Research Article

Detection of nail oncometabolite SAICAR in oral cancer and its molecular interactions with PKM2 enzyme

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CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.
ETHICAL STATEMENT: This study obtained ethical approval from the Institutional Ethics Committee, Dr. D. Y. Patil Vidyapeeth, Pune

ABSTRACT

Background

Oncometabolites are known to drive metabolic adaptations in oral cancer. These oncometabolites serve as biomarkers for early detection of oral cancer. Among potential oncometabolite, SAICAR is one of them that support growth and invasiveness of cancer cells. SAICAR has been reported to activate Pyruvate Kinase M2 (PKM2) enzyme, which in turn favors the survival of cancer cells in low glucose tumor microenvironment. There is a significant gap in detection of SAICAR in biological fluids/materials including nails of oral cancer patients.

Methods

This study includes metabolite identification of SAICAR in nails of oral cancer assisted by a novel vertical tube gel electrophoresis (VTGE) in combination with LC-HRMS. Further molecular docking and molecular dynamics simulations (MDS) were employed to determine the nature of molecular interactions of SAICAR (CHEBI ID:18319) with PKM2 (PDB ID: 4G1N). Here, we have performed Molecular docking of SAICAR (CHEBI ID:18319) against Pyruvate kinase M2 (PDB ID: 4G1N) according to available literature.

Results

Data suggest the presence of oncometabolite SAICAR in nails of oral cancer. Molecular docking of SAICAR with PKM2 showed appreciable binding affinity (-8.0 kcal/mol) with residues including ASP407, THR405, GLU410, ARG443, GLY321, ARG436, HIS439, LYS266 and TYR466. Furthermore, MDS confirmed the specific binding of SAICAR within the activator site of PKM2 and stability of SAICAR and PKM2 molecular interactions.

Conclusion

In conclusion, we report SAICAR as an oncometabolite biomarker in nails of oral cancer. Additionally, we addressed the activation potential of SAICAR with the PKM2 enzyme.

Keywords:

Oral Cancer, Metabolite biomarkers, SAICAR, Pyruvate Kinase M2, i*n silico* studies.

INTRODUCTION

Oral cancer is considered as the 6th most prevalent cancer type at global level (1)(2)(3). India has the highest cases of oral cancer with the rate of approximately 30% of all new cases yearly. Higher rate of prevalence in developing countries is due to their regional habit of chewing tobacco and betel nuts (2)(3)(4).

Biomarkers are the biological molecules including oncometabolites, which have a vital role in differentiating diseased and normal states. Biomarkers include proteins, antibodies, nucleic acid, peptides, enzymatic changes, lipids, metabolites and carbohydrates (5) (6). Among these biomarkers, metabolites as a biomarker are currently focused on the study of oral cancer. Study of metabolites from different cancer cells and tissues have application in diagnose cancer at an early stage by identifying these metabolites as a biomarker for oral cancer and precancerous lesions.

Altered metabolism and survival under stressed conditions is one of the hallmarks of cancer. Pyruvate kinase enzyme has a vital role in the regulation of both the conditions. Generally, mammal's pyruvate kinase has four types of isomers including protein kinase M (PKM)2. PKM2 is known to be significantly expressed in highly proliferating cells (7)(8)(9)(10). PKM2 is reported as a catalyst that converts phosphoenolpyruvate into pyruvate along with the ATP in cancer cells. Expression of PKM2 has been observed to be significantly increased in some types of cancer including oral cancer (8) (11) (12).

In tumor cells, PKM2 works as a dimer. Several studies have revealed that oncometabolites namely SAICAR (succinylaminoimidazolecarboxamide ribose-5′-phosphate), fructose-1,6-P2 and serine can lead to the formation of tetramer from dimer of PKM2 (13)(14)(15)(16)(17). In fact, SAICAR, an intermediate product of the *de novo* purine nucleotide synthesis pathway acts as an oncometabolite that supports the growth cancer cells in nutrient limited medium (15)(16)(17). Limited studies revealed the ability of SAICAR to modulate the kinase activity of PKM2 (16)(17).

Based on the existing data, there is a significant gap in the detection of SAICAR in biological materials of oral cancer including nails. Furthermore, molecular docking and molecular dynamics simulation (MDS) data on SAICAR interactions with PKM2 is needed for better understanding. Hence, we attempt to detect SAICAR in nails of oral cancer by using a novel VTGE assisted methodology and performed molecular docking and MDS to collect data on molecular interactions between SAICAR and PKM2.

MATERIALS AND METHODS

Study Population

In this study, oral cancer patients (n=5) and healthy controls (n=6) were recruited at Dr. D. Y. Patil Dental College and Hospital, Pune, India. The participating subjects in both OSCC and healthy subjects were in the age group of 30-60 years. Before commencement of this study, Institutional Ethics Committee approval was obtained. In this study, participants were detailed on the purpose of the study and informed consent was collected.

VTGE assisted purification of nail metabolites

We report here an approach assisted by a novel vertical tube gel electrophoresis (VTGE) system to detect SAICAR in the nail metabolite lysate of oral cancer patients (Figure S1). Fingernail clippings of oral cancer patients were dissolved in 800 µl of nail lysis buffer (5M Urea, 2.6M thiourea, Tris-HCl (20mM, pH-8.5) and beta-mercaptoethanol). Further, nail lysates were purified by the help of VTGE metabolite purification system (35, 36). Then, purified nail metabolites of oral cancer patients were detected by using LC-HRMS. During LC, RPC18 column (Zorbax, 2.1 X 50 mm, 1.8 µm) was employed. Then metabolites submitted to MS Q-TOF Quadrupole time-of-flight mass spectrometry (Q-TOF-MS) with positive electrospray ionization (ESI) M-H mode.

Molecular Docking

SAICAR was detected as a potential oncometabolite in the nails of oral cancer patients, so we have proceeded to *in silico* studies on SAICAR. To perform molecular docking, potential oncometabolite SAICAR (CHEBI ID:18319) was retrieved as a ligand from ChEBI database in SDF format. Then OpenBabel software was employed for the conversion of ligands in SDF format into PDB format. Energy minimization of ligand before performing molecular docking is an important step to obtain stable conformation of the ligand. Avogadro software was used for energy minimization of the ligand by selecting the steepest descent method and MMFF94s force field (18). Pyruvate kinase M2 (PDB ID: 4G1N) was considered as the target receptor protein. It was downloaded in PDB format from Protein Data Bank (PDB). In order to free all the binding pockets on the receptor, we have removed bound ligands by deleting hetatoms from the pdb file. Then protein pdb file was opened in AutoDock Tool 4.2. To perform the steps of protein preparation, steps consist removal of water molecules, bond correction, assigning AD4 type atoms, addition of polar hydrogens and Kollman charges (19) to the receptor. We have

used AutoDock Vina Software for molecular docking of SAICAR oncometabolite with PKM2 protein (20).

AutoDock Vina has inbuilt automatic grid maps (20). First, we have performed blind docking to confirm the active binding sites of the ligand. Blind docking includes the covering of the whole receptor protein with a grid box of appropriate size. The docking includes organized conformational expansion of the ligand and a further interaction of oncometabolite to the active site residues of the receptor occurs. The visualization of the binding position of SAICAR into the binding pockets of the PKM2 was performed by using a discovery studio visualizer.

Molecular Dynamics Simulations (MDS)

We have used Desmond software for 10ns Molecular Dynamics (MD) simulation of oncometabolite SAICAR - PKM2 complex to confirm the binding stability and strength of the complex (21). Desmond software comes with the features of adding pressure, temperature, volume system and many other functions to complete the protein-ligand binding. Protein-Ligand complex was immersed in a water-filled orthorhombic box of 10 Å spacing (22). The SAICAR-PKM2 complex system is solvated by 21066 water molecules using an extended three-point water model (TIP3P) with periodic boundary conditions. These studies were performed with a run of 10ns and temperature 300K, considering certain parameters such as integrator as MD. The conformational changes upon binding of SAICAR with PKM2 were recorded with the help of 1000 trajectories frames generated during 10ns MD simulation. Root Mean Square Deviation (RMSD) was calculated to confirm the deviation in the conformation of ligand and protein.

RESULTS

Identification of Oncometabolite from nails of Oral Cancer Patient

Previous findings on Oral cancer and precancerous lesions have reported that it does not only involve proteins and expressed genes but also contains endogenous metabolites (23) (24) (25) (26). Recent studies on oral cancer, metabolite biomarker is a focus for the early diagnosis of oral cancer. By taking these findings in consideration, we explored nail metabolome by the purification of metabolites from nails of oral cancer subjects with the help of novel VTGE tools (27) (28).

Elutes of nail metabolites prepared by VTGE were identified by using LC-HRMS in a positive ESI mode. Based on the metabolite profiles, we report SAICAR as an oncometabolite

in the nails of oral cancer patients. Here, detected oncometabolite SAICAR showed distinctive mass ion spectra with chemical formula ($C_{13}H_{19}N_4O_{12}P$) showed m/z (436.0626) and mass (454.0726) in a positive ESI mode (Figure 1). At the same time, SAICAR is present in the nail lysates of healthy controls.

Molecular Docking

As per the previous studies on SAICAR-Pyruvate Kinase M2, it is understood that SAICAR binds to the dimeric form of PKM2 and converts it into trimetric form (15) (16). Which further plays a role in progression of tumor by activating inactive PKM2 (17). There is a lack of clear molecular interactions studies about binding of SAICAR to PKM2 that leads to the activation of enzymes.

Here, the authors have performed molecular docking to understand the binding pattern of SAICAR upon PKM2. Autodock Vina was utilized to carry out molecular docking experiments. To find out the binding amino acid residues, we have used Discovery studio visualizer software. It also shows the no. of polar bonds between ligand and amino acid residues with bond distance.

Molecular docking of SAICAR with PKM2 (PDB ID: 4G1N) revealed appreciable binding affinity (-8.0 kcal/mol) (Figure 2A). Upon the detailed visualization of interactive amino residues, SAICAR binds through 13 polar bonds to the binding residues including ASP407, THR405, GLU410, ARG443, GLY321, ARG436, HIS439, LYS266 and TYR466 of PKM2 protein (Figure 2B 2C and Figure 2D).

Molecular Dynamics Simulations (MDS)

MDS was performed for the duration of 10ns to examine the stability of the ligand-protein complex. Root mean square deviation (RMSD) of ligand and protein was calculated during the 10ns of simulation period. RMSD was aimed to measure the average change in the displacement of C-α atoms for 1000 frames concerning a reference frame (initial docked conformation). The RMSD plot of PKM2 protein indicates that displacement of protein is up to 2.5Å, which is totally acceptable within range (1-3Å) for stability of protein throughout the simulation period. The RMSD plot of the SAICAR-PKM2 illustrates that initial displacement in the conformation of the complex up to 4ns, afterwards it maintains the equilibration state (Figure 3).

In this graphical image, the left axis depicts the RMSD plot for PKM2 enzyme regarding changes in structural conformation during 10ns simulation time. The changes in the protein

RMSD value are observed in the range of 1-3Å. The right-Y axis denotes the RMSD value of bound SAICAR (CHEBI ID:18319) to PKM2 and this value is not significantly larger than the RMSD value of PKM2 enzyme (PDB ID: 4G1N). Thus, the RMSD plot describes the stable ligand-protein complex.

MD Simulation study also comprises the graphical presentation of protein-ligand contacts, categorized by type of bonds. It explains the availability of interaction between ligand and protein over course of simulation. This graph shows that SER406 and ASP407 have hydrogen bonds for 60% to 90% of simulation time (Figure 4A). Another graph for protein-ligand interaction depicts the interaction of each residue with ligand in each time frame of the simulation. There is a darker shade of orange in the graph, which describes that some residues have more than one specific contact with the ligand. Simulation data suggested that ASP407, GLU285, SER 406, ASP407 and GLU410 have more than one bond with ligand. MD simulation also provides the schematic diagram of detailed ligand interaction with residues of protein, it shows the interaction which generates for more than 30% of the simulation time of the selected 0.00 to 10.00 ns trajectory (Figure 4B). Amino acid residues such as ASP407, THR405, SER406 and ARG443 have been shown to interact with ligands in a schematic diagram with the nature of the residues in different colors. Altogether, data collected from molecular docking and MD simulation suggests a strong evidence of SAICAR-PKM2 interaction in cancer cells.

DISCUSSION

PKM2 is described as one of the limiting enzymes in glycolysis and this enzyme induces formation of pyruvate and ATP from phosphoenolpyruvate (PEP) and ADP in proliferating cancer cells (29) (30). PKM2 is present in the dimeric form with higher *K*m value for the phosphoenolpyruvate (PEP) substrate, accordingly dimeric form of PKM2 is in inactive state at normal physiological state (31)(32).

There are reports that SAICAR concentration in cancer cells increases gradually during starvation of glucose and eventually stimulates PKM2 for cancer progression (15) (16). Another study reveals that PKM2 expression promotes the uptake of glucose upon inhibition of oxygen and expression of low active PKM2 dimer bring the accumulation metabolites such as serine, phosphoenolpyruvate (PEP) and glucose-6-phosphate (33)(34). Serine binds to PKM2 and activates it by maximizing the use of glucose and in glucose deprived condition switches to SAICAR for activation of dimeric PKM2 independent of FBP (35)(36)(38).

Furthermore, the clinical relevance of PKM2 enzyme is linked with oral cancer and this enzyme supports the growth and proliferation during abnormal glucose metabolism (1) (39) (40).

Besides existing views, there is a complete gap in the clinical relevance of SAICAR as an oncometabolite in oral cancer. In the present work, detection of SAICAR in nails of oral cancer is attributed to a novel VTGE assisted novel approach that helped in a clear detection of SAICAR. On the other hand, SAICAR is not detectable in healthy control.

Here, SAICAR is detected as an oncometabolite and there is evidence on the overexpression of PKM2 enzyme in oral cancer. Therefore, we attempted to reveal the molecular interaction of SAICAR with the PKM2 enzyme by using molecular docking and MDS. Besides the strong binding affinity of SAICAR, interactive amino acid residues including ASP407, THR405, GLU410, ARG443, GLY321, ARG436, HIS439, LYS266 and TYR466 of PKM2 the presence of a good number of 13 polar bonds. Apart from molecular docking data, MD simulation study of SAICAR-PKM2 complex confirmed the key amino acid residues ASP407, THR405, SER406 and ARG443. In literature, THR405, SER406 and ASP407 residues are spanning within the key pocket of allosteric activator sites in PKM2 enzyme for activator oncometabolites such as F16BP and serine (33) (41). Therefore, present molecular interaction studies provide additional information on the activation binding sites of SAICAR upon PKM2 and support the existing *in vitro* and *in vivo* evidence on SAICAR as an activator of PKM2.

Taken together, detection of SAICAR as an oncometabolite in nails of oral cancer is a novel report and additional molecular interaction data strongly support the activation potential against PKM2 that may contribute towards the growth and proliferation of oral cancer. Besides optimistic sides on SAICAR as an oncometabolite, reported PKM2 overexpression and a clear ability of SAICAR to activate PKM2 that help oral cancer cells, a valid question needs to be resolved whether SAICAR-PKM2 relevance is applicable to all oral cancer patients as a biomarker or limited by heterogeneity factor among oral cancer patients that may be influenced by the dietary patterns. The authors propose that SAICAR as a metabolite biomarker in nails of oral cancer patients with overexpression of PKM2 and dietary patterns of oral cancer patients.

CONCLUSION

In conclusion, we suggest the potential of SAICAR as oncometabolite biomarkers in nails of oral cancer patients. Furthermore, SAICAR shows effective allosteric activation binding upon PKM2 enzyme. This study may be helpful for future therapeutic approaches that can

block the generation of SAICAR oncometabolite and disrupt the SAICAR-PKM2 interaction that led to activation of this enzyme in oral cancer.

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Details of Figures and their legends:

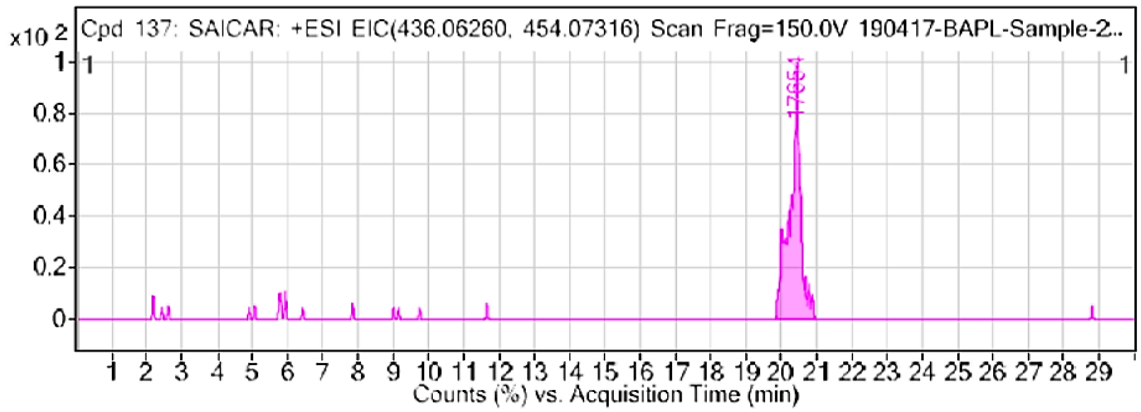

Figure 1. An oncometabolite SAICAR is detected in nails of oral cancer patients.

A positive ESI Extracted Ion Chromatogram (EIC) of SAICAR that was detected during LC-HRMS of nail lysates purified by the help of a novel VTGE tool.

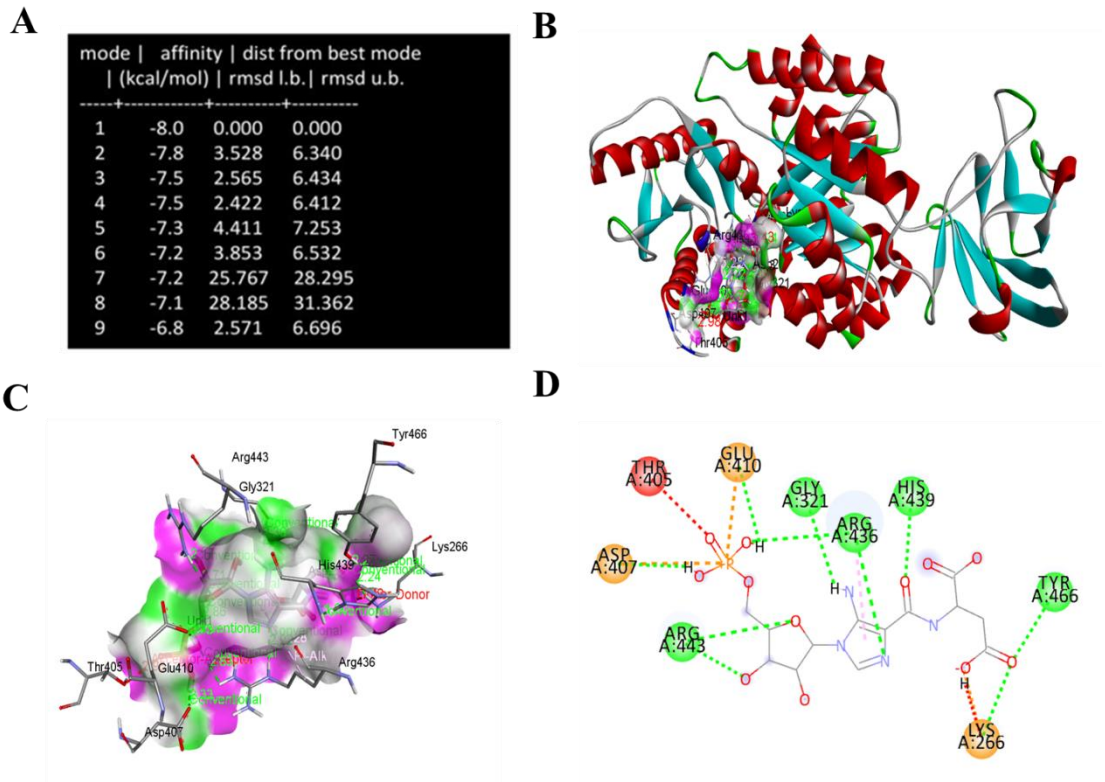

Figure 2. An oncometabolite SAICAR (CHEBI ID: 18319) shows strong molecular binding with Pyruvate Kinase M2 (PKM2) enzyme (PDB ID: 4G1N).

Molecular docking and interaction of SAICAR with PKM2 was visualized with the help of Discovery Studio Visualizer.

(A). Molecular docking affinity estimated by AutoDock Vina. (B). 3D view of Interaction between SAICAR and PKM2 with binding residues, bond distances and types of bonds. (C). Docked molecular structure between SAICAR and PKM2 visualized in 3D image depicting H-Bond interactions (Acceptor in Green and Donor in Pink color) (D). 2-D Image of docked molecular structure between SAICAR and PKM2 derived from Discovery Studio Visualizer.

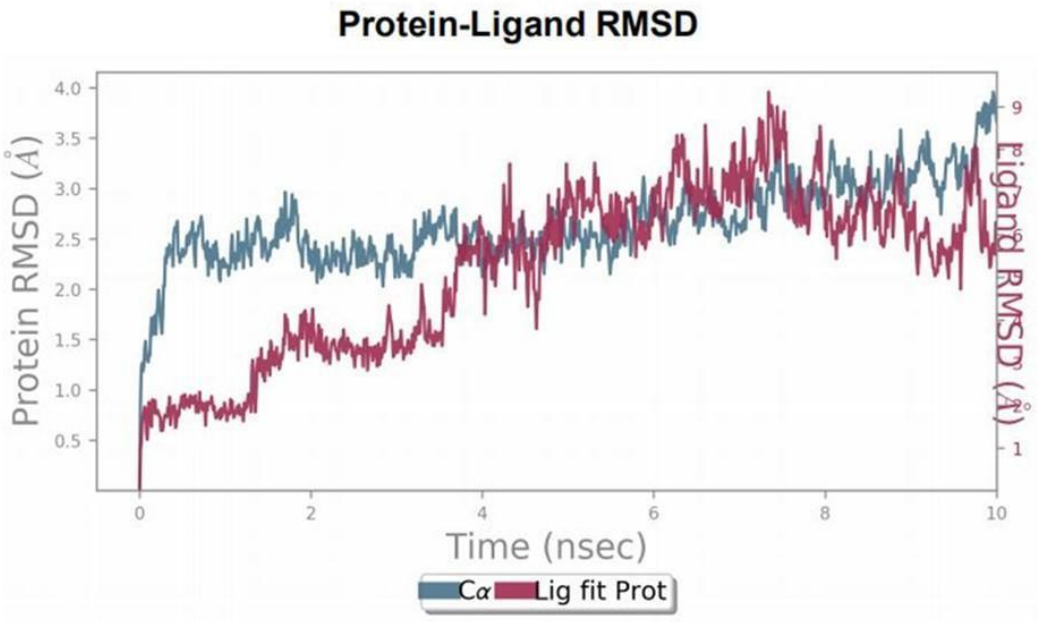

Figure 3. An oncometabolite SAICAR displays stable complex with PKM2

PKM2-SAICAR Room Mean Square Deviation (RMSD) Plot for 10ns of time frame showing the stability of the complex between SAICAR depicts strong and specific binding to PKM2.

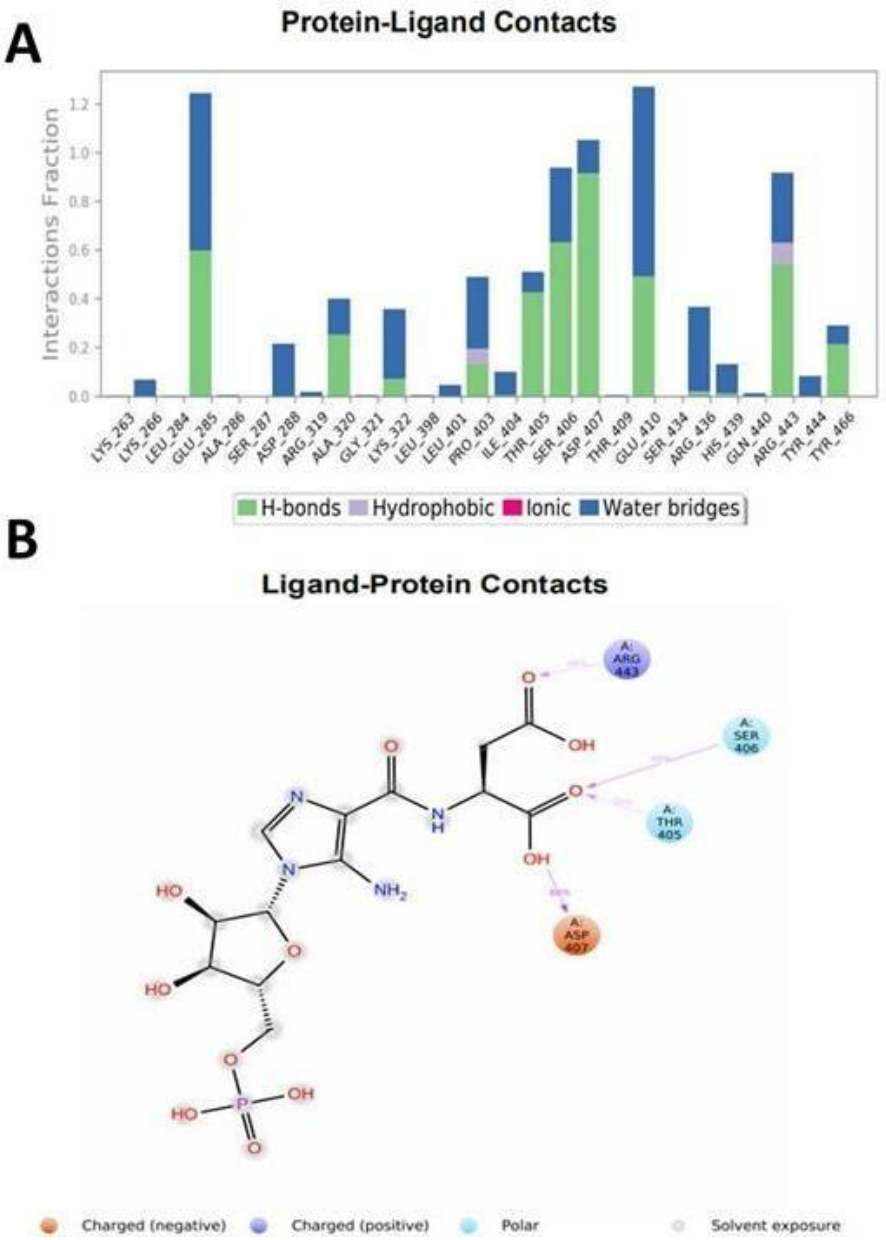

Figure 4. SAICAR shows specific contacts within the activation site of PKM2.

(A) PKM2 and SAICAR interaction plot on interaction between amino acid residues and ligand remained during 10ns simulation. On the Y-axis, interaction fraction shows the time of established interaction between key amino acid and ligand through different types of bonds such as hydrogen bonds, hydrophobic, ionic and water bridges. (B) Schematic diagram on interaction of ligand with amino acid residues, which has remained for more than 30% of interaction time of simulation. Here, various color combinations are used to represent the extent and nature of ligands to enzyme atomic interactions including ionic, hydrophobic, polar, water and solvent exposure.

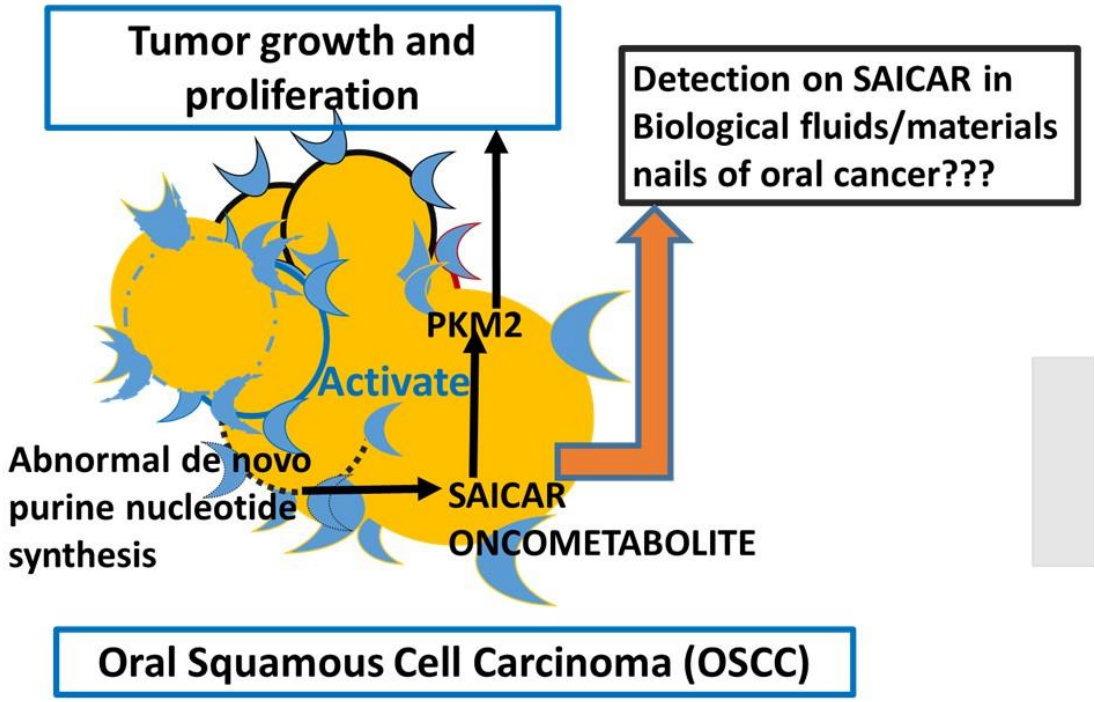

Figure 5. A proposed model on the role of SAICAR oncometabolite as a nail metabolite biomarker.

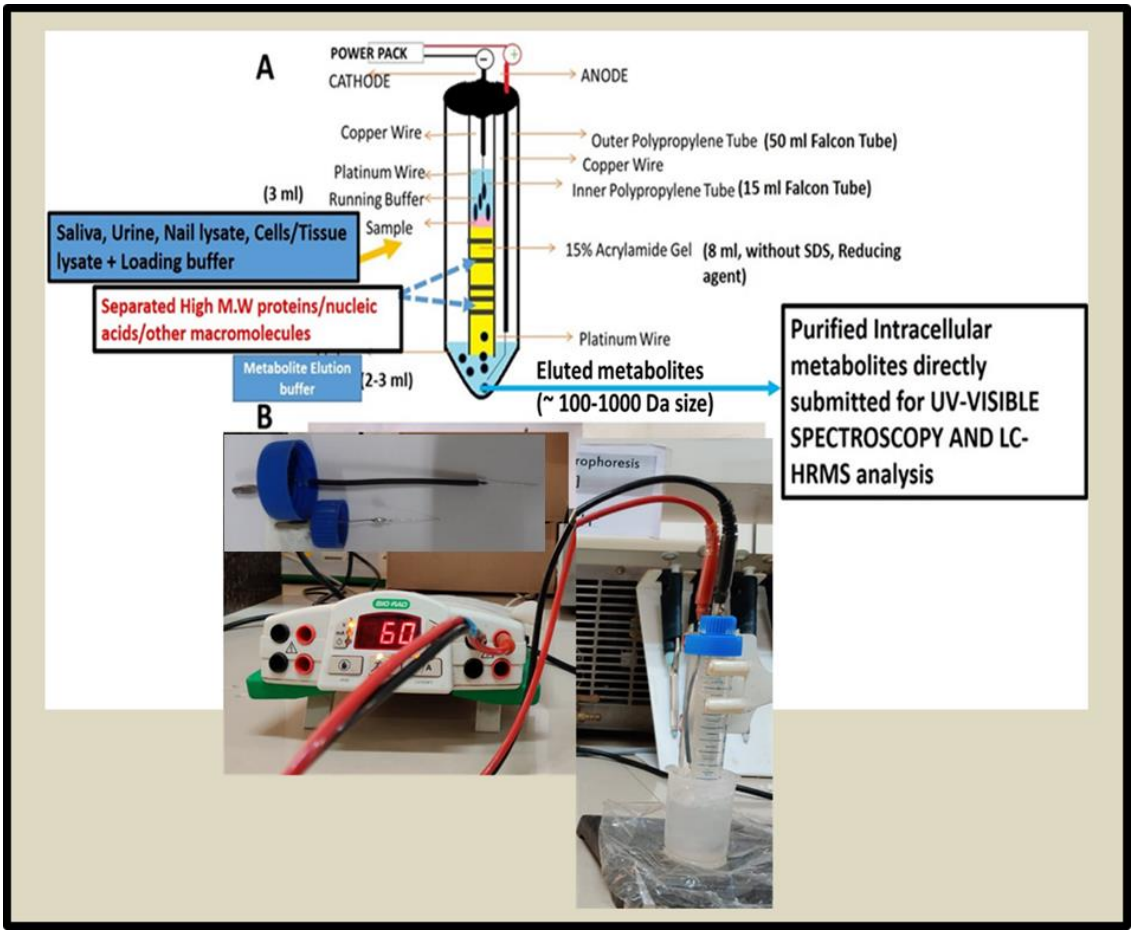

Figure S1. A model on a novel vertical tube gel electrophoresis (VTGE) system. (A) A flow diagram of VTGE system (B) A running model on VTGE system.