-L1 in various solid tumors, including NSCLC, CC, and NPC. Further exploration revealed that CD38 facilitates the stability and expression of PD-L1 protein through the ADO-cAMP-GSK3β signaling axis. Additionally, we identified PRMT5 as an interacting partner of CD38, which influences the symmetric dimethylation of arginine at the R58 site of CD38. Notably, we found that the CD38 R58 site, via the ADO-cAMP-GSK3β signaling axis, enhances the expression of PD-L1 in tumor cells, thereby promoting tumor immune evasion. Our findings shed light on the regulatory role of symmetric dimethylation at the CD38 R58 site in modulating tumor cell immune escape through the ADO-cAMP-GSK3β-mediated regulation of PD-L1 expression. Importantly, this study offers a novel strategy for enhancing the efficacy of tumor immunotherapy by targeting CD38.

2. Materials and methods

2.1. Cell lines and culture

We utilized the human NSCLC cell line A549, CC cell line CaSki, NPC cell line HNE2, and human renal epithelial cell line HEK293T, all preserved in our laboratory. The cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10 % fetal bovine serum (Gibco) and 1 % penicillin-streptomycin (Macgene). Cultures were maintained in a humidified atmosphere with 5 % CO2 at 37 °C. Mycoplasma testing was performed routinely.

2.2. Reagents and antibodies

The proteasome inhibitor MG132, protein synthesis inhibitor actinomycin (CHX), and PRMT5 inhibitor GSK591 (S8111) were procured from Selleck, while the PKA inhibitor H-89 (HY-15979) was sourced from MCE. For immunoprecipitation, we utilized Pierce™ Protein A/G plus agarose (Thermo Fisher, USA), anti-FLAG beads (F2426), and anti-HA beads (IP0010) from Sigma Aldrich, USA. All antibodies utilized in this study are detailed in Supplementary Table S1.

2.3. siRNA and plasmid transfection

siRNAs targeting PRMT5, CD38, GSK3β, and a negative control were procured from RiboBio, with detailed information available in Supplementary Table S2. Transfections were performed using the riboFECT CP Transfection Kit (RIBOBIO Co., China) or Neofect DNA Transfection Reagent (Neofect Biotech, China) in accordance with the respective manufacturer's instructions.

2.4. RNA extraction and quantitative real-time PCR

Total RNA extraction was carried out using the Total RNA extraction reagent (Invitrogen, 15596026). Subsequently, the isolated RNA was reverse transcribed to cDNA using HiScript II Q RT SuperMix (Vazyme, R323-01). Quantitative real-time PCR (qRT-PCR) was performed with Universal SYBR qPCR Master Mix (Vazyme, Q511-02). GAPDH served as the internal control, and data normalization was accomplished using the 2-ΔΔCt method. Primer sequences are detailed in online Supplementary Table S3.

2.5. Western blot analysis and immunoprecipitation

Protein blot analysis was conducted following previously established procedures [23]. For immunoprecipitation, cells were harvested using IP lysis buffer supplemented with protease and/or phosphatase inhibitors. The supernatant lysate was immunoprecipitated overnight at 4 °C using designated primary antibodies and Protein A/G agarose beads (Thermo Fisher, USA, 20423). On the following day, the immune complex underwent six washes with IP lysis buffer. The proteins were then boiled for 10 min in sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer at 95 °C, followed by protein imprinting analysis. Quantitative analysis was performed using Image J software (V1.52). Detailed information on antibodies is available in online Supplementary Table S1.

2.6. Liquid-chromatography coupled to tandem mass spectrometry (LC-MS/MS)

The purified protein complex eluate underwent concentration using IP beads (Bimake) and subsequent separation by SDS-PAGE. Staining with Coomassie Brilliant Blue (Beyotime) allowed the identification of bands different from the control, which were excised. In-gel reduction, alkylation, and overnight trypsin digestion (Gibco) at 37 °C followed. The resulting peptides were dried, resuspended, and analyzed in MS-compatible buffer. For protein interaction identification, mixtures were subjected to analysis using LTQ Orbitrap Velos MS (Thermo Fisher) coupled with an UltiMate RSLC Nano LC System (Dionex). Proteins were identified using Proteome Discoverer 1.4 software (Thermo Fisher), with data imported for exploration of the UniProtKB/Swiss-Prot database. Mass tolerances for precursors and fragments were set at 10 ppm and 0.8 Da, respectively. Peptide data with a false discovery rate <1 % (q < 0.01) were discarded. For identifying protein modification sites, peptide segments were separated by an ultra-high performance liquid chromatography system and injected into an NSI ion source before analysis by Thermo Scientific™ Q Exacte™ Plus mass spectrometry, according to the instrument specifications.

2.7. Pulse-chase assay

To assess the impact of CD38 on the stability of PD-L1 protein, A549 cells transfected with specific siRNA were subjected to a pulse-chase assay. Cells were treated with the protein synthesis inhibitor CHX (50 μg/mL) for a designated duration before collection, followed by subsequent analysis through Western blot.

2.8. Immunofluorescence staining

Cells were seeded on glass coverslips at approximately 50 % density and fixed with 4 % paraformaldehyde for 20 min at room temperature. After permeabilization with 0.3 % Triton X-100, cells were blocked with 5 % bovine serum albumin (BSA) for 1 h at room temperature. For tumor tissue, fresh xylene was used to deparaffinize the paraffin sections, which were then hydrated in gradient alcohol. The citric acid buffer was used for antigen retrieval at 90 °C–100 °C for 30 min to which an appropriate amount of endogenous peroxidase blocker was added. The standard goat serum working solution was sealed at room temperature for 15 min. Primary antibodies were diluted in a closed buffer and incubated overnight at 4 °C. Subsequently, the corresponding secondary antibodies were added to the samples and incubated at room temperature for 30 min. To visualize cell nuclei, 4,6-diamidino-2-phenylindole (DAPI) was used. Cell imaging was performed using a confocal laser scanning microscope (UltraView Vox; PerkinElmer, USA) and analyzed with Volocity software version 6.1.1 (PerkinElmer). Detailed information on antibodies is provided in Supplementary Table S1.

2.9. Determination of 5-ethynyl-2′-deoxyuridine (EdU)

Cells were exposed to the 5-ethynyl-2′-deoxyuridine assay (EdU; Ribobio) for 2 h. Following the manufacturer's guidelines, cells underwent three washes with PBS. Subsequently, cells were treated with a 0.5 % Triton X-100 solution for 20 min at room temperature, shielded from light. For the EdU reaction, cells were incubated with 100 μL of a 1X Apollo reaction mixture away from light for 30 min. After three additional washes with PBS, resuspended cells were prepared for flow cytometry detection and analysis.

2.10. Preparation and culture of primary T cells

Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll separation solution (Cytova, 45-001-749). CD3/CD28 amplification magnetic beads (Mitenyi Biotec) were introduced to induce T lymphocyte proliferation. Activated T lymphocytes were cultivated in 1640 complete culture medium supplemented with 15 ng/mL IL-2 (Invitrogen, PHC0026), 5 ng/mL IL-7 (Invitroen, PHC0073), and 10 mL IL-15 (Invitroen, PHC9151).

2.11. Flow cytometry analysis

T Cell Apoptosis Detection: Co-culture transfected tumor cells with activated primary human T cells. T cell apoptosis was assessed following the manufacturer's instructions using the Apoptosis Detection Kit (Biotech, AP101) and fluorescently labeled CD3 and CD8 antibodies. This kit stains Annexin V and PI, allowing identification of early apoptotic cells (Annexin V+, PI-) and late apoptotic cells (Annexin V+, PI+). Detection was conducted using DxPAthenaTM flow cytometry (Cytek, USA), and FlowJo software (RRID: SCR:008520) was employed for data analysis. Antibodies are detailed in Supplementary Table S1.

Reactive Oxygen Species Assay: Tumor cells were incubated with the DCFH-DA fluorescent probe. Collected cells were analyzed for reactive oxygen species using DxPAthenaTM flow cytometry (Cytek, USA).

2.12. Enzyme-linked immunosorbent assay (ELISA)

Cytokine Assay: Transfected tumor cells were co-cultured with activated T cells, and culture supernatants were collected for cytokine assays. The ELISA kit from JiangLai Biologicals was utilized for detecting IL-2, GZMB, IFN-γ, and PRF1. Measurements were performed using a plate reader with SoftMax® Pro 7 software, version 7.1.0 (Molecular Devices). All experiments adhered to the manufacturer's instructions.

Determination of cAMP Level, NAD Level, and ADO Content: Levels of cAMP, NAD, and adenosine (ADO) were determined
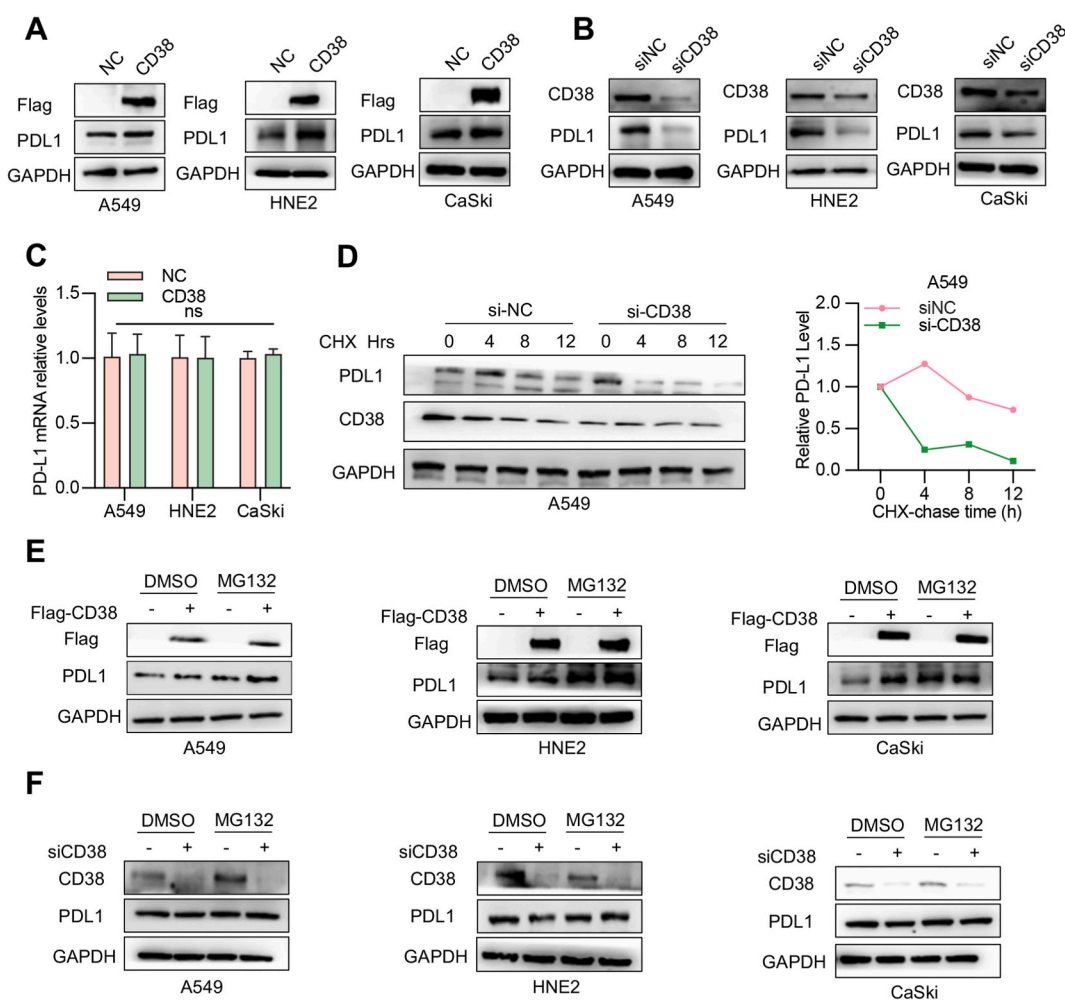

Fig. 1. CD38 upregulates PD-L1 expression in tumor cells by inhibiting proteasome-mediated protein degradation
(A) Immunoblotting analysis of PD-L1 expression in A549, HNE2, and CaSki cells transfected with NC or Flag-CD38. (B) Immunoblotting analysis of PD-L1 expression in A549, HNE2, and CaSki cells transfected with siNC or siCD38. (C) qPCR analysis of *PD-L1* mRNA expression levels in A549, HNE2, and CaSki cells transfected with NC or Flag-CD38. (D) Immunoblotting analysis of PD-L1 expression in A549 cells with or without CD38 knockdown treated with CHX. (E) Immunoblotting analysis of PD-L1 expression in A549, HNE2, and CaSki cells transfected with NC or Flag-CD38 and treated with or without MG132. (F) Immunoblotting analysis of PD-L1 expression in A549, HNE2, and CaSki cells treated with or without MG132 and transfected with siCD38 or siNC. Results are expressed as mean ± SD (n = 3).

following the manufacturer's protocols. The Human Cyclic Adenosine Monophosphate (cAMP) ELISA Kit from Jianglai Biologicals, the NAD$^+$/NADH Assay Kit (Biocloud, S0175), and the Human Adenosine ELISA Kit from Jianglai Biologicals were employed for these assessments.

2.13. Animal studies

Transfected tumor cells (3×10^6) were subcutaneously injected into female BALB/c nude mice in a volume of 200 μL. Measurements of tumor size and body weight were recorded on indicated dates, with tumor volume calculated as length $\times$ width$^2 \times$ (π/6). At the experiment's conclusion, subcutaneous tumors were excised, weighed, photographed, and subjected to further analysis.
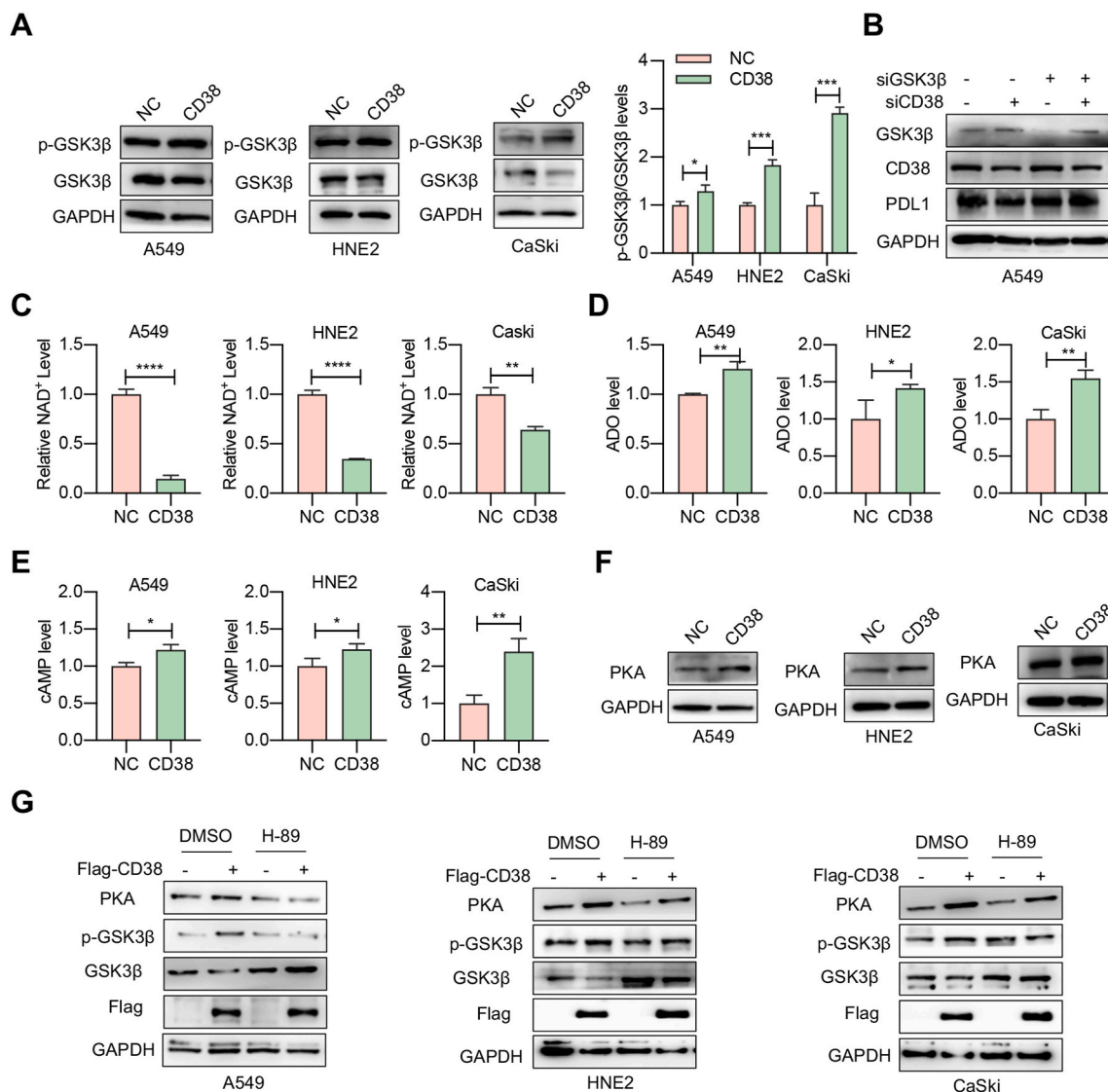

Fig. 2. CD38 promotion of PD-L1 protein stability is mediated by ADO-cAMP promotion of GSK3β phosphorylation

(A) Immunoblotting analysis of p-GSK3β/S9 and GSK3β expression in A549, HNE2, and CaSki cells transfected with NC or Flag-CD38. (B) Immunoblotting analysis of p-GSK3β/S9 and CD38 expression in A549 cells expressing siNC or siCD38 with or without siGSK3β. (C) Detection of NAD$^+$ levels in A549, HNE2, and CaSki cells transfected with NC or Flag-CD38. (D) Detection of adenosine levels in A549, HNE2, and CaSki cells transfected with NC or Flag-CD38. (E) Detection of cAMP levels in A549, HNE2, and CaSki cells transfected with NC or Flag-CD38. (F) Immunoblotting analysis of PKA expression in A549, HNE2, and CaSki cells transfected with NC or Flag-CD38. (G) Immunoblotting analysis of PKA expression in A549, HNE2, and CaSki cells transfected with NC or Flag-CD38 and treated with or without PKA inhibitor H-89. Results are expressed as mean $\pm$ SD (n = 3).

2.14. Statistical analysis

Statistical charts were generated using GraphPad Prism 8 software. T-tests were employed to assess significant differences between any two groups, while One-Way ANOVA was used to analyze variations in data among two or more groups. Each experiment was conducted a minimum of three times. A significance level of $P < 0.05$ was considered statistically significant, denoted as follows: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

3. Results

3.1. CD38 upregulates PD-L1 expression in tumor cells by inhibiting proteasome-mediated protein degradation

In our preliminary research, we observed aberrant expression of CD38 in solid tumors such as NPC [19–22]and CC [23–25]. Furthermore, studies have indicated that NSCLC treated with PD-1/PD-L1 blocking antibodies can develop resistance, attributed to all-trans retinoic acid (ATRA) and interferon-beta (IFN-β) inducing upregulation of CD38. This upregulated CD38 subsequently inhibits the function of CD8$^+$ T cells via adenosine receptor signaling [14]. However, the precise molecular mechanism through which CD38 regulates PD-L1 expression in solid tumor cells remains unexplored. To elucidate the relationship between CD38 and PD-L1 in solid tumor cells, we initially examined the effect of CD38 on PD-L1 expression. Our results demonstrated that overexpression of CD38 significantly enhanced PD-L1 protein expression in NSCLC, CC, and NPC cells (Fig. 1A). Conversely, knockdown of CD38 led to a notable decrease in PD-L1 protein expression (Fig. 1B). Notably, overexpression of CD38 did not significantly alter the mRNA levels of *PD-L1* in tumor cells (Fig. 1C), suggesting that CD38 regulates PD-L1 expression at the post-transcriptional level. Subsequently, to delineate the impact of CD38 on PD-L1 stability, we inhibited the synthesis of new proteins by adding actinomycin (CHX) and monitored PD-L1 expression at various time points. Our findings revealed that knockdown of CD38 significantly shortened the half-life of PD-L1 (Fig. 1D), indicating that CD38 modulates the stability of PD-L1 protein. Additionally, treatment with the proteasome inhibitor MG132 augmented the CD38-induced upregulation of PD-L1 in tumor cells (Fig. 1E), and MG132 reversed the CD38-induced downregulation of PD-L1 (Fig. 1F). Collectively, these results suggest that CD38 promotes PD-L1 expression in tumor cells by inhibiting proteasome-mediated protein degradation.

3.2. CD38 promotes PD-L1 protein stability by phosphorylating GSK3β through ADO-cAMP

Previous studies have demonstrated that PD-L1 is phosphorylated by GSK3β, leading to subsequent degradation by the proteasome. Phosphorylation of GSK3β results in its own inactivation, thereby stabilizing PD-L1 expression [29,30]. Therefore, we initially investigated whether CD38 influenced GSK3β phosphorylation levels in A549, CaSki, and HNE2 cells. Our results revealed that overexpression of CD38 significantly upregulated phosphorylation of GSK3β at serine 9 (S9), accompanied by a decrease in total GSK3β protein levels (Fig. 2A). Additionally, we assessed the impact of CD38 on *GSK3β* mRNA levels via qRT-PCR and found no significant alteration induced by CD38 (Supplementary Fig. 1A). To further elucidate whether GSK3β mediates the effect of CD38 on PD-L1 protein expression, we designed siRNA targeting GSK3β and confirmed its knockdown efficiency through protein blotting and qRT-PCR experiments (Supplementary Fig. 1B). Subsequently, we transfected si-GSK3β into A549 cells with or without CD38 knockdown and assessed PD-L1 protein expression levels. Remarkably, silencing of GSK3β rescued the reduction in PD-L1 induced by CD38 knockdown (Fig. 2B). These findings strongly support the notion that CD38 enhances PD-L1 protein stability through the phosphorylation of GSK3β.

To investigate the potential mechanisms underlying CD38-mediated effects on GSK3β phosphorylation, we initially explored whether CD38 physically interacts with GSK3β. However, immunoprecipitation coupled with protein blotting analysis revealed no interaction between CD38 and GSK3β (Supplementary Fig. 1C). Given CD38's role as an extracellular enzyme in adenosine production, we hypothesized that CD38-mediated GSK3β phosphorylation might be regulated through the cAMP-PKA axis. Adenosine, generated by CD38, binds to G protein-coupled receptors, leading to the production of the second messenger cAMP, which activates PKA to phosphorylate its substrates, including GSK3β [31]. To test this hypothesis, we initially examined the effects of CD38 on extracellular NAD levels and adenosine content in A549, HNE2, and CaSki cells. Our results indicated that CD38 overexpression decreased extracellular NAD levels while increasing adenosine content (Fig. 2C and D). Furthermore, qRT-PCR analysis demonstrated that CD38 overexpression inhibited *ADORA1* mRNA levels but promoted *ADORA2a* and *ADORA2b* mRNA levels (Supplementary Fig. 1D). Given adenosine's role in stimulating cAMP generation through G protein-coupled receptors [32], we assessed the effect of CD38 on cAMP levels in A549, CaSki, and HNE2 cells. Remarkably, CD38 overexpression significantly increased cAMP production (Fig. 2E). Additionally, protein blotting experiments revealed that CD38 overexpression upregulated PKA protein expression in A549, CaSki, and HNE2 cells (Fig. 2F). Further, treatment with the PKA inhibitor H-89 blocked CD38-mediated GSK3β phosphorylation (Fig. 2G), suggesting that CD38-mediated phosphorylation of GSK3β is regulated through the cAMP-PKA axis. Collectively, these findings confirm that CD38 upregulates PD-L1 expression in malignant tumor cells through the ADO-cAMP-GSK3β signaling axis.

3.3. Impact of symmetric dimethylation of CD38 R58 site on PD-L1 expression

To delve deeper into the molecular mechanism underlying CD38's influence on PD-L1 expression in tumor cells, we conducted immunoprecipitation assays after overexpressing Flag-CD38 in CaSki cells, followed by mass spectrometry analysis to identify CD38-interacting proteins (Fig. 3A). Notably, we observed a distinct band at 72 kDa, revealing 34 potentially interacting molecules

(Supplementary Table S4). Among these, five specific proteins were detected in the Flag lane, with PRMT5 exhibiting the highest abundance, capturing our interest (Fig. 3B–Supplementary Table S5). To validate the interaction between CD38 and PRMT5, we conducted immunoprecipitation assays in various cancer cell lines, including A549, CaSki, HNE2, and 293T cells. Protein blotting results confirmed the interaction between CD38 and PRMT5 (Fig. 3C). Co-transfection of Flag-CD38 and HA-PRMT5 into CaSki cells for immunoprecipitation further supported this interaction (Fig. 3D). Additionally, immunofluorescence experiments demonstrated evident co-localization between CD38 and PRMT5 (Fig. 3E). In conclusion, the above results provide experimental evidence for the existence of interaction between CD38 and PRMT5 in a variety of cancer cells.To explore the reciprocal regulatory relationship between CD38 and PRMT5, we designed PRMT5 siRNA sequences and identified the most effective knockdown sequence through protein blotting (Supplementary Fig. 2A). Subsequent knockdown of PRMT5 in A549, CaSki, and HNE2 cells led to a significant reduction in CD38 protein expression (Fig. 3F). Conversely, overexpression of PRMT5 in A549 cells significantly upregulated CD38 protein expression (Supplementary Fig. 2B). However, alterations in CD38 expression did not significantly affect PRMT5 protein levels in A549, CaSki, and HNE2 cells (Fig. 3G). Similarly, CD38 knockdown in A549 cells did not impact PRMT5 protein expression (Supplementary Fig. 2C). Additionally, qRT-PCR analysis revealed that PRMT5 did not affect *CD3*8 mRNA levels (Supplementary Fig. 2D). Overall, our findings suggest that PRMT5 is a novel interacting partner of CD38 and modulates CD38 protein expression levels, shedding light on a previously unrecognized regulatory axis in tumor cells.

PRMT5, a protein methyltransferase, predominantly catalyzes intracellular arginine symmetric dimethylation (SDMA) modifications [33]. To elucidate the significance of PRMT5's methyltransferase activity in CD38 protein expression, we treated A549, CaSki, and HNE2 cells with GSK591, a potent and selective PRMT5 chemical inhibitor [34]. Remarkably, inhibition of PRMT5 methylation activity led to a significant downregulation of CD38 protein levels (Fig. 4A), with CD38 protein expression decreasing with increasing
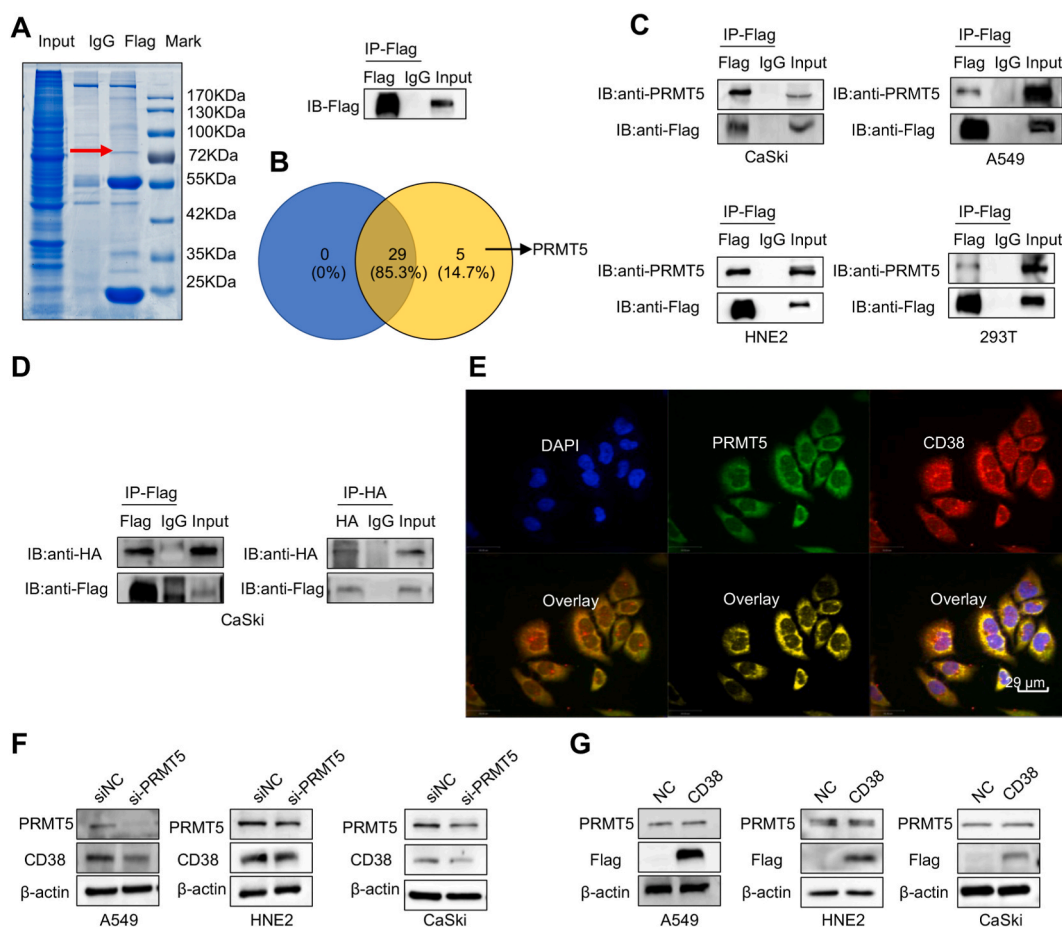

Fig. 3. Physical interaction of CD38 with PRMT5
(A) Immunopurification of cell extracts of CaSki cells expressing Flag-CD38 using an anti-Flag affinity column followed by mass spectrometry analysis. (B) Molecular distribution maps and detailed results of mass spectrometry analysis. (C) Immunoblotting analysis of Flag expression in various cell lines. (D) Co-transfection of Flag-CD38 and HA-PRMT5 into CaSki cells followed by immunoblotting using anti-HA and anti-Flag antibodies. (E) Co-localization analysis of exogenous Flag-CD38 and HA-PRMT5 in CaSki cells using a laser confocal microscope. (F) Immunoblotting analysis of PRMT5 and CD38 expression in A549, HNE2, and CaSki cells transfected with siNC or siPRMT5. (G) Immunoblotting analysis of PRMT5 and CD38 expression in A549, HNE2, and CaSki cells transfected with NC or Flag-CD38.

GSK591 concentration (Fig. 4B). These findings underscore the pivotal role of PRMT5 methyltransferase activity in modulating CD38 protein expression. To ascertain whether PRMT5 symmetrically dimethylates CD38, we performed immunoprecipitation using SYM10 antibody (specific for symmetric dimethylation) and observed that CD38 can indeed be symmetrically dimethylated by PRMT5 (Fig. 4C). To further exploration into the specific arginine residues methylated by PRMT5 in CD38, we introduced si-NC and si-PRMT5 into Flag-CD38-overexpressing CaSki cells to confirm knockdown efficiency, and revealed a dimethylation site at arginine 58 (R58) through mass spectrometry analysis (Fig. 4D and E). Subsequently, we constructed a CD38-R58G mutation vector which was replaced the arginine at this site with glycine (Fig. 4F). Immunoprecipitation results demonstrated that knocking down PRMT5 in cells transfected with the CD38 vector led to a decrease in CD38 symmetrical dimethylation levels. Similarly, in cells transfected with the CD38-R58G vector, the level of CD38 symmetrical dimethylation decreased. Interestingly, after PRMT5 knockdown, the level of CD38 symmetrical dimethylation remained unchanged (Fig. 4G). These results elucidate PRMT5's role in inducing symmetric dimethylation at the R58 site of CD38.

To assess the impact of symmetric dimethylation at the R58 site of CD38 on tumor progression, we treated CD38-overexpressing tumor cells with GSK591, a PRMT5 inhibitor. Remarkably, GSK591 treatment significantly suppressed cell proliferation activity (Supplementary Fig. 2E), indicating a potential role of PRMT5-mediated CD38 dimethylation in promoting tumor cell growth. Similarly, tumor cells transfected with the CD38-R58G mutation exhibited significantly inhibited proliferation ability (Supplementary Fig. 2F), suggesting that disruption of R58 dimethylation attenuates the oncogenic potential of CD38. Moreover, analysis of cell apoptosis and reactive oxygen species (ROS) levels revealed that tumors overexpressing CD38-R58G displayed increased cell apoptosis and a rightward shift in ROS compared to those overexpressing CD38-WT (Supplementary Figs. 2G and 2H). In addition, in vivo experiments found that compared with the CD38-WT group, the CD38-R58G group significantly inhibited tumor volume and tumor weight (Fig. 4H–J). The immunofluorescence results showed that CD38 promoted the expression of PDL1, while CD38-R58G inhibited this effect (Fig. 4K and L). This indicates that CD38 R58 significantly inhibits tumor growth in vivo. These findings collectively suggest that PRMT5-mediated CD38-R58 methylation enhances its carcinogenic function, highlighting the potential significance of this post-translational modification in tumor progression.

To investigate whether symmetric dimethylation of the CD38 R58 site influences PD-L1 protein expression, we transfected cells with empty, Flag-CD38, and Flag-CD38-R58G vectors, followed by protein blotting analysis. The results revealed that CD38 undergoes symmetric dimethylation via the R58 site, consequently affecting the protein expression of PD-L1 (Fig. 4M). These findings provide compelling experimental evidence supporting the notion that PRMT5 specifically methylates CD38 at the Arg-58 residue, thereby influencing the protein expression level of PD-L1.

3.4. CD38 R58 upregulates PD-L1 expression and promotes tumor cell immune escape via the ADO-cAMP-GSK3β signaling axis

Given the observed impact of CD38 R58 methylation on PD-L1 expression, we sought to confirm whether CD38 R58 upregulates PD-L1 expression through the ADO-cAMP-GSK3β signaling axis, thereby promoting tumor cell immune escape. To investigate this, we examined the effect of CD38 R58 on extracellular NAD levels and adenosine content in tumor cells. Our results demonstrated that the CD38-R58G mutation inhibited the reduction of extracellular NAD levels and the increase of adenosine content compared to the overexpression of CD38-WT (Fig. 5A and B). Furthermore, we evaluated the effect of CD38 R58 on intracellular cAMP concentration in tumor cells, revealing that the CD38 R58G mutation suppressed cAMP production compared to CD38-WT overexpression (Fig. 5C). Notably, the CD38 R58G mutation restored the protein expression of PKA, leading to reduced GSK3β phosphorylation levels and restored total GSK3β levels (Fig. 5D). To further elucidate the role of CD38 R58 in affecting PD-L1 protein expression via GSK3β, we transfected si-GSK3β into A549, CaSki, and HNE2 cells overexpressing CD38-WT and CD38-R58G, and assessed the expression level of PD-L1. The results indicated that GSK3β silencing rescued the reduction in PD-L1 caused by CD38-R58G (Fig. 5E and F). In summary, our findings support the notion that CD38 R58 mediates PD-L1 expression through the ADO-cAMP-GSK3β signaling axis, thereby promoting tumor cell immune evasion.

The binding of PD-L1 on tumor cells to PD-1 on T cells inhibits the effector function of T cells [35,36]. To determine whether CD38 R58 induces T cell apoptosis by promoting PD-L1 expression, we transfected CD38-WT and CD38-R58G into A549, CaSki, and HNE2 cells, and then co-cultured them with activated human primary T cells. The results demonstrated that CD38 WT increased the degree of apoptosis in primary T cells, while CD38 R58 could restore the apoptosis rate of primary T cells (Fig. 6A and B). T cells can secrete a large number of cytokines (such as IFN-γ, IL-2, and IL-10) to promote the cytotoxic activity of CD8$^+$ T cells to kill tumor cells [35,37]. Therefore, we detected the levels of IFN-γ, TNF-α, PRF1, and GZMB in T cells. The results showed that CD38 WT reduced the secretion of these cytokines from T cells, while CD38-R58G reversed this effect (Fig. 6C–F). Additionally, we evaluated the mRNA levels of *IFN-γ*, *IL-2*, and *GZMB* in T cells co-cultured with tumor cells. The results indicated that CD38-WT reduced the mRNA levels of *IFN-γ*, *IL-2*, and *GZMB* in T cells, while CD38-R58G could reverse this effect (Fig. 6G). In summary, our findings suggest that CD38 R58 upregulates PD-L1 expression and promotes tumor cell immune escape through the ADO-cAMP-GSK3β signaling axis.

4. Discussion

CD38, traditionally recognized as an immune molecule due to its expression on activated immune cells, has recently gained attention for its role in solid tumors such as NSCLC, NPC, and CC. Our preliminary investigations revealed aberrant expression of CD38 in these malignancies [19–25]. Notably, we observed that CD38 facilitates NPC cell proliferation, promotes cell cycle progression from G0-G1 to S phase, and modulates intracellular ATP, lactate, and cyclic adenosine monophosphate levels. Additionally, CD38 influences the expression of key metabolic pathway molecules and exerts its pro-cancer effects in NPC by affecting ZAP70 [19–22]. Similarly, in
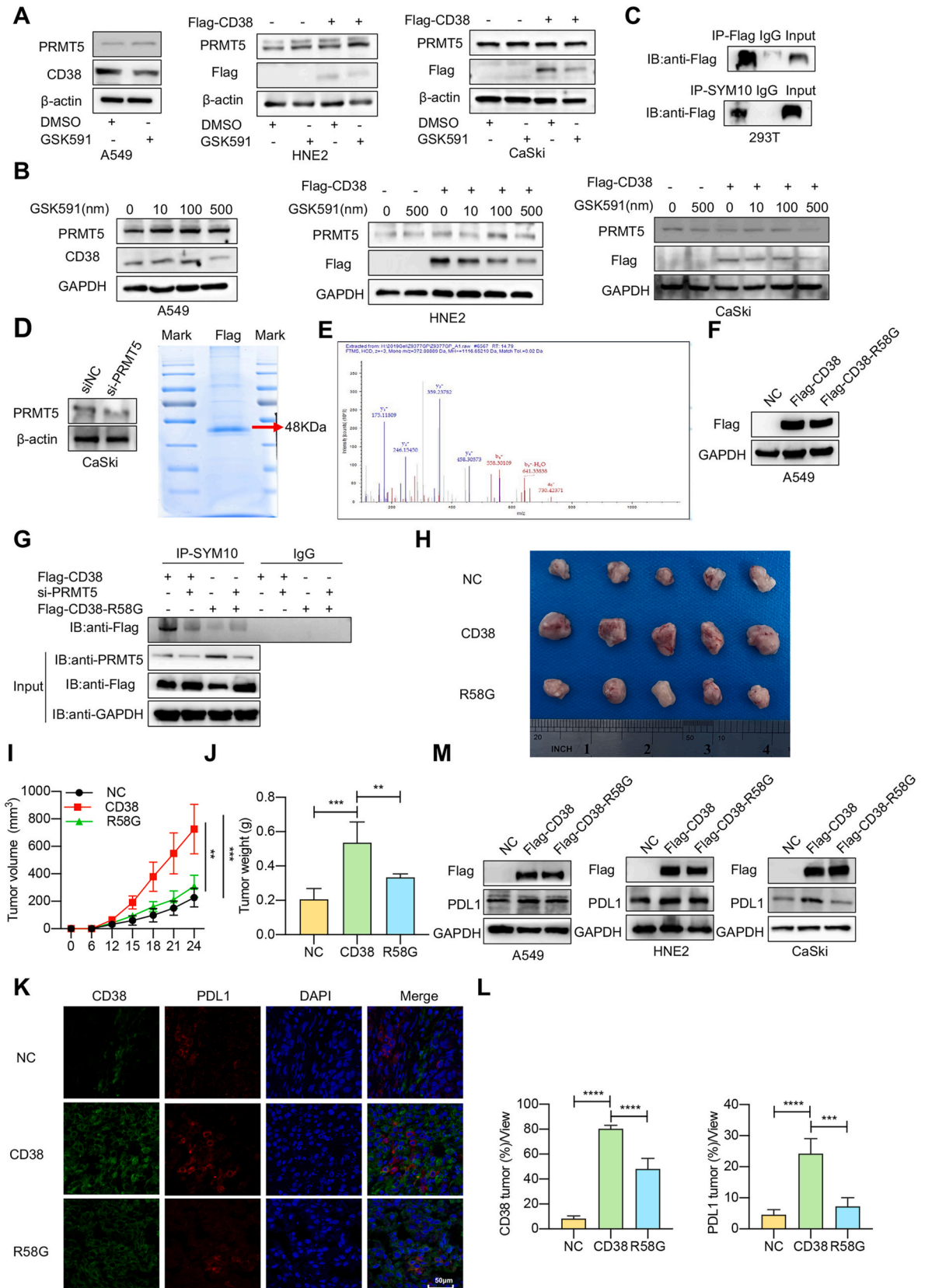
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Fig. 4. Symmetric dimethylation of the CD38 R58 site affects PD-L1 expression

(A) Immunoblotting analysis of CD38 expression in A549, HNE2, and CaSki cells treated with GSK591 for 48 h. (B) Immunoblotting analysis of CD38 expression in A549, HNE2, and CaSki cells treated with different concentrations of GSK591 for 48 h. (C) Immunoprecipitation in 293T cells using anti-Flag or anti-SYM10 antibodies. (D) Immunoblotting analysis of PRMT5 and CD38 expression in CaSki cells expressing NC or Flag-CD38 transiently transfected with siNC or siPRMT5. (E) Mass spectrometry identification of methylation at CD38 R58. (F) Immunoblotting analysis of Flag expression in A549 cells transfected with NC or Flag-CD38 or Flag-CD38 R58G. (G) Immunoblotting analysis of symmetric dimethylation of CD38 in A549 cells transfected with siNC or siPRMT5 and NC or Flag-CD38 or Flag-CD38 R58G. Results are expressed as mean ± SD (n = 3). (H) Construction of a subcutaneous tumor model in BALB/c nude mice and measurement of tumor volume (n = 5). (I, J) Construction of the tumor growth curve and measurement of tumor volume. (K, L) Immunofluorescence of mouse tumor tissue. (K) Representative image. (L) Statistical chart. (M) Immunoblotting analysis of Flag and PD-L1 expression in A549, HNE2, and CaSki cells transfected with NC or Flag-CD38 or Flag-CD38 R58G. Results are expressed as mean ± SD (n = 3).

CC, CD38 promotes cell proliferation, inhibits cellular senescence, alters NAD(H) and NADP(H) concentrations, enhances glucose consumption, and fosters lactate accumulation [23–25]. These findings underscore the pivotal role of CD38 in the pathogenesis of malignant tumors, including nasopharyngeal and CC. Moreover, in NSCLC treated with anti-PD-L1 antibodies, increased all-trans retinoic acid (ATRA) levels induce CD38 expression in tumor cells through the regulation of IRF1 and downstream IFN-β. Subsequently, CD38 inhibits cytotoxic T-cell function via adenosine receptor signaling [14]. However, the precise mechanism underlying CD38-mediated regulation of PD-L1 expression in solid tumor cells remains elusive in existing literature. In this study, we demonstrated a positive correlation between CD38 and PD-L1 levels and elucidated that CD38 promotes the stability and expression of PD-L1 protein. This novel finding sheds light on the interplay between CD38 and PD-L1 in malignant tumors and provides insights into the mechanism of immune evasion by CD38-expressing tumor cells.

GSK3β was initially identified for its role in glycogen metabolism, specifically in the regulation of glycogen synthase activity and glycogen synthesis [38]. However, it has since emerged as a critical component of various signaling pathways, including the Wnt/β-catenin pathway, with implications in tumorigenesis, invasion, and metastasis [39–41]. Notably, GSK3β-mediated
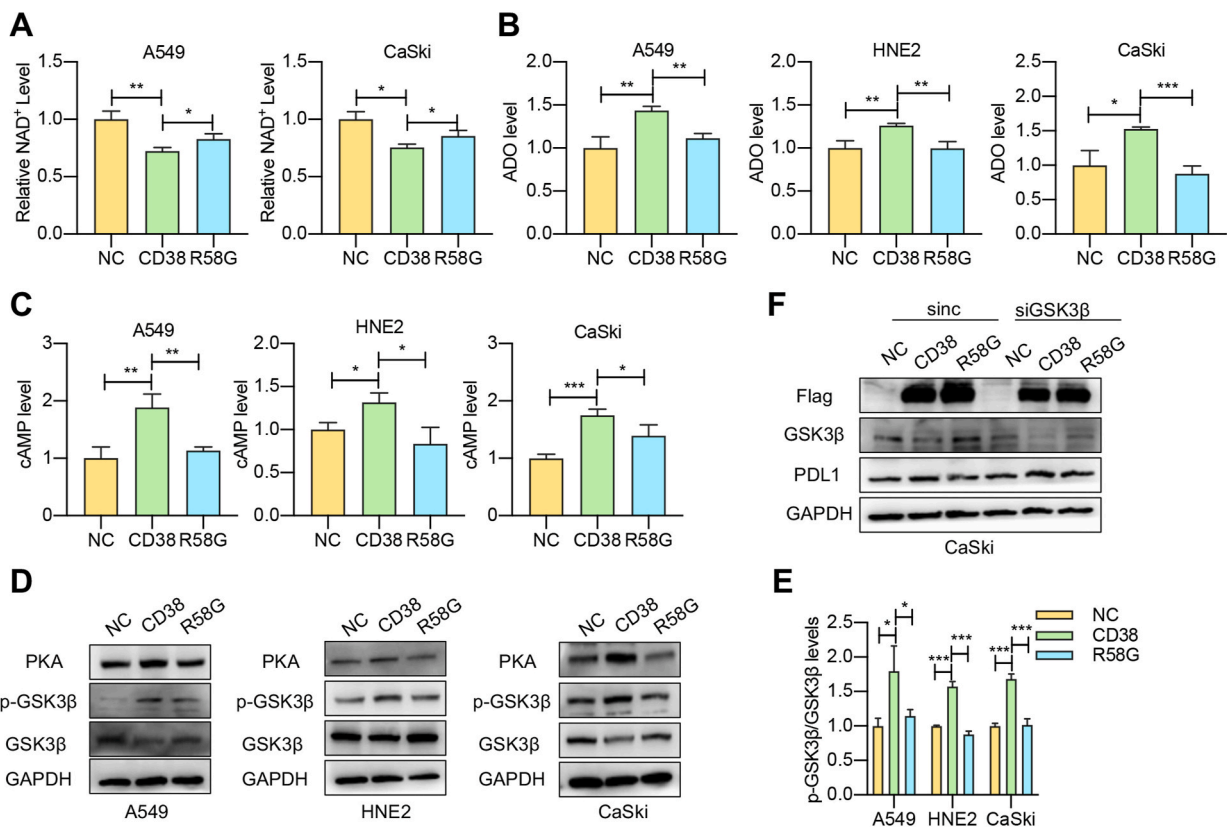
Fig. 5. CD38 R58 via ADO-cAMP GSK3β Signal axis upregulates PD-L1 expression

(A) Detection of NAD$^+$ levels in A549 and CaSki cells transfected with NC, Flag-CD38, or Flag-CD38 R58G. (B) Detection of adenosine levels in A549, HNE2, and CaSki cells transfected with NC, Flag-CD38, or Flag-CD38 R58G. (C) Detection of cAMP levels in A549, HNE2, and CaSki cells transfected with NC, Flag-CD38, or Flag-CD38 R58G. (D) Immunoblotting analysis of PKA, p-GSK3β/S9, and GSK3β expression in A549, HNE2, and CaSki cells transfected with NC, Flag-CD38, or Flag-CD38 R58G. (E) Quantitative analysis. (F) Immunoblotting analysis of PDL1, Flag, and GSK3β expression in CaSki cells. Results are expressed as mean ± SD (n = 3).
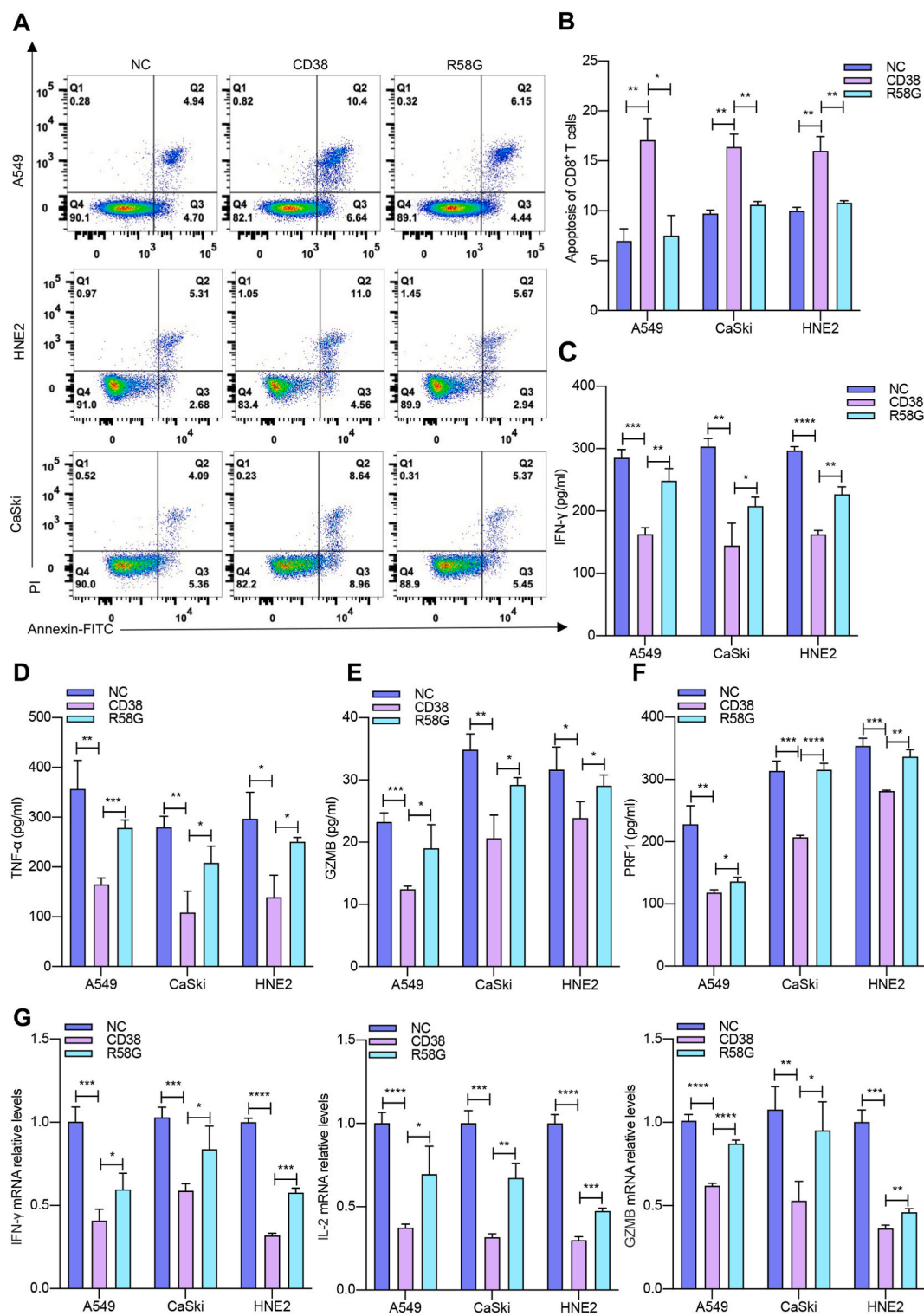
Fig. 6. CD38 R58 upregulates PD-L1 expression and promotes tumor cell immune escape through ADO-cAMP- GSK3β signaling axis
(A, B) Flow cytometry analysis of primary T cell apoptosis co-cultured with A549, HNE2, and CaSki cells transfected with NC or Flag-CD38 or Flag-CD38 R58G. (C–F) ELISA analysis of IFN-γ, TNF-α, PRF1, and GZMB secretion in primary T cells co-cultured with A549, HNE2, and CaSki cells transfected with NC or Flag-CD38 or Flag-CD38 R58G. (G) qRT-PCR analysis of *IFN-γ*, *IL-2*, and *GZMB* mRNA levels in primary T cells co-cultured with A549, HNE2, and CaSki cells transfected with NC or Flag-CD38 or Flag-CD38 R58G. Results are expressed as mean ± SD (n = 3).

phosphorylation of specific substrates promotes their recognition by ubiquitin E3 ligases, leading to ubiquitination-mediated degradation [42]. Recent studies have unveiled the involvement of GSK3β in modulating programmed death ligand 1 (PD-L1) expression and stability. Specifically, eukaryotic elongation factor 2 kinase (eEF2K) has been shown to enhance PD-L1 stability and expression by inactivating GSK3β. Consequently, cancer cells exploit this mechanism to evade immune surveillance. Inhibition of eEF2K through specific inhibitors or RNA interference results in reduced PD-L1 expression, enhanced tumor cell killing by CD8$^+$ T cells, and synergistic effects with programmed cell death protein 1 (PD-1) blockade [43]. In our study, we demonstrated that CD38 influences GSK3β phosphorylation at residue S9, leading to GSK3β inactivation. This observation prompted us to investigate whether CD38 mediates PD-L1 expression through GSK3β. Consistent with our hypothesis, our experimental findings confirmed that CD38-mediated inactivation of GSK3β promotes PD-L1 stability and expression. This elucidates a refined molecular mechanism underlying the regulation of PD-L1 expression by CD38 in tumor cells. By delineating the intricate interplay between CD38 and GSK3β in modulating PD-L1 expression, our study contributes to a deeper understanding of the immunoregulatory functions of CD38 in the tumor microenvironment. Moreover, these insights may pave the way for the development of novel therapeutic strategies targeting CD38-GSK3β-PD-L1 axis to enhance anti-tumor immune responses and improve the efficacy of immune checkpoint blockade therapies.

Adenosine, a mononucleoside that accumulates in the tumor microenvironment, has been implicated in promoting tumor progression by fostering immune evasion mechanisms. Extracellular adenosine serves as a ligand that binds to adenosine receptors, initiating downstream signaling pathways that contribute to tumor-mediated immune evasion. For instance, activation of the adenosine A2B receptor (ADORA2B) triggers signaling cascades involving adenylate cyclase (AC) activation, leading to increased intracellular cyclic AMP (cAMP) levels. Subsequently, protein kinase A (PKA) is activated, exerting immunosuppressive effects [44–47]. Additionally, the adenosine A1 receptor (ADORA1) has been implicated in immune escape in melanoma by regulating tumor PD-L1 expression through ATF3 [48]. Notably, the non-classical adenosine pathway, guided by CD38/CD203a, facilitates the generation of substrates for CD73, thus promoting the production of potent immunosuppressive adenosine and regulating tumor immunity [27, 28,49]. CD38, known to promote adenosine production, participates in tumor-mediated immune evasion through adenosine receptors. This led us to hypothesize that CD38 may mediate PD-L1 expression via adenosine activation of the cAMP signaling pathway. Our experimental findings indeed confirm that CD38 promotes the protein stability and expression of PD-L1 through the ADO-cAMP signaling axis. These results shed light on the immunosuppressive role of CD38 in regulating the tumor immune microenvironment. Consequently, CD38 emerges as a promising target for cancer immunotherapy, with potential implications for developing novel therapeutic interventions aimed at modulating the tumor immune response and enhancing anti-tumor immunity.

The main targets of cyclic AMP (cAMP) signaling include protein kinase A (PKA), exchange protein directly activated by cAMP 1 (Epac1), and Epac2, among others. Upon activation, cAMP stimulates PKA, which subsequently phosphorylates various substrates, including CREB, Raf, Bad, and glycogen synthase kinase 3 beta (GSK3β) [32,50]. GSK3β plays a critical role in regulating PD-L1 expression, as it interacts with PD-L1 and induces phosphorylation-dependent proteasomal degradation of PD-L1 via β-TrCP. Inhibition of GSK3β leads to PD-L1 stabilization, thereby inhibiting T cell activity [29,51,52]. Therefore, cAMP-mediated phosphorylation of GSK3β through PKA activation can regulate PD-L1 protein expression. In our study, we observed that CD38 activates the phosphorylation of GSK3β through the ADO-cAMP-PKA axis, resulting in the inactivation of GSK3β and subsequent stabilization of PD-L1 expression. These findings provide additional insights into the mechanism by which CD38 influences T cell function. CD38-mediated tumor immune evasion not only directly affects T cells through adenosine signaling but also regulates PD-L1 expression in tumor cells via adenosine signaling. This further underscores the pivotal role of CD38 in tumor immunotherapy and highlights its potential as a
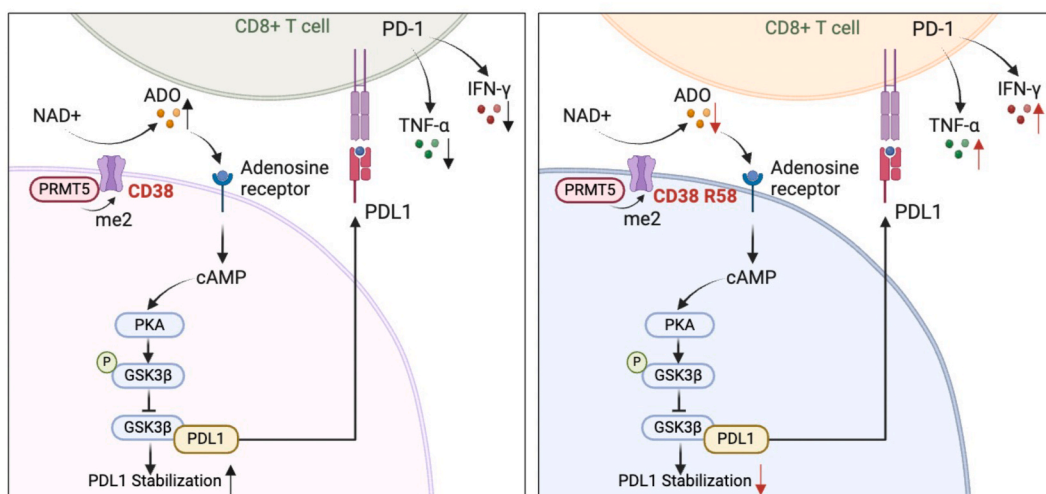

Fig. 7. A working model of CD38 symmetric dimethyl site R58 promoting immune escape.
Schematic representation of CD38 symmetric dimethyl site R58 promoting immune escape from malignant tumor cells by regulating the cAMP-GSK3β-PD-L1 axis.

therapeutic target for modulating the tumor immune microenvironment and enhancing anti-tumor immune responses.

In this study, we employed immunoprecipitation mass spectrometry analysis to identify proteins interacting with CD38, revealing a novel interaction between CD38 and protein arginine methyltransferase 5 (PRMT5). PRMT5, a member of the protein arginine methyltransferase family, is known to symmetrically dimethylate both histone and non-histone proteins [53–55]. Studies have highlighted the crucial role of PRMT5 in immune cell development, as its specific deficiency in T cells has been shown to lead to a reduction in various T cell populations [56–58]. Furthermore, inhibition of PRMT5 has been demonstrated to promote CD8$^+$ T cell apoptosis by upregulating P53 expression and reducing AKT pathway activity [59], underscoring its significance in immune regulation. Our study uncovered that PRMT5 induces symmetric dimethylation at the R58 site of the CD38 protein. This modification, mediated through the ADO-cAMP-GSK3β signaling axis, upregulates the expression of PD-L1 in tumor cells, ultimately facilitating tumor immune escape. Thus, our findings reveal a novel molecular mechanism governing PD-L1 expression and shed light on the inhibitory role of CD38 protein R58 site symmetric dimethylation in anti-tumor immunity. These results highlight the multifaceted impact of CD38 in tumor immune evasion. Notably, CD38 can exert its influence not only through direct effects on CD8$^+$ T cells via adenosine signaling but also by promoting tumor immune escape through modulation of PD-L1 expression on tumor cells. This comprehensive understanding provides valuable insights into the intricate interplay between immune cells and tumor cells within the tumor microenvironment, offering potential avenues for therapeutic intervention targeting CD38-mediated immunosuppression.

5. Conclusion

In summary, our findings indicate that symmetric dimethylation of the R58 site of the CD38 protein plays a critical role in enhancing PD-L1 stability and expression through the ADO-cAMP-GSK3β signaling axis. This mechanism ultimately inhibits CD8$^+$ T cell-mediated tumor cell killing, facilitating cancer cell evasion of immune surveillance. Given these insights, CD38 emerges as a promising molecular target for anti-tumor therapies aimed at bolstering anti-cancer immunity (Fig. 7).

Ethics declarations

1. Animal procedures were approved by the Animal Experimentation Ethics Committee of Central South University (2021sydw0092).
2. There are no conflicts of interests.

Funding

CRediT authorship contribution statement

Lin Liang: Writing – original draft, Validation, Data curation. **Chunxue Yue:** Validation, Methodology. **Wentao Li:** Project administration, Methodology, Formal analysis. **Jingqiong Tang:** Validation, Formal analysis. **Qian He:** Resources, Methodology. **Feng Zeng:** Methodology, Investigation. **Jiaying Cao:** Software, Formal analysis. **Siyi Liu:** Validation, Investigation. **Yan Chen:** Resources, Methodology. **Xin Li:** Software, Formal analysis. **Yanhong Zhou:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37958.

References

[1] B.B.Y. Ma, W.T. Lim, B.C. Goh, E.P. Hui, K.W. Lo, A. Pettinger, et al., Antitumor activity of nivolumab in recurrent and metastatic nasopharyngeal carcinoma: an international, multicenter study of the mayo clinic phase 2 consortium (NCI-9742), J. Clin. Oncol. 36 (14) (2018) 1412–1418.

[2] D.R. Adkins, R.I. Haddad, Clinical trial data of Anti-PD-1/PD-L1 therapy for recurrent or metastatic nasopharyngeal Carcinoma: a review, Cancer Treat Rev. 109 (2022) 102428.

[3] F.H. Wang, X.L. Wei, J. Feng, Q. Li, N. Xu, X.C. Hu, et al., Efficacy, safety, and correlative biomarkers of toripalimab in previously treated recurrent or metastatic nasopharyngeal carcinoma: a phase II clinical trial (POLARIS-02), J. Clin. Oncol. 39 (7) (2021) 704–712.

[4] M. Yi, X. Zheng, M. Niu, S. Zhu, H. Ge, K. Wu, Combination strategies with PD-1/PD-L1 blockade: current advances and future directions, Mol. Cancer 21 (1) (2022) 28.

[5] Q. Tang, Y. Chen, X. Li, S. Long, Y. Shi, Y. Yu, et al., The role of PD-1/PD-L1 and application of immune-checkpoint inhibitors in human cancers, Front. Immunol. 13 (2022) 964442.

[6] D. Ren, Y. Hua, B. Yu, X. Ye, Z. He, C. Li, et al., Correction to: predictive biomarkers and mechanisms underlying resistance to PD1/PD-L1 blockade cancer immunotherapy, Mol. Cancer 19 (1) (2020) 31.

[7] S. Chen, G.A. Crabill, T.S. Pritchard, T.L. McMiller, P. Wei, D.M. Pardoll, et al., Mechanisms regulating PD-L1 expression on tumor and immune cells, J Immunother Cancer 7 (1) (2019) 305.

[8] Y. Jiang, M. Chen, H. Nie, Y. Yuan, PD-1 and PD-L1 in cancer immunotherapy: clinical implications and future considerations, Hum Vaccin Immunother 15 (5) (2019) 1111–1122.

[9] M. Yi, M. Niu, L. Xu, S. Luo, K. Wu, Regulation of PD-L1 expression in the tumor microenvironment, J. Hematol. Oncol. 14 (1) (2021) 10.

[10] Y. Wei, X. Xiao, X.M. Lao, L. Zheng, D.M. Kuang, Immune landscape and therapeutic strategies: new insights into PD-L1 in tumors, Cell. Mol. Life Sci. 78 (3) (2021) 867–887.

[11] M.R. Pitter, W. Zou, Uncovering the immunoregulatory function and therapeutic potential of the PD-1/PD-L1 Axis in cancer, Cancer Res. 81 (20) (2021) 5141–5143.

[12] M. Wu, Q. Huang, Y. Xie, X. Wu, H. Ma, Y. Zhang, et al., Improvement of the anticancer efficacy of PD-1/PD-L1 blockade via combination therapy and PD-L1 regulation, J. Hematol. Oncol. 15 (1) (2022) 24.

[13] C.M. Fares, E.M. Van Allen, C.G. Drake, J.P. Allison, S. Hu-Lieskovan, Mechanisms of resistance to immune checkpoint blockade: why does checkpoint inhibitor immunotherapy not work for all patients? Am Soc Clin Oncol Educ Book 39 (2019) 147–164.

[14] L. Chen, L. Diao, Y. Yang, X. Yi, B.L. Rodriguez, Y. Li, et al., CD38-Mediated immunosuppression as a mechanism of tumor cell escape from PD-1/PD-L1 blockade, Cancer Discov. 8 (9) (2018) 1156–1175.

[15] K.A. Hogan, C.C.S. Chini, E.N. Chini, The multi-faceted ecto-enzyme CD38: roles in immunomodulation, cancer, aging, and metabolic diseases, Front. Immunol. 10 (2019) 1187.

[16] F. Zeng, J. Zhang, X. Jin, Q. Liao, Z. Chen, G. Luo, et al., Effect of CD38 on B-cell function and its role in the diagnosis and treatment of B-cell-related diseases, J. Cell. Physiol. 237 (7) (2022) 2796–2807.

[17] W. Li, Y. Li, X. Jin, Q. Liao, Z. Chen, H. Peng, et al., CD38: a significant regulator of macrophage function, Front. Oncol. 12 (2022) 775649.

[18] W. Li, L. Liang, Q. Liao, Y. Li, Y. Zhou, CD38: an important regulator of T cell function, Biomed. Pharmacother. 153 (2022) 113395.

[19] D. Zheng, S. Liao, G. Zhu, G. Luo, S. Xiao, J. He, et al., CD38 is a putative functional marker for side population cells in human nasopharyngeal carcinoma cell lines, Mol. Carcinog. 55 (3) (2016) 300–311.

[20] Y. Ge, Y. Long, S. Xiao, L. Liang, Z. He, C. Yue, et al., CD38 affects the biological behavior and energy metabolism of nasopharyngeal carcinoma cells, Int. J. Oncol. 54 (2) (2019) 585–599.

[21] D. Zheng, G. Zhu, S. Liao, W. Yi, G. Luo, J. He, et al., Dysregulation of the PI3K/Akt signaling pathway affects cell cycle and apoptosis of side population cells in nasopharyngeal carcinoma, Oncol. Lett. 10 (1) (2015) 182–188.

[22] Z. He, C. Yue, X. Chen, X. Li, L. Zhang, S. Tan, et al., Integrative analysis identified CD38 as a key node that correlates highly with immunophenotype, chemoradiotherapy resistance, and prognosis of head and neck cancer, J. Cancer 14 (1) (2023) 72–87.

[23] S. Liao, L. Liang, C. Yue, J. He, Z. He, X. Jin, et al., CD38 is involved in cell energy metabolism via activating the PI3K/AKT/mTOR signaling pathway in cervical cancer cells, Int. J. Oncol. 57 (1) (2020) 338–354.

[24] S. Liao, S. Xiao, H. Chen, M. Zhang, Z. Chen, Y. Long, et al., CD38 enhances the proliferation and inhibits the apoptosis of cervical cancer cells by affecting the mitochondria functions, Mol. Carcinog. 56 (10) (2017) 2245–2257.

[25] S. Liao, S. Xiao, G. Zhu, D. Zheng, J. He, Z. Pei, et al., CD38 is highly expressed and affects the PI3K/Akt signaling pathway in cervical cancer, Oncol. Rep. 32 (6) (2014) 2703–2709.

[26] F. Malavasi, S. Deaglio, A. Funaro, E. Ferrero, A.L. Horenstein, E. Ortolan, et al., Evolution and function of the ADP ribosyl cyclase/CD38 gene family in physiology and pathology, Physiol. Rev. 88 (3) (2008) 841–886.

[27] A.L. Horenstein, A. Chillemi, R. Zini, V. Quarona, N. Bianchi, R. Manfredini, et al., Cytokine-induced killer cells express CD39, CD38, CD203a, CD73 ectoenzymes and P1 adenosinergic receptors, Front. Pharmacol. 9 (2018) 196.

[28] F. Costa, B. Dalla Palma, N. Giuliani, CD38 expression by myeloma cells and its role in the context of bone marrow microenvironment: modulation by therapeutic agents, Cells 8 (12) (2019).

[29] C.W. Li, S.O. Lim, W. Xia, H.H. Lee, L.C. Chan, C.W. Kuo, et al., Glycosylation and stabilization of programmed death ligand-1 suppresses T-cell activity, Nat. Commun. 7 (2016) 12632.

[30] B.W. Doble, J.R. Woodgett, GSK-3: tricks of the trade for a multi-tasking kinase, J. Cell Sci. 116 (Pt 7) (2003) 1175–1186.

[31] H. Zhang, Q. Kong, J. Wang, Y. Jiang, H. Hua, Complex roles of cAMP-PKA-CREB signaling in cancer, Exp. Hematol. Oncol. 9 (1) (2020) 32.

[32] M.B. Ahmed, A.A.A. Alghamdi, S.U. Islam, J.S. Lee, Y.S. Lee, cAMP signaling in cancer: a PKA-CREB and EPAC-centric approach, Cells 11 (13) (2022).

[33] C. Cai, S. Gu, Y. Yu, Y. Zhu, H. Zhang, B. Yuan, et al., PRMT5 enables robust STAT3 activation via arginine symmetric dimethylation of SMAD7, Adv. Sci. 8 (10) (2021) 2003047.

[34] P. Sachamitr, J.C. Ho, F.E. Ciamponi, W. Ba-Alawi, F.J. Coutinho, P. Guilhamon, et al., PRMT5 inhibition disrupts splicing and stemness in glioblastoma, Nat. Commun. 12 (1) (2021) 979.

[35] H. Tang, Y. Liang, R.A. Anders, J.M. Taube, X. Qiu, A. Mulgaonkar, et al., PD-L1 on host cells is essential for PD-L1 blockade-mediated tumor regression, J. Clin. Invest. 128 (2) (2018) 580–588.

[36] C. Blank, T.F. Gajewski, A. Mackensen, Interaction of PD-L1 on tumor cells with PD-1 on tumor-specific T cells as a mechanism of immune evasion: implications for tumor immunotherapy, Cancer Immunol. Immunother. 54 (4) (2005) 307–314.

[37] A. Chow, K. Perica, C.A. Klebanoff, J.D. Wolchok, Clinical implications of T cell exhaustion for cancer immunotherapy, Nat. Rev. Clin. Oncol. 19 (12) (2022) 775–790.

[38] N. Embi, D.B. Rylatt, P. Cohen, Glycogen synthase kinase-3 from rabbit skeletal muscle. Separation from cyclic-AMP-dependent protein kinase and phosphorylase kinase, Eur. J. Biochem. 107 (2) (1980) 519–527.

[39] G. Augello, M.R. Emma, A. Cusimano, A. Azzolina, G. Montalto, J.A. McCubrey, et al., The role of GSK-3 in cancer immunotherapy: GSK-3 inhibitors as a new frontier in cancer treatment, Cells 9 (6) (2020).

[40] J.S. Saleh, S.R. Abd El Hadi, H.S. Ibrahim, E.Z. Elrazaz, K.A.M. Abouzid, Fragment merging approach for design, synthesis, and biological assessment of urea/acetyl hydrazide clubbed thienopyrimidine derivatives as GSK-3 beta inhibitors, BMC Chem 17 (1) (2023) 127.

[41] H.Y. Pan, M. Valapala, Regulation of autophagy by the glycogen synthase kinase-3 (GSK-3) signaling pathway, Int. J. Mol. Sci. 23 (3) (2022).

[42] R. Mancinelli, G. Carpino, S. Petrungaro, C.L. Mammola, L. Tomaipitinca, A. Filippini, et al., Multifaceted roles of GSK-3 in cancer and autophagy-related diseases, Oxid. Med. Cell. Longev. 2017 (2017) 4629495.

[43] X. Chen, K. Wang, S. Jiang, H. Sun, X. Che, M. Zhang, et al., eEF2K promotes PD-L1 stabilization through inactivating GSK3beta in melanoma, J Immunother Cancer 10 (3) (2022).

[44] C. Xia, S. Yin, K.K.W. To, L. Fu, CD39/CD73/A2AR pathway and cancer immunotherapy, Mol. Cancer 22 (1) (2023) 44.

[45] Z. Kurago, G. Guo, H. Shi, R.J. Bollag, M.W. Groves, J.K. Byrd, et al., Inhibitors of the CD73-adenosinergic checkpoint as promising combinatory agents for conventional and advanced cancer immunotherapy, Front. Immunol. 14 (2023) 1212209.

[46] C. Giacomelli, S. Daniele, C. Romei, L. Tavanti, T. Neri, I. Piano, et al., The A(2B) 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.

[47] R.D. Leone, L.A. Emens, Targeting adenosine for cancer immunotherapy, J Immunother Cancer 6 (1) (2018) 57.

[48] H. Liu, X. Kuang, Y. Zhang, Y. Ye, J. Li, L. Liang, et al., ADORA1 inhibition promotes tumor immune evasion by regulating the ATF3-PD-L1 Axis, Cancer Cell 37 (3) (2020) 324–339 e8.

[49] E. Ferretti, A.L. Horenstein, C. Canzonetta, F. Costa, F. Morandi, Canonical and non-canonical adenosinergic pathways, Immunol. Lett. 205 (2019) 25–30.

[50] A. Kilanowska, A. Ziolkowska, P. Stasiak, M. Gibas-Dorna, cAMP-dependent signaling and ovarian cancer, Cells 11 (23) (2022).

[51] H. Bai, A.S. Padron, Y. Deng, Y.J. Liao, C.J. Murray, C. Ontiveros, et al., Pharmacological tumor PDL1 depletion with chlorambucil treats ovarian cancer and melanoma: improves antitumor immunity and renders anti-PDL1-resistant tumors anti-PDL1-sensitive through NK cell effects, J Immunother Cancer 11 (2) (2023).

[52] J.M. Hsu, C.W. Li, Y.J. Lai, M.C. Hung, Posttranslational modifications of PD-L1 and their applications in cancer therapy, Cancer Res. 78 (22) (2018) 6349–6353.

[53] H. Kim, Z.A. Ronai, PRMT5 function and targeting in cancer, Cell Stress 4 (8) (2020) 199–215.

[54] H. Shailesh, Z.Z. Zakaria, R. Baiocchi, S. Sif, Protein arginine methyltransferase 5 (PRMT5) dysregulation in cancer, Oncotarget 9 (94) (2018) 36705–36718.

[55] A. Motolani, M. Martin, M. Sun, T. Lu, The structure and functions of PRMT5 in human diseases, Life 11 (10) (2021).

[56] Y. Abe, T. Sano, N. Tanaka, The role of PRMT5 in immuno-oncology, Genes 14 (3) (2023).

[57] J. Zheng, B. Li, Y. Wu, X. Wu, Y. Wang, Targeting arginine methyltransferase PRMT5 for cancer therapy: updated progress and novel strategies, J. Med. Chem. 66 (13) (2023) 8407–8427.

[58] R. Hu, B. Zhou, Z. Chen, S. Chen, N. Chen, L. Shen, et al., PRMT5 inhibition promotes PD-L1 expression and immuno-resistance in lung cancer, Front. Immunol. 12 (2021) 722188.

[59] C.D. Strobl, S. Schaffer, T. Haug, S. Volkl, K. Peter, K. Singer, et al., Selective PRMT5 inhibitors suppress human CD8(+) T cells by upregulation of p53 and impairment of the AKT pathway similar to the tumor metabolite MTA, Mol. Cancer Therapeut. 19 (2) (2020) 409–419.