Cell Cycle Activity Correlates with Increased Anti-Tumor Immunity in Diverse Cancers

Shanmei Jiang
China Pharmaceutical University
Yin He
China Pharmaceutical University
Mengyuan Li
China Pharmaceutical University
Xiaosheng Wang (xiaosheng.wang@cpu.edu.cn)
China Pharmaceutical University https://orcid.org/0000-0002-7199-7093

Research

Keywords: Cell cycle, Anti-tumor immunity, PD-L1 expression, Cancer genomics, Cancer immunotherapy

Posted Date: January 29th, 2020

DOI: https://doi.org/10.21203/rs.2.21975/v1

Version of Record: A version of this preprint was published at Clinical and Translational Medicine on June 1st, 2020. See the published version at https://doi.org/10.1002/ctm2.98.

Abstract

Objectives

The cell cycle pathway regulating cell proliferation is overactivated in various cancers. Immune evasion is another important mechanism for tumor cell hyperproliferation. Nevertheless, the relationship between cell cycle and tumor immunity remains not fully understood.

Materials and Methods

Using the cancer genomics datasets for 10 cancer cohorts from the Cancer Genome Atlas (TCGA) program, we investigated the association between cell cycle activity (CCA) and anti-tumor immune signatures. We also explored the association between CCA and PD-L1 expression in these cancer cohorts. Moreover, we investigated the association between CCA and immunotherapy response in several cancer cohorts receiving immunotherapy.

Results

CCA likely exhibited positive associations with anti-tumor immune signatures (CD8+ T cell infiltration and immune cytolytic activity) in these cancer cohorts. The strong positive associations of CCA with DNA damage repair pathways and with tumor mutation load may explain the positive associations between CCA and anti-tumor immune signatures. Moreover, CCA displayed significant positive correlations with PD-L1 expression. Finally, we found that the enhanced CCA tended to be associated with unfavorable clinical outcomes in the TCGA cancer cohorts, though such association was not observed in the cancer cohorts receiving immune checkpoint blockade therapy.

Conclusions

CCA has significant positive associations with both anti-tumor immune signatures and tumor immune-suppressive signatures in diverse cancer types. Our findings provide new insights into cancer biology and potential clinical implications for cancer immunotherapy.

1. Background

Recently, cancer immunotherapies, such as the immune checkpoint blockade (ICB)[1] and chimeric antigen receptor (CAR) T cell immunotherapy[2], have achieved success in treating a wide range of cancers. However, only a subset of cancer patients currently can respond to such therapies[3]. To this end, certain biomarkers associated with cancer immunotherapy response have been identified, including PD-L1 expression[4], defective DNA mismatch repair (dMMR)[5], and tumor mutation burden (TMB)[6]. In addition, to improve cancer immunotherapy response, the combination of other therapeutic strategies with immunotherapy has been investigated[7].

The cell cycle pathway, which regulates cell growth, replication, and division, is often overactivated in cancer cells[8, 9]. Immune evasion is another important mechanism for cancer-associated hyperproliferation[10]. Nonetheless, the relationship between cell cycle and tumor immunity remains not fully understood, although a recent study demonstrated that inhibition of the cell cycle pathway could enhance anti-tumor immunity[11].

With the recent advances in genomic technologies and bioinformatics methods, an investigation of the association between cell cycle and tumor immunity based on large-scale cancer genomics datasets is viable. In this study, we investigated the association between the cell cycle pathway and anti-tumor immunity across 10 cancer cohorts by the bioinformatics analysis of the Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov) datasets. We also explored the associations of the cell cycle pathway with PD-L1 expression as well as with the ICB therapy response. Our data showed that cell cycle activity (CCA) has significant positive associations with both anti-tumor immune signatures and tumor immune-suppressive signatures in these cancer cohorts. Our findings furnish potential clinical implications for cancer immunotherapy.

2. Results

2.1. Cell cycle activity is positively associated with anti-tumor immune signatures in cancer

In the 10 TCGA cancer cohorts, including BLCA, BRCA, COAD, HNSC, KIRC, LIHC, LUAD, PRAD,THCA, and UVM, CCA consistently positively correlated with CD8 + T cell infiltration levels (Pearson's correlation test, FDR < 0.05) (Fig. 1A). Moreover, in 9 of the 10 cancer cohorts (except LIHC), CCA positively correlated with immune cytolytic activity (Pearson's correlation test, FDR < 0.05) (Fig. 1A). In pan-cancer of the 10 cancer cohorts, CCA positively correlated with both CD8 + T cell infiltration levels and immune cytolytic activity (Pearson's correlation test, P < 0.001). Moreover, we found that the ratios of immune-stimulatory to immune-inhibitory signatures (CD8+/CD4 + regulatory T cells, M1/M2 macrophages, and pro-/anti-inflammatory cytokines) were significantly higher in the tumors with higher CCA than in the tumors with lower CCA in most of individual cancer types (Mann-Whitney U test, one-sided FDR < 0.05) (Fig. 1B). Notably, CCA positively correlated with the expression levels of numerous human leukocyte antigen (HLA) genes in cancer. For example, HLA-B, L, DOB, DPB2, DQA1, DQA2, DQB2 & DRA had significant positive expression correlations with CCA in pan-cancer and in at least 5 individual cancer types (Pearson's correlation test, FDR < 0.05) (Fig. 1C). Collectively, these results demonstrate that CCA is likely to positively correlate with anti-tumor immunity in these cancer cohorts.

Furthermore, we identified the KEGG[14] pathways that were more highly enriched in the tumors with higher CCA versus the tumors with lower CCA in each of the 10 cancer types by GSEA[17]. We found that a number of immune-related pathways were upregulated in higher-CCA tumors, e.g., the pathways of natural killer cell-mediated cytotoxicity, primary immunodeficiency, allograft rejection, autoimmune

thyroid disease, chemokine signaling, cytokine-cytokine receptor interaction, graft versus host disease, intestinal immune network for IgA production, systemic lupus erythematosus, T cell receptor signaling, Toll-like receptor signaling, hematopoietic cell lineage, leishmania infection, and NOD-like receptor signaling, which were highly enriched in higher-CCA tumors in at least 5 individual cancer types (Fig. 1D). Again, these results indicate a positive correlation between CCA and anti-tumor immune signatures in cancer.

In addition, we found many genes in the cell cycle pathway showing a positive expression correlation with anti-tumor immune signatures in pan-cancer and in multiple individual cancer types, e.g., CDK1, CDK6, CDC6, CDC7, and CDC20 (Pearson's correlation test, FDR < 0.05) (Fig. 1E). All together, these data demonstrated that CCA tends to be positively associated with anti-tumor immune activity in various cancers.

2.2. Prediction of anti-tumor immune signatures in cancer using CCA, TMB, and aneuploidy

To investigate the predictability of CCA for anti-tumor immune signatures, we performed logistic regression analyses to estimate the contributions of CCA in predicting CD8 + T cell infiltration levels and immune cytolytic activity. Because both TMB[6] and tumor aneuploidy[16] have been associated with anti-tumor immune signatures, we utilized three predictors (CCA, TMB, and aneuploidy) to predict the tumor samples with high (upper third) versus low (bottom third) immune signature scores in each logistic regression model.

In pan-cancer, all the predictors CCA (β coefficient: $\beta = 0.41$, $P = 4.06 \times 10^{-6}$), TMB ($\beta = 0.39$, $P = 0.001$), and aneuploidy (β=-0.73, $P = 1.52 \times 10^{-13}$) displayed significant contributions in predicting CD8 + T cell infiltration levels (Fig. 2). The similar results were observed in predicting immune cytolytic activity (Fig. 2). These results indicate that anti-tumor immune signatures have significant positive associations with CCA and TMB while they have a significant negative association with tumor aneuploidy level. This is in accordance with the results from previous studies[6, 16].

In the 10 individual cancer types, CCA was a significant positive predictor for CD8 + T cell infiltration levels and immune cytolytic activity in 7 and 6 cancer types, respectively ($P < 0.05$) (Fig. 2). In these cancer types, although TMB also displayed the potential as a positive predictor, few of the correlations were statistically significant (Fig. 2). In contrast, aneuploidy was a significant negative predictor for CD8 + T cell infiltration levels and immune cytolytic activity in 6 and 5 cancer types, respectively ($P < 0.05$) (Fig. 2). Collectively, these results suggest that CCA has stronger predictability for anti-tumor immune signatures than TMB and aneuploidy in these cancer types.

2.3. CCA positively correlates with PD-L1 expression

Strikingly, we observed significant positive correlations between PD-L1 expression levels and CCA in pan-cancer (Pearson's correlation test, $R = 0.29$, $P = 5.37 \times 10^{-97}$) and in 9 of the 10 individual cancer types

(Pearson's correlation test, FDR < 0.05) (Fig. 3A). Moreover, 61 cell cycle pathway genes showed significant positive expression correlations with PD-L1 in at least 5 individual cancer types (Pearson's correlation test, FDR < 0.05), including CCNA1, CCNB3, CCND2, CCNE2, CDC14A, CDC23, CDC25B, CDC27, CDC7, CDK2, and CDK6 (Fig. 3B). These results suggest that increased CCA correlates with enhanced tumor immune-suppressive signatures since PD-L1 can inhibit anti-tumor immune response[20] .

3. Discussion

Using the bioinformatics approach, we investigated the association between CCA and anti-tumor immune signatures in 10 cancer cohorts. Our data demonstrate that CCA may enhance the expression of anti-tumor immune signatures in these cancer cohorts. One possible reason why CCA can promote anti-tumor immunity could be that CCA is capable of increasing TMB[21]. Indeed, we observed significant positive correlations between CCA and TMB in pan-cancer and 5 individual cancer types, including BRCA, LUAD, PRAD, BLCA, and COAD (Spearman's correlation test, FDR < 0.01) (Fig. 4A). Moreover, in addition to many immune-related pathways (Fig. 1D), we found several cancer-associated pathways which were highly enriched in higher-CCA tumors in at least 5 individual cancer types, including homologous recombination, DNA replication, p53 signaling, mismatch repair, base excision repair, nucleotide excision repair, and spliceosome. Evidently, these pathways are DNA damage repair relevant. Correlation analyses showed that CCA likely had strong positive correlations with the activity of these pathways in pan-cancer and in most of individual cancer types (Pearson's correlation test, FDR < 0.01) (Fig. 4B). It indicates that the positive association between CCA and anti-tumor immune signatures is DNA damage repair-mediated.

Surprisingly, the positive association between CCA and anti-tumor immune signatures did not result in positive associations between CCA and clinical outcomes in these cancer cohorts. In contrast, the tumors with higher CCA tended to have unfavorable clinical phenotypes compared to the tumors with lower CCA. For example, CCA had strong positive correlations with the expression levels of MKi67 (a marker for tumor cell proliferation) in pan-cancer and in all the 10 individual cancer types (Pearson's correlation test, R > 0.5, FDR < 0.001) (Fig. 5A). Moreover, the tumors with higher CCA displayed significantly worse survival prognosis (OS, disease-specific survival (DSS), progression-free interval (PFI), and/or disease-free interval (DFI)) than the tumors with lower CCA in pan-cancer and in multiple individual cancer types (log-rank test, P < 0.05) (Fig. 5B). A possible explanation could be that CCA promotes the expression of tumor immune-suppressive signatures (such as PD-L1) as well, thereby counteracting the effect of elevated anti-tumor immune signatures in these cancer cohorts. In fact, we found that the ratios of the mean expression levels of CD8A (CD8 + T cell marker) to PD-L1 were significantly lower in higher-CCA tumors than in lower-CCA tumors in pan-cancer and in 4 individual cancer types (Mann-Whitney U test, one-sided FDR < 0.05) (Fig. 5C). It indicates that CCA has a stronger positive correlation with the tumor immune-suppressive signature (PD-L1) than with the anti-tumor immune signature (CD8 + T cells) in these cancer cohorts.

A recent study[11] showed that inhibition of CCA could increase tumor immunogenicity and enhance anti-tumor immune response in breast cancer. However, our data demonstrate that CCA may promote anti-

tumor immune response in diverse cancers, including breast cancer. In addition, our data showed that CCA had a significant positive association with PD-L1 expression, suggesting that combining cell cycle inhibitors with anti-PD-1/PD-L1 immunotherapies, as suggested in[11], may not be a viable strategy for treating these cancers. In fact, we found that in Snyder cohort[12] receiving anti-PD-L1 therapy, higher-CCA cancer patients showed favorable survival (overall survival (OS) and Progression free survival (PFS)) trends compared to lower-CCA patients (Fig. 6A). Moreover, we found CDK7, a member of the cyclin-dependent protein kinase (CDK) family, whose upregulation was significantly associated with better survival in this cancer cohort (log-rank test, P = 0.002, 0.004 for OS and PFS, respectively) (Fig. 6B). The positive correlation between CDK7 expression and survival prognosis could be attributed to the elevated anti-PD-L1 response rate in the cancers with higher CDK7 expression levels versus the cancers with lower CDK7 expression levels (Fisher's exact test, P = 0.097, odds ratio = 5.41) (Fig. 6B). In addition, in Samstein cohort receiving anti-PD-1/PD-L1/CTLA-4 therapy[13], we found a number of cell cycle pathway genes whose mutations were associated with better OS (log-rank test, $P < 0.05$) (Fig. 6C). These genes included CDKN2A, CDK6, CCNE1, ABL1, ATM, ATR, and CREBBP. Among them, CDKN2A and ATM are tumor suppressor genes and play important roles in promoting cell cycle arrest for response to DNA damage. Their mutations can enhance CCA. CDK6 are key cell division protein kinases and their mutations also likely promote CCA. Collectively, these results suggest that enhanced CCA may promote anti-PD-1/PD-L1/CTLA-4 therapy response. Consequently, the suggestion of combining cell cycle inhibitors with immunotherapies for cancer therapy[11] should be considered with caution.

4. Conclusion

CCA has significant positive associations with both anti-tumor immune signatures and tumor immune-suppressive signatures in diverse cancer types. Our findings provide new insights into cancer biology and potential clinical implications for cancer immunotherapy.

5. Materials And Methods

5.1. Materials

We downloaded RNA-Seq gene expression profiles (RSEM normalized, Level 3), gene somatic mutations (Level 3), and clinical data for 10 TCGA cancer cohorts from the genomic data commons data portal (https://portal.gdc.cancer.gov/). The pan-cancer RNA-Seq gene expression profiling dataset (batch effects normalized mRNA data) was downloaded from UCSC (https://xenabrowser.net/datapages/). We downloaded a bladder cancer dataset (Snyder cohort) containing gene expression profiles, anti-PD-L1 therapy response, and survival prognosis data[12] from the website http://doi.org/10.5281/zenodo.546110. In addition, we downloaded a pan-cancer dataset (Samstein cohort receiving anti-PD-1/PD-L1/CTLA-4 therapy), which contained gene somatic mutations and survival prognosis data, from the publication[13]. A summary of these datasets is shown in Table 1. We collected gene lists in pathways from KEGG[14], and the lists of marker genes for immune signatures from several

publications, including CD8 + T cells[15], immune cytolytic activity[15], CD4 + regulatory T cells[15], pro-/anti-inflammatory cytokines[16], and M1/M2 macrophages[16]. These gene lists for pathways and immune signatures are presented in the Supplementary Table S1.

Table 1
The datasets analyzed

Cancer type	Full name	Sample size		
		Total	High-CCA tumors	Low-CCA tumors
BRCA	breast invasive carcinoma	408	136	136
BLCA	bladder urothelial carcinoma	1100	367	367
COAD	colon adenocarcinoma	287	96	96
HNSC	head and neck squamous cell carcinoma	522	174	174
KIRC	kidney renal clear cell carcinoma	534	178	178
LIHC	liver hepatocellular carcinoma	373	124	124
LUAD	lung adenocarcinoma	517	172	172
PRAD	prostate adenocarcinoma	498	166	166
THCA	thyroid carcinoma	509	170	170
UVM	uveal melanoma	80	27	27
Pan-cancer (TCGA)	pan-cancer	4828	1609	1609
BLCA (Snyder cohort)	bladder urothelial carcinoma	24	12	12
Pan-cancer (Samstein cohort)	pan-cancer	1610	NA	NA

5.2. Definition of pathway or immune activity in tumor

For a pathway or immune signature, we defined its activity (or score) in a tumor sample as the average expression level (log2 transformed) of all genes in the pathway or immune signature. In the TCGA datasets, we classified a tumor sample into the higher-CCA group if its CCA score was in the upper third in pan-cancer or each individual cancer type, and a tumor sample into the lower-CCA group if its CCA score was in the bottom third. In the dataset with immunotherapy response data, the median of CCA scores was used to define the higher- and lower-CCA groups considering the small sample size (n = 24) in this dataset. We defined the ratio of immune signature A to immune signature B in a tumor sample as the

log2-transformed ratio of the geometric mean expression level of all marker genes of A to that of B in the tumor sample.

5.3. Gene-set enrichment analysis

In each individual cancer type, we used GSEA[17] to identify highly enriched KEGG[14] pathways in higher- and lower-CCA tumors, respectively, using a FDR (adjusted P values) cutoff < 0.05.

5.4. Evaluation of TMB and tumor aneuploidy level

We defined the TMB of a tumor sample as the total number of somatic mutations occurred in the tumor. The tumor aneuploidy level was the ploidy score of the tumor evaluated by the ABSOLUTE algorithm[18].

5.5. Correlation analyses

We assessed the correlation between two variables using the Pearson's or Spearman's correlation test. To adjust for multiple tests, the false discovery rate (FDR) was calculated by the Benjamini and Hochberg (BH) method[19]. The cutoff FDR < 0.05 was used to define the significance of correlations.

5.6. Logistic regression analyses

To evaluate the contributions of CCA, TMB, and aneuploidy level in predicting anti-tumor immune signatures, we built logistic regression predictive models with the three predictors to predict CD8 + T cell infiltration levels and immune cytolytic activity. The dependent variable had the binary values of "high" (upper third) versus "low" (bottom third) immune signature scores. We performed logistic regression analyses in R programming environment. The R function "glm" was utilized to fit the prediction model, and the R function "lm.beta" in R package "QuantPsyc" was used to calculate the regression coefficients (β values).

5.7. Survival analyses

We compared the survival (OS, DSS, PFI and DFI) between the higher- and lower-CCA tumors in pan-cancer and 10 individual cancer types. In Snyder cohort[12], we compared OS and disease-free survival (DFS) between the higher- and lower-CCA tumors as well as between higher- (> median) and lower-CDK7-expression-level (< median) tumors. In Samstein cohort[13], we compared OS between gene-mutated and gene-wildtype tumors with respect to cell cycle pathway genes. We used Kaplan-Meier curves to display the survival time differences and the log-rank test to evaluate the significance of survival differences. The R package "survival" was used to perform the survival analyses.

Abbreviations

BH Benjamini and Hochberg; CAR Chimeric antigen receptor; CCA Cell cycle activity; CDK Cyclin-dependent protein kinase; dMMR Defective DNA mismatch repair; DFI Disease-free interval; DSS Disease-specific survival; FDR False discovery rate; HLA Human leukocyte antigen; ICB Immune

checkpoint blockade; PFI Progression-free interval; PFS Progression free survival; OS Overall survival; TCGA The Cancer Genome Atlas; TMB Tumor mutation burden

Declarations

Ethics approval and consent to participate

Ethical approval and consent to participate was waived since we used only publicly available data and materials in this study.

Consent for publication

Not applicable.

Availability of data and materials

The TCGA datasets were downloaded from the genomic data commons data portal (https://portal.gdc.cancer.gov/). The pan-cancer RNA-Seq gene expression profiling dataset was downloaded from UCSC (https://xenabrowser.net/datapages/). The bladder cancer dataset (Snyder cohort) containing anti-PD-L1 therapy response data were downloaded from the website http://doi.org/10.5281/zenodo.546110. The pan-cancer dataset (Samstein cohort receiving anti-PD-1/PD-L1/CTLA-4 therapy) were obtained from its associated publication.

Competing interests

The authors declare that they have no competing interests.

Funding

This study was funded by the China Pharmaceutical University (grant number 3150120001).

Authors' contributions

SJ performed data analyses and helped prepare for the manuscript. YH performed data analyses and helped prepare for the manuscript. ML performed data analyses and helped prepare for the manuscript.

XW conceived the research, designed analysis strategies, and wrote the manuscript. All the authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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Supplementary Files

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