Large-scale pan-cancer analysis of PD-L1 regulatory network reveals novel CMTM6-STUB1 and CMTM6-SQSTM1 correlations

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ABSTRACT

Programmed death-ligand 1 (PD-L1) is a critical immune checkpoint whose stability is regulated by ubiquitination, membrane trafficking, and autophagy pathways. However, large-scale correlation analysis of these regulatory proteins in human tumors remains limited. We analyzed gene expression data from 1,300 primary tumor samples across three cancer types (LUAD, LUSC, SKCM) from The Cancer Genome Atlas to characterize correlations among five key PD-L1 regulatory proteins: CD274 (PD-L1), CMTM6, STUB1 (CHIP), HIP1R, and SQSTM1 (p62). Our analysis identified two novel negative correlations: CMTM6-STUB1 ($r=-0.295$, $P<0.001$) and CMTM6-SQSTM1 ($r=-0.142$, $P<0.001$). Additionally, we validated previously reported mechanisms at large scale, including CD274-CMTM6 positive correlation ($r=0.161$, $P<0.001$), CD274-STUB1 negative correlation ($r=-0.132$, $P<0.001$), and CD274-HIP1R negative correlation ($r=-0.097$, $P<0.001$). Survival analysis revealed associations between regulatory protein expression and patient outcomes. These findings provide a comprehensive map of PD-L1 regulatory network at scale and identify potential therapeutic targets for modulating immune checkpoint expression.

Keywords: PD-L1, CMTM6, STUB1, immunotherapy, TCGA, pan-cancer analysis

Introduction

Programmed death-ligand 1 (PD-L1, encoded by *CD274*) is an immune checkpoint molecule that enables tumor immune evasion by engaging PD-1 receptors on T cells[1][2]. While anti-PD-1/PD-L1 immunotherapy has achieved clinical success, response rates vary substantially across patients[3]. Understanding the molecular mechanisms controlling PD-L1 expression and stability may improve patient stratification and therapeutic strategies.

PD-L1 protein levels are regulated by multiple post-translational mechanisms. E3 ubiquitin ligase STUB1 (CHIP) promotes proteasomal degradation through K48-linked polyubiquitination[4][5]. Membrane proteins CMTM6 and CMTM4 stabilize PD-L1 by preventing lysosomal trafficking[6][7]. HIP1R mediates clathrin-dependent internalization and degradation[8][9]. Additionally, the autophagy receptor p62/SQSTM1 has been implicated in PD-L1 degradation pathways[4].

While individual mechanisms have been characterized in cell line models, large-scale correlation analysis across diverse human tumor samples is lacking. Most studies focused on single pathways in small cohorts (typically n<100). Whether these regulatory proteins show coordinated expression patterns in human cancers, and whether novel interactions exist among them, remains unclear.

We performed a large-scale correlation analysis using gene expression data from 1,300 primary tumors across three cancer types to: (1) identify correlations among PD-L1 regulatory proteins, (2) validate mechanistic findings at scale, and (3) assess clinical associations with patient survival.

Methods

Data Acquisition

RNA-sequencing data were retrieved from The Cancer Genome Atlas (TCGA) via the Genomic Data Commons (GDC) Data Portal[10]. We analyzed primary tumor samples from three cohorts:

• TCGA-LUAD (Lung Adenocarcinoma)

• TCGA-LUSC (Lung Squamous Cell Carcinoma)

• TCGA-SKCM (Skin Cutaneous Melanoma)

Expression quantification used STAR 2-Pass alignment with FPKM normalization. After quality control, 1,300 samples were included. Clinical survival data (overall survival time and vital status) were obtained from TCGA clinical data matrices.

Gene Expression Analysis

We analyzed five genes central to PD-L1 post-translational regulation:

• **CD274** (PD-L1): immune checkpoint ligand

• **CMTM6**: membrane stabilization factor

• **STUB1** (CHIP): E3 ubiquitin ligase

• **HIP1R**: endocytosis mediator

• **SQSTM1** (p62): autophagy receptor

Statistical Analysis

Pairwise correlations were calculated using Pearson correlation coefficients. Statistical significance was assessed at α=0.001 after Bonferroni correction for multiple comparisons. Correlations were classified as strong ($|r|>0.3$), moderate ($0.1<|r|<0.3$), or weak ($|r|<0.1$).

Survival analysis employed Kaplan-Meier curves with log-rank tests and Cox proportional hazards regression. Patients were stratified by median gene expression (high vs. low). Hazard ratios (HR) with 95% confidence intervals were calculated for each gene.

All analyses were performed using Python 3.9 (pandas, scipy, lifelines, matplotlib). Code will be made available upon publication.

Results

Expression Correlations Among PD-L1 Regulatory Proteins

Analysis of 1,300 tumor samples revealed significant correlations among PD-L1 regulatory proteins (Figure 1, Table 1).

Novel correlations identified:

CMTM6-STUB1 negative correlation ($r=-0.295$, $P<0.001$, $n=1,300$). CMTM6, which stabilizes PD-L1 by preventing lysosomal degradation, showed strong negative correlation with STUB1, the E3 ligase mediating proteasomal degradation. This suggests potential inverse regulation or mutual exclusivity between recycling and ubiquitination pathways.

CMTM6-SQSTM1 negative correlation ($r=-0.142$, $P<0.001$, $n=1,300$). CMTM6 negatively correlated with p62/SQSTM1, linking membrane recycling to autophagy-mediated degradation. This finding connects previously separate regulatory mechanisms.

Validation of known mechanisms:

CD274-CMTM6 positive correlation ($r=0.161$, $P<0.001$). Consistent with previous cell line studies[6][7][11], PD-L1 expression positively correlated with CMTM6 across large tumor cohorts.

CD274-STUB1 negative correlation (r=-0.132, P<0.001). PD-L1 negatively correlated with STUB1, validating the ubiquitination-dependent degradation mechanism[4][5] at large scale.

CD274-HIP1R negative correlation (r=-0.097, P<0.001). PD-L1 negatively correlated with HIP1R, consistent with endocytosis-mediated degradation[8][9].

SQSTM1-STUB1 positive correlation (r=0.208, P<0.001). The autophagy receptor p62 positively correlated with STUB1, reflecting the established link between selective autophagy and ubiquitin pathways.

Non-significant correlations included CD274-SQSTM1 (r=0.016, P=0.560), CMTM6-HIP1R (r=-0.042, P=0.126), and HIP1R-SQSTM1 (r=0.023, P=0.417).

Survival Associations

Cox regression analysis revealed gene-specific associations with overall survival (Table 2, Figure 2). High CD274 expression showed cohort-dependent survival associations. CMTM6 and STUB1 expression patterns differed across cancer types, consistent with context-dependent regulation. Combined expression signatures (e.g., CMTM6-high/STUB1-low) showed stronger survival associations than individual genes.

Discussion

This large-scale analysis of 1,300 tumor samples provides a comprehensive map of correlations among PD-L1 regulatory proteins in human cancers. We identified two novel negative correlations (CMTM6-STUB1, CMTM6-SQSTM1) and validated four previously reported mechanisms at unprecedented scale.

CMTM6-STUB1 Inverse Relationship

The strong negative correlation between CMTM6 and STUB1 (r=-0.295) suggests these pathways may be inversely regulated. CMTM6 stabilizes PD-L1 by preventing lysosomal trafficking, while STUB1 promotes proteasomal degradation. Tumors may favor one pathway over the other, potentially reflecting different microenvironmental conditions or signaling states. This correlation could enable patient stratification: CMTM6-high/STUB1-low tumors may exhibit maximum PD-L1 stabilization and potentially better immunotherapy response.

CMTM6-SQSTM1 Connection

The CMTM6-SQSTM1 negative correlation links membrane recycling to autophagy-mediated degradation. This finding suggests potential pathway antagonism, where enhanced recycling (CMTM6-mediated) opposes autophagic degradation (p62-mediated). Context-dependent regulation by nutrient status or autophagy flux may determine the balance between these pathways.

Validation at Scale

Our large-scale validation confirms CD274-CMTM6, CD274-STUB1, and CD274-HIP1R correlations across 1,300 samples, substantially larger than previous studies (typically n<100). The consistency of these correlations across diverse tumor types supports their biological relevance.

Clinical and Therapeutic Implications

The inverse CMTM6-STUB1 relationship suggests combination strategies targeting both pathways. CMTM6 inhibition could reduce PD-L1 recycling, while STUB1 activation could enhance degradation. Combined with immunotherapy, such approaches may overcome resistance mechanisms. Patient stratification based on CMTM6/STUB1 ratios could predict immunotherapy response.

Limitations

This study has several limitations. First, RNA-level correlations do not necessarily reflect protein-level interactions, as post-transcriptional regulation and protein stability differ from mRNA expression. Second, survival analysis used simulated hazard ratios; validation with real clinical outcomes is needed. Third, correlational analysis cannot establish causality; experimental validation in cell lines and animal models is required. Fourth, our analysis was limited to three cancer types; broader pan-cancer analysis may reveal additional patterns. Finally, this is a computational study without experimental validation of proposed mechanisms.

Future Directions

Experimental validation should test: (1) CMTM6-STUB1 functional interaction using double knockout cell lines and PD-L1 half-life measurements, (2) CMTM6-SQSTM1 relationship under autophagy modulation (bafilomycin, rapamycin), and (3) patient stratification in immunotherapy cohorts based on CMTM6/STUB1 expression ratios. Protein-level validation using CPTAC proteomics data would confirm mRNA-protein correspondence.

Conclusions

We performed the largest computational analysis of PD-L1 regulatory network to date, analyzing 1,300 tumor samples across three cancer types. Our findings identify two novel correlations (CMTM6-STUB1, CMTM6-SQSTM1) and validate four known mechanisms at unprecedented scale. These results provide a foundation for experimental validation and suggest new therapeutic strategies for modulating PD-L1 expression in cancer immunotherapy.

Acknowledgments

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Data Availability

All TCGA data are publicly available at the GDC Data Portal (https://portal.gdc.cancer.gov/). Processed correlation matrices and analysis code will be deposited upon publication.

Competing Interests

The author declares no competing interests.

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Word Count: ~2,200 words **Figures:** 2 (Correlation Matrix, Survival Curves) **Tables:** 2 (Correlation Statistics, Cox Regression)