Preclinical Targeting of the PGRMC1-CK2 Axis with Silmitasertib: A Potential Strategy for Lung Adenocarcinoma Therapy

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ABSTRACT

Progesterone receptor membrane component 1 (PGRMC1) is a pleiotropic protein over-expressed in lung adenocarcinoma (LUAD). The precise molecular mechanisms underlying the signature motif of Casein kinase (CK2) presence in PGRMC1 and their role in LUAD remain unclear. X-ray crystallographic structure for CK2 and PGRMC1 from the PubChem database was obtained and subjected to protein-protein interaction (PPI) analysis to identify their interactions. In addition, the CK2 inhibitor – Silmitasertib was also utilised to understand the interaction between PGRMC1-CK2. The PPI complex (PGRMC1-CK2) and the PPI-ligand interaction analysis and their Molecular Dynamics (MD) studies revealed the stability of their interactions and critical amino acid contacts within the 5Å vicinity of the CK2 signature motif "T/S-x-x-E/D". Moreover, *in-vitro* colony formation assay, migration assay, and gene expression analysis using quantitative Real-time PCR revealed that Silmitasertib (IC_{50}–2.5 µM) was highly influential in suppressing the PGRMC1-CK2 expression axis. In conclusion, our study infers that PGRMC1-CK-2 axis inhibition could be a potential therapeutic option to limit the promotion and progression of lung cancer.

Introduction

Progesterone receptor membrane component 1 (PGRMC1) is a well-known heme-binding, membrane-associated progesterone receptor (MAPR) family protein responsible for cell proliferation and tumour growth in many cancers, including lung cancer. Among the lung cancer histological subtypes, lung adenocarcinoma (LUAD > 40 % diagnosed cases) belongs to the predominant Non-Small Cell Lung Cancer (NSCLC) kind with a poor survival rate. PGRMC1 is more inclined as a signal hub protein with a wide range of associations with various signaling pathways [1]. PGRMC1 is highly over-expressed in LUAD and facilitates drug resistance and cell proliferation. However, despite extensive research, the molecular insights on PGRMC1's role in LUAD progression remain unclear. Notably, this protein's ancestral form predates the emergence of metazoans [2]. This evolutionary aspect of the protein accounts for many of the functions attributed to it that are deeply entrenched in fundamental metabolic processes, including cell proliferation, apoptosis, and responses to DNA damage [2, 3]. Previous reports in NSCLC have shown the ability of PGRMC1 to control and regulate binding partners such as the Cytochrome members (CYP51A1,

CYB5), Epidermal Growth Factor Receptor (EGFR) and Progesterone 4 (P4). In addition, we believe that PGRMC1 plays a crucial role in LUAD progression through its ability to regulate other interacting partners, such as Casein Kinase 2 (CK2), a critical player in the β-Catenin pathway. The coming of age of the PGRMC1 – CK2 axis and its role in the LUAD provided the impetus for pursuing this study using the small molecule inhibitor – Silmitasertib [4]. Currently, Silmitasertib is being studied in clinical trials as a supplementary treatment to chemotherapy for cholangiocarcinoma, which is a form of cancer that affects the bile duct. Additionally, it is undergoing phase I and II clinical trials for treating recurrent Sonic Hedgehog (SHH) medulloblastoma. It is in the preclinical development stage for various other forms of cancer, such as haematological and lymphoid malignancies [5–6].

Material and Methods

Predicting domains and motifs of wild-type PGRMC1

Using two different tools, my hits motif scan and Scanprosite tools detected the presence of CK2 sites on full length wild-type PGRMC1 (195 amino acids) sequence of homosapiens were retrieved from UniProtKB ID: O0026. These tools allow scanning of the protein sequences for matches against a specific pattern from signature databases [7, 8].

Evolutionary conservation analysis

The ConSurf server was used to identify evolutionarily conserved amino acids of PGRMC1. It estimates the conserved amino acids in each protein by analysing the evolutionary relationship between homologous sequences using an empirical Bayesian inference and assigns conservation scores [9].

Molecular Docking

Hdock, a standalone online webserver, was used to perform the protein-protein interaction of PGRMC1 and CK2 to understand the binding affinity with the 3D X-ray crystallography structure of PGRMC1 and CK2 (PDB ID: 4X8Y, 6HMB). The standard parameters were applied to perform the protein-ligand docking. The interaction between them was analysed using Ligplot + /Dimplot [10].

Molecular Simulation

Molecular Dynamics (MD) has become a valuable tool for investigating the function and dynamics of protein-protein and protein-ligand complexes. The docked complexes were subjected to a 100 nanosecond (ns) MD simulation using the Desmond software integrated with Schrödinger 2021–1. MD simulations began with the standard protocol [11, 12].

Cell culture, cell line maintenance and other reagents

The A549 cell line was obtained from the National Center for Cell Science (NCCS) in Pune. A549 cells were cultured DMEM and supplemented with 10 % FBS and 1 % antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). Cells were maintained in a humidified atmosphere with 5 % CO_2 at a temperature of 37 °C. CX-4945, also known as Silmitasertib, a CK2 inhibitor, was purchased from Med Chem Express (catalogue number HY-50855) with a high purity level of 99.3 %.

Scratch assay

The scratch assay was created by gently scraping the cell monolayer using the tip of a 100 µl pipette. The detached cells were subsequently removed by washing with PBS. Images of the scratched area were captured at three different time points: 0 hours, 24 hours, and 48 hours. The scratched area was quantitatively assessed using Image J software.

Colony formation assay

A549 cells, the cells were treated with Silmitasertib (IC_{50}–2.5 µM) after 48 hours of incubation, the culture medium was replaced, and the cells were further incubated for ten days. After incubation, colonies were firmly fixed using a 10 % formalin solution and stained with 1 % crystal violet dissolved in 10 % methanol. Images of the stained colonies were captured and were determined using ImageJ software. Graphical representations of the data were generated using GraphPad Prism.

Quantitative Real Time-PCR

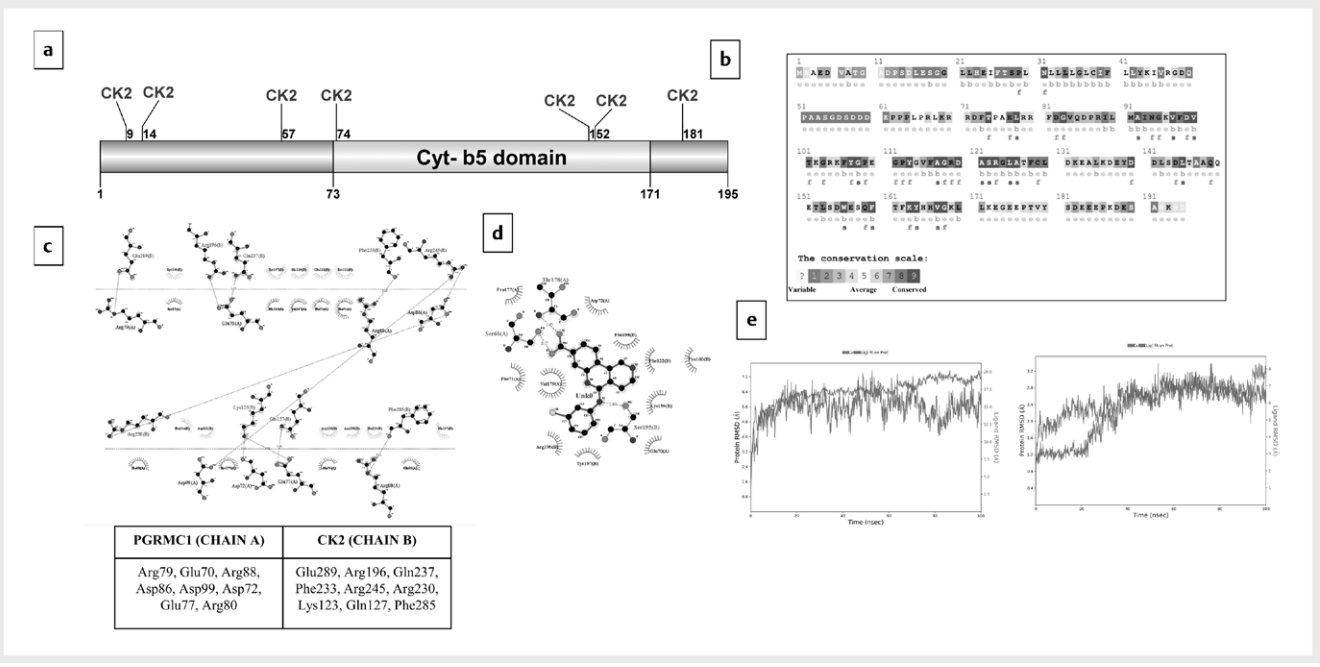
Relative gene expression of PGRMC1 and CK2 were detected using quantitative real-time PCR (qRT-PCR: QuantStudio 5). The A549 cells were seeded in 6-well plates and allowed to reach approximately 80 % confluence; the cells were treated with Silmitasertib at the mentioned concentration, respectively. Total RNA extraction was conducted employing TRIzol reagent from Gibco under the manufacturer's instructions (**Supplementary Table 1**) [13].

Statistical Analysis

All experiments were carried out in triplicate, and results were analysed statistically using an unpaired student's t-test. The data is displayed as Mean ± S.D. Differences were deemed statistically significant at $^{*}p < 0.05$.

Results and Discussion

Earlier mutation studies have reported the existence of the CK2 binding motif pattern "T/S-x-x-E/D" at S57 and S181 amino acid positions in PGRMC1 [14]. However, no study has reported the existence of multiple other CK2 signature motifs in PGRMC1 or its associated role in lung cancer promotion and progression. The presence of numerous CK2 signature motif "T/S-x-x-E/D", in PGRMC1 were identified at 9, 14, 74, 146, and 152, in addition to 57 and 181 amino acid positions using bioinformatics tools: Motif Scan-My Hits and ScanProsite [7, 8] in this study (▶ **Fig. 1a**). The CK2 signature motifs were observed to be located on the functional and exposed region [9] of the PGRMC1 protein sequence, which is highly conserved. The protein-protein interaction (PPI) analysis plays a fundamental role in understanding the potential binding contacts of PGRMC1 – CK2 and their biological function [10]. Silmitasertib – a CK2 inhibitor, to understand the bonding and non-bonding interactions between PGRMC1 [PDB ID:4X8Y] and its potential binding partner – CK2 [PDB ID:6HMB]. The HDOCK confidence score and free energy calculation for PGRMC1-CK2 PPI interaction analysis were 0.8662 and − 243.49 Kcal/mol, respectively. This confidence



▶ **Fig. 1** **a**) Protein Structure of PGRMC1 with identified CK2 binding sites **b**) Evolutionary conservancy of PGRMC1 produced by Consurf. **c**) Interaction diagram of protein–protein & protein-ligand. Ligand interactions of (**a**) PGRMC1-CK2, (**b**) PGRMC1-CK2-Silmitasertib using Ligplus. **e**) RMSD plot of protein-protein (PGRMC1-CK2) and protein-ligand (PGRMC1-CK2 – silmitasertib) complexes.

score suggests they are likely to bind to each other (▶ **Fig. 1b**). The acidic residues PGRMC1 were found to have hydrogen bonding with basic residues of CK2 (▶ **Fig. 1b**). These results suggest that the acidic residues of PGRMC1 present adjacent to the CK-2 "T/S-x-x-E/D" signature motif starting at amino acid position Thr 74 A can undergo post-translational modifications like the phosphorylation leading to the control and functional regulation of PGRMC1.

Interestingly, the confidence score value 0.6 was obtained for the Silmitasertib docking to the PGRMC1-CK2 PPI complex. Silmitasertib was found to be evident that interacting with PGRMC1 – Ser 68 A and Thr 178 A, which is very close to the 5Å vicinity of the CK2 signature motif at Thr 74 A and Thr 181 amino acid positions of the PGRMC1 sequence, confirming their potential ability to interact with both CK-2 motif and PGRMC1.

Furthermore, the molecular dynamics (MD) simulation studies provided information on the stability of interacting residues occurring between the CK2 (Cyan) and PGRMC1 (Red). However, the stability of the MD-based interaction analysis was weaker between PPI (CK2 and PGRMC1). MD simulation analysis from the 20 ns to 100 ns of the PPI-Silmitasertib interaction complex showed improved bonded and nonbonded interactions stability (▶ **Fig. 1d**). The results obtained for Root Mean Square Fluctuations (RMSF) that indicate the fluctuations of individual amino acid residues during the period of MD simulation studied correlated well with observations of the RMSD [11, 12].

Experimentally, scratch assays results demonstrate that Silmitasertib impedes the migration of LUAD cells compared to the A549 control group (▶ **Fig. 2a-c**). Consequently, using the colony growth assay when A549 cells were exposed to Silmitasertib resulted in significant suppression of the growth of the colonies (▶ **Fig. 2d**) and also substantially controls the proliferation and their ability to form colonies. Gene expression studies using real-time PCR were also performed for the control versus Silmitasertib-treated cells. The expression analysis revealed that Silmitasertib was highly influential in suppressing the metastatic potential of LUAD cells, and this could be attributed to its ability to regulate the PGRMC1-CK2 axis (▶ **Fig. 2e**). Nonetheless, from our computational and experimental *in-vitro* studies, we firmly believe that Silmitasertib suppressed the PGRMC1-CK2 signaling axis. From all the above, the substrate kinase combination of the PGRMC1-CK2 axis confidently entrenches the importance of its functional and signaling attributes toward LUAD progression. In conclusion, our study infers the PGRMC1-CK2 axis that appears to be an essential target to combat LUAD.

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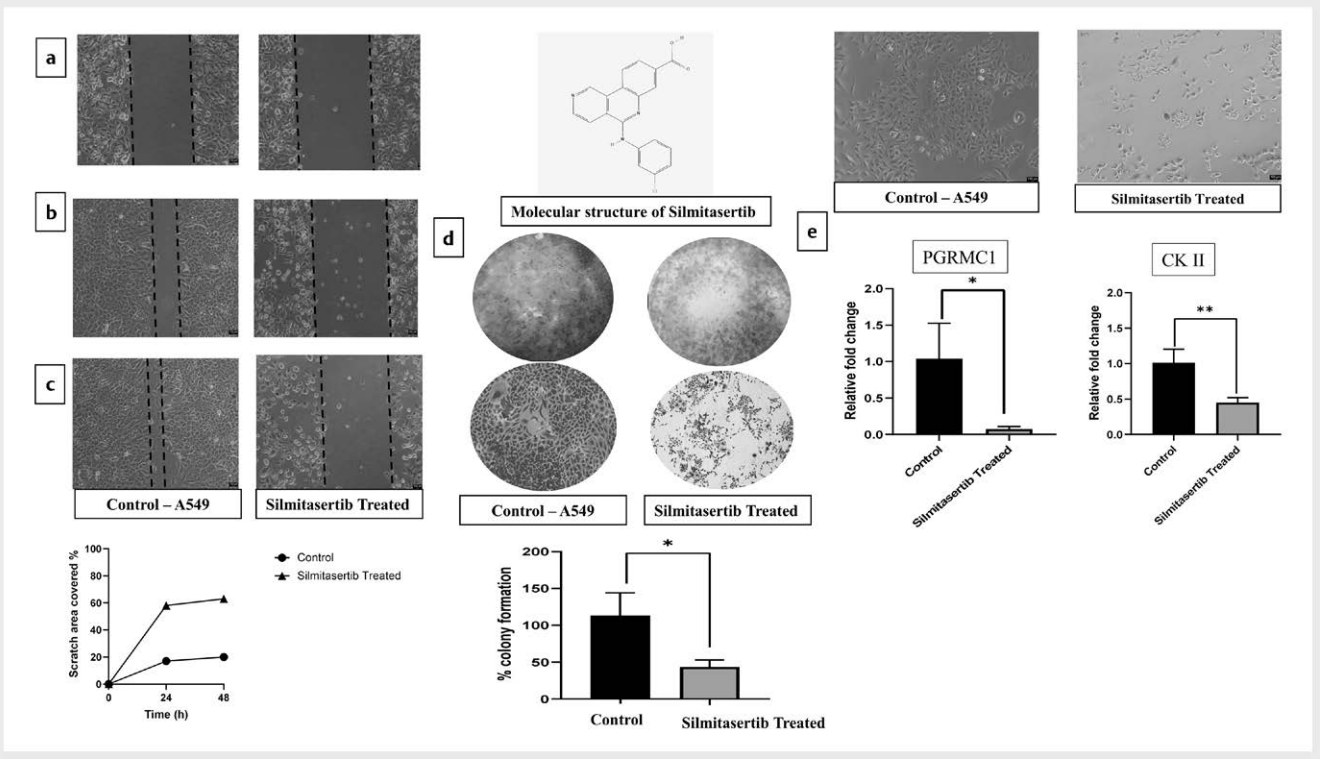

▶ **Fig. 2** : (**a-c**) Silmitasertib prevents the migration of cells compared to control. Pictures are representative of 20X magnification. (**d**) Silmitasertib treated decreases the number of colony-forming units (CFU). Images of the Petri plate with colonies are shown here. (**e**) Real-time PCR expression analysis of PGRMC1 and CK2 in CK2 inhibitor silmitasertib treated LUAD cells.

Conflict of Interest

The authors declare that they have no conflict of interest.

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