Artificial Intelligence Aided Drug Discovery of Pyruvate Kinase Modulators

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Introduction

- Pyruvate kinase (PK) is a crucial enzyme that plays an essential role in the final step of glycolysis catalyzing the transfer of a phosphate group from phosphoenolpyruvate (PEP) to adenosine diphosphate (ADP) to generate pyruvate and adenosine triphosphate (ATP).
- PK has four different isoenzymes: L, R, M1 and M2. The L/R isoenzymes (PKLR) are homologous and expressed in the liver and blood.
- PKLR is involved in 2,3-Bisphosphoglycertae (2,3-BPG) homeostasis, a critical contributor to sickle cell disease (SCD) pathogenesis.
- Cancer cells primarily depend on ATP for proliferation and inhibition of PKLR is known to suppress the Warburg effect in hepatocellular carcinoma (HCC).
- PKLR is thus a potential target for both sickle cell disease and HCC.

Hypothesis: *"Discovery of PKLR small molecule allosteric modulators could translate to novel therapies for several diseases such as SCD and cancer"*

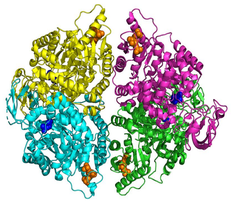

Figure 1. X-ray crystal structure of PKLR (PDB ID: 2VGB)

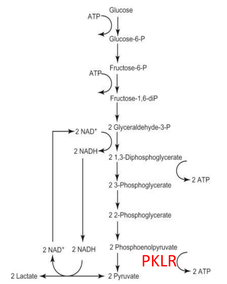

Figure 2. Role of PKLR the glycolytic pathway

Methods

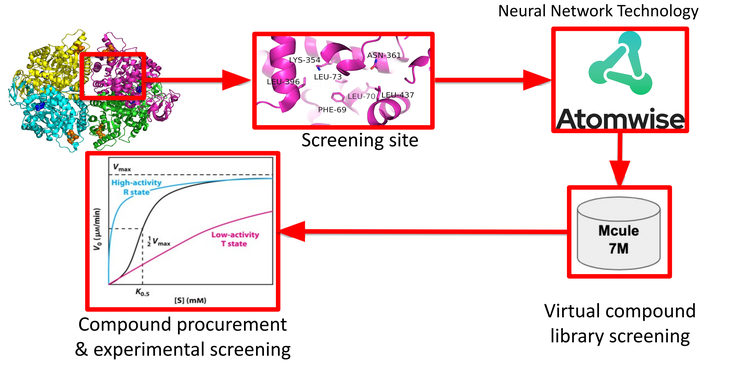

Figure 3. The overall virtual screening strategy to identifying PKLR modulators.

- A virtual screening campaign was carried out to identify potential allosteric modulators of PKLR using the AtomNet® technology.
- Enzyme activity was determined by LDH coupled assay, which measures the decrease in UV/VIS absorbance at 340 nM as a result of NADH oxidation.
- Initial screening of the compounds was preformed in the presence of 1.0 mM PEP (substrate) and compared to the activator (FBP) and inhibitor (L-PHE).
- Detailed kinetic experiments were carried out for five promising compounds with different PEP concentrations (0.25 - 3.0 mM).

Results

- Top scoring 70 compounds were tested.
- Out of the 70 compounds, 5 hits were identified, including 3 inhibitors and 2 activators of PKLR (Figure 4A; Table 1).
- The V_{max} of the compounds ranged from 2.8e(-5) to 7.9e(-5) mM•S^{-1} vs. 8.8e(-5) mM•S^{-1} for PEP (Table 1).
- The K_m for the compounds ranged from 0.35 to 3.4 mM vs. 0.91 mM for PEP (Table 1).
- The 3 identified inhibitors DA1-3 exhibited different types of inhibition mechanisms, which are uncompetitive, non-competitive and completive mechanisms, respectively (Table 1).
- DA1 and DA2 showed antiproliferative activity in the CCK8 cell-based assay using hepatocellular carcinoma (Hep3B) cell line (Figure 5A-B).

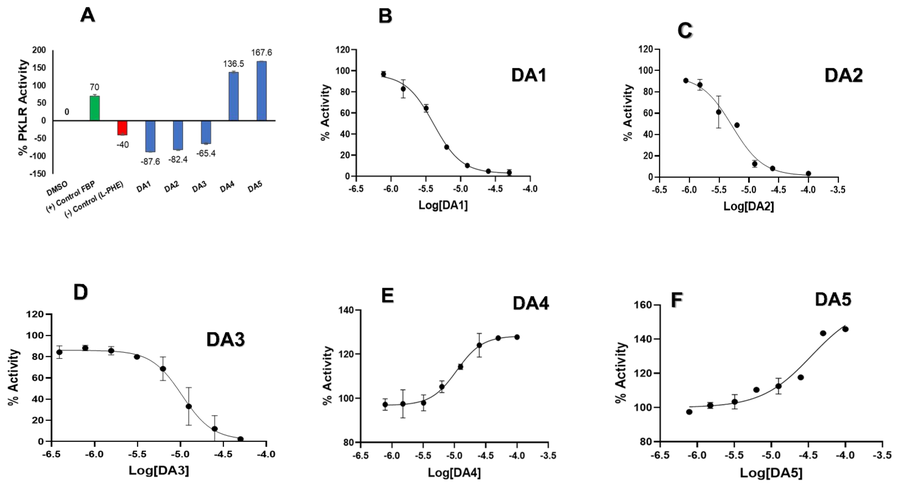

Figure 4. Screening results. A) The effect of the 5 identified hits on the activity of PKLR at 50 µM concentration in the presence of 1mM PEP (substrate). Results are compared to positive control (FBP) and negative control (L-PHE). B) through D) Dose–response curves for the identified inhibitors DA1-3, respectively. E) and F) Dose–response curves for the 2 identified activators DA4 and DA5, respectively.

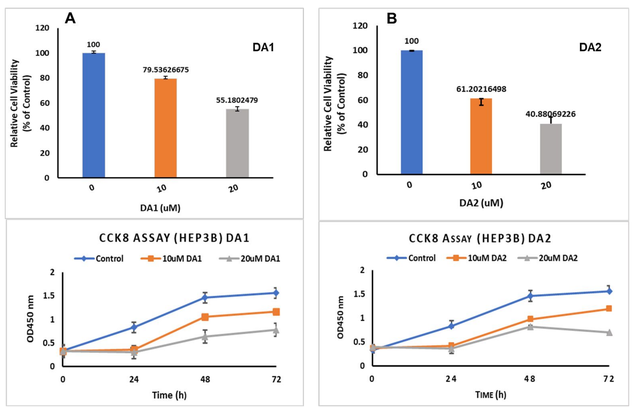

Figure 5. Cell proliferation assay results measured by CCK-8 cell viability assay. A) and B) Treatment with PKLR inhibitors, DA1 and DA2 respectively, inhibits cell viability of Hep3B cells.

Table 1. Detailed kinetic parameters of the identified hits.

Comp ID	V_{max} (mM) . S^{-1}	K_m (mM)	nH	IC_{50}/EC_{50} (µM)	Type of Modulation
PEP	8.8e(-5) ±0.9e(-5)	0.91 ± 0.014	3.2 ±0.2	NA	Control
FBP	13 e(-5)±2.1e(-5)	0.84 ± 0.013	0.9 ±0.1	NA	Activator
DA1	2.8e(-5) ±0.45e(-5)	0.35 ± 0.021	2.5±0.29	4.0 ± 0.33	Un-Competitive inhibitor
DA2	1.3e(-5) ± 0.8e(-5)	0.76 ± 0.19	0.9±0.2	5.2 ± 0.74	Non-Competitive inhibitor
DA3	7.7e(-5) ±0.9e(-5)	3.4 ± 0.71	1.6±0.1	23.6 ±2.0	Competitive inhibitor
DA4	7.4e(-5) ±4.0e(-5)	0.89 ± 0.13	2.6±0.6	10.8 ±0.81	Activator
DA5	7.9e(-5) ±1.7e(-5)	0.67 ± 0.035	2.2±0.29	34 ±1.9	Activator

Conclusions

- PKLR is a crucial enzyme that plays an essential role in the final step of glycolysis.
- PKLR has been identified as a potential target for SCD and HCC.
- A virtual screening campaign using an artificial intelligence aided approach was carried out to identify potential small molecule modulators of PKLR.
- Top scoring 70 compounds were obtained and experimentally tested for PKLR modulation.
- 3 inhibitors and 2 activators were identified.

Ongoing/future experiments:

- Determine K_D values for the identified PKLR modulators.
- Establish structural activity relationships for these compounds.
- *In vivo* studies to measure effects of the identified PK modulators in both sickle cell disease and liver cancer animal models
- Co-crystallize PKLR with the identified modulators

References

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