

Research Article

Molecular docking and simulation studies predict lactyl-CoA as the substrate for p300 directed lactylation

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ETHICAL STATEMENT: This study does not involve any ethical concern.

ABSTRACT

Background

According to Warburg effects, cancer cells are known to produce lactate as an end product instead of pyruvate and accumulation of lactate is linked to metabolic reprogramming. However, substantial exist in terms of the non-metabolic role of lactate including modification of histones during epigenetic regulation, in particular.

Methods

This study employs *in silico* molecular docking and molecular dynamics study to determine the potential mechanisms of lactylation on histone proteins that achieve epigenetic changes in cancer and non-cancer cells. Here, we tested three potential substrate sources for lactylation, namely lactate (CHEBI ID:24996), lactyl-CoA (CHEBI ID:15529) and (R)-S-lactoylglutathione (PubChem ID-440018). A histone acetyltransferase p300 (HAT p300) enzyme (PDB ID: 6GYR) was considered as a potential candidate for the lactylation process.

Results

Among the studied substrates for the lactylation process, molecular docking reveals a highly efficient binding affinity (docking energy -8.6 Kcal/Mol) of lactyl-CoA with p300 enzyme. On the other hand, lactate and (R)-S-Lactoylglutathione did not shown any significant and specific binding to HAT p300 enzyme. Furthermore, molecular dynamics simulation study suggests a stable binding of lactyl-CoA at the substrate-binding site of p300 with amino acid residues ASP-1399, HIS1402, ARG-1410, THR1411, TYR1414, TRP1436, ASP1454 and LYS1456.

Conclusion

In conclusion, our data support that lactyl-CoA is a potential substrate for lactylation carried out by the HAT p300 enzyme. However, lactyl-CoA is not detected at the physiological level in cancer and non-cancer supporting cells such as macrophage. Based on our data and existing views on lactylation, the authors propose an involvement of pro-tumor bacteria in this that converts lactate to lactyl-CoA and lactyl-CoA is shuttled to the macrophage within the tumor microenvironment. Due to lactyl-CoA entry into macrophages (anti-tumorigenic)s, lactylation process allow the transcriptional changes and achieve the M1 to M2 macrophage polarization (pro-tumor) and in turn, promotes the tumor growth and survival.

Keywords: Metabolic reprogramming, Glycolysis, Lactic acid, Epigenetic modification, Histone modification, Warburg effect.

INTRODUCTION

The relevance of the glycolytic product lactate is widely appreciated as an oxidative and gluconeogenic metabolite, a signaling molecule that drives both genetic and epigenetic landscape in various types of cells including cancer cells (1-4). The tumor contains various kinds of cancer cells and non-cancer cells including immune cells, stromal cells and in the close vicinity, microbiotas. There are strong suggestions that intracellular and intercellular shuttling of lactate may be required to meet the diverse metabolic, signaling and epigenetic regulations of cells (5-11).

Hence, a strong possibility of intercellular shuttling of lactate and lactate derived products such as lactyl-CoA is anticipated. Besides the clear role of lactate in metabolic requirements of growing cancer cells, there is a gap in our understanding of lactate mediated non-metabolic implications including epigenetic modifications of histones. A recent understanding supports the role of lactate as a non-metabolic signaling molecule that promote lactylation, post-translational modification of a lysine residue on histone proteins (12-18). At the same time, lactylation is reported in certain types of cells including macrophages and lactylation is linked to the transcriptional changes that help the M1 macrophages to be changed into M2 macrophages (15-18). In essence, M2 macrophages are shown to share similar attributes to that of tumor-associated macrophages.

In this way, an indirect role of lactylation in cancer growth and proliferation is proposed by activating the M1 macrophage in the tumor microenvironment and in turn transformed into pro-tumor macrophages. Here it is important to mention that metabolomic analysis of tumor tissues and cells does not support the presence of lactyl-CoA, a potential substrate derived from lactate for lactylation at the physiological concentration. Therefore, clear evidence on the source of lactyl-CoA that may participate in the lactylation process at the cellular level is missing.

Furthermore, no experimental evidence *in silico*, *in vitro* or *in vivo* is available that shows the nature of enzymes that carry out the lactylation process by using lactyl-CoA as a substrate. Recent finding by Zhang et al (2019)¹⁷ that claimed the novel evidence on the lactylation process did not bring clear proof on the nature of the enzyme and the source of lactyl-CoA in physiological conditions. At the same time, a suggestion was made on the involvement of histone acetyltransferase (HAT) p300 that is well-known as a regulator of transcription of genes by chromatin remodelling (19-27).

In this direction, we attempt to determine binding affinity of lactyl-CoA with a potential enzyme HAT p300 by using molecular docking and dynamics calculations. Based on our results, we also propose a model on the source of lactyl-CoA and the enzymatic process of lactylation that supports tumor growth and proliferation.

MATERIALS AND METHODS

Molecular Docking Study on Oncometabolite Biomarker and Proteins

Potential oncometabolites including lactate (CHEBI ID:24996), lactyl-CoA (CHEBI ID:15529), (R)-S-lactoylglutathione (PubChem ID-440018) were retrieved from as ligands for molecular docking. The ChEBI (<https://www.ebi.ac.uk/chebi/>) database was used to download the structure of ligands in SDF format. Then conversion of ligands into PDB format took place using the software OpenBable. Before performing molecular docking, both ligands were energy minimized to obtain stable conformation using Avogadro software (28) with the steepest descent method and MMFF94s force field. Protein Data Bank (PDB) (<https://www.rcsb.org>) was used to download the receptor protein. Here, HAT p300 (PDB ID-6GYR) was taken as the target receptor protein. Hetatoms are removed from the protein before performing docking. This protein was subjected to the AutoDock Tool 4.2. to perform the steps of protein preparation, which includes removal of water molecules, bond correction, assigning AD4 type atoms, add polar hydrogens and adding Kollman charges (29). AutoDock Vina Software was used in our *in silico* study, to perform molecular docking of oncometabolites with proteins (30).

AutoDock Vina comes with the feature of the calculation of grid maps automatically (30). Docking of oncometabolite with the active site residues of the receptor proteins was performed to ensure the binding conformations. First, we have performed blind docking for all docking studies, which includes the covering of the whole receptor with a grid box of adequate size. The docking procedure involves the organized conformational enlargement of the ligand, which further includes the binding of Oncometabolites to the binding sites of the receptor protein. After the successful docking, confirmation of the binding position of oncometabolite into the receptor protein and calculation of bond distance has been done by PyMol (www.pymol.org). PyMol allows the clear visualization of binding of ligand-protein with its polar bonds as well as bond distance.

Molecular Dynamics Simulations

The 10ns Molecular Dynamics (MD) simulation of metabolite lactyl-CoA (CHEBI ID:15529) with HAT p300 (PDB ID: 6GYR) was performed with the help of Desmond software to confirm the binding stability and strength of the complex (31). Desmond has inbuilt

functions to add pressure, volume system, temperature and many functionalities to accomplish protein-ligand binding. Ligand-protein complex was plunged in a water-filled orthorhombic box of 10 Å spacing (32). The lactoyl-CoA-HAT p300 complex had 30352 water molecules with help of an extended three-point water model (TIP3P) with periodic boundary conditions. MD simulation study was carried out with a run of 10ns at a temperature of 300K in considering certain parameters such as integrator as MD. The conformational changes upon binding of lactyl-CoA with HAT p300 were recorded by using the 1000 trajectories frames generated during the 10ns MD simulation and the Root Mean Square Deviation (RMSD) was calculated to reveal the binding stability of lactyl-CoA.

RESULTS

Molecular docking of lactate and lactate metabolized products with HAT p300

With reference to the recent discovery of an epigenetic lactylation process Zhang et al. (2019)⁽¹⁷⁾, there is no concrete evidence on the nature of substrate and the enzyme which is known to play a vital role in lactylation for histone modification. However, suggestions are put forth on the involvement of lactate derived metabolites such as lactyl-CoA and (R)-S-lactoylglutathione during lactylation process (12-18). There is a complete lack of *in silico*, *in vitro* and *in vivo* proof on the suitability and availability of these metabolites to take part in lactylation process.

In this direction, we have employed *in silico* approach to understanding the epigenetic modification by the process of lactylation. Autodock Vina was used to performing molecular docking experiments due to its better accuracy of predicting binding patterns, less run time, higher reproducibility and its ability of powerful searching of potential energy surfaces (30).

The molecular binding patterns of lactate (CHEBI ID:24996), lactyl-CoA (CHEBI ID:15529) and (R)-S-Lactoylglutathione (PubChem ID-440018) against HAT p300 is presented in Table 1. PyMol was used after molecular docking to find the binding residues and the number of polar bond with their bond distance. PyMol is functionally accurate in showing ligand-protein interaction.

Among all the performed docking experiments, docking of lactyl-CoA with the HAT p300 has shown appreciable binding energy of -8.6 (Figure 1A). Lactyl-CoA binds through 12 polar bonds to the binding residues ASP1399, LYS1407, CYS1408, ARG1410, THR1411, LYS1456, TRP1466, LYS1469 of HAT p300 protein (Figure 1B and Figure 1C, Table 1). Furthermore, docking abilities of lactate (-4.2 Kcal/Mol) and (R)-S-Lactoylglutathione (-7.3 Kcal/Mol) with

HAT p300 are not strong and non-specific to the catalytic site as shown in case of lactyl-CoA (Table 1).

Molecular Dynamics Simulation

To analyze the stability of the ligand-protein complex, the molecular dynamics simulation was carried out for the duration of 10ns. We have selected a complex of lactyl-CoA-HAT p300 for the simulation study due to its appreciable docking affinity/energy and specific amino acid residues at the catalytic site as displayed by PyMol view in Figure 1B and 1C in PyMol view. We have analyzed the conformation of the protein-ligand complex obtained during the simulation period of 10ns. Root mean square deviation (RMSD) was calculated during the simulation trajectory of 10ns for the ligand and protein. It was calculated to measure the average change in displacement of C- α atoms for 1000 frames concerning a reference frame (initial docked conformation). The RMSD evolution plot of HAT p300 protein on the Y-axis suggests that the changes in the RMSD of protein are within the order of 1-3Å that is well accepted for the stability of protein during the simulation. The RMSD plot for the lactyl-CoA-HAT p300 explains that initial deviation in the conformation in the complex observed till 9ns, which further stabilizes in the production phase. It also indicates the continuation in the stabilization of conformation beyond 10ns (Figure 2).

Molecular dynamics simulation study also depicts the plot of protein-ligand contacts and explains the interaction fraction of the protein residue with the ligand, which means how much % of the simulation time the specific interaction is maintained between ligand and receptors complexes. In this, LYS1456 and ARG1410 have shown the highest interaction fraction of 1.75 or more than it with more than 1 bond of Hydrogen and water bridges type (Figure 3). There is another graph for protein-ligand interactions which explains the contacts each residue makes with ligands in the time frame of the simulation. Some residues make more than one specific contact with the ligand, which is shown in a darker shade of orange. LYS-1456, ARG-1410, THR-1411, ARG-1462 have been shown to make more than one contact with ligands (Figure 4). A detailed schematic diagram of protein-ligand interaction has also resulted from dynamic simulation which explains that interactions that occur more than 30% of the simulation time in the 0.00 through 10.00ns are shown. This simulation study depicts that the amino acid residues such as ARG1410, THR1411, HIS1402, ASP1454, LYS1456, TYR1414, TRP1436, ASP1399 within the catalytic site of HAT p300 enzyme can interact with lactyl-CoA with the help of a different type of interactions (Figure 5). There might be many residues that occurred after 10nsec MD simulation time due to their multiple interactions with the ligand, which are not shown in the above diagram because we have performed simulation

for the duration of 0.00 to 10.00 ns. We have also recorded a video of 10ns MD simulation showing the conformation changes of protein after of ligand at different conformations of protein (Supplementary Material 1). The simulation recording in video appreciably suggests the strong and specific association within the catalytic site of HAT p300 enzyme. Take together, data collected from molecular docking, simulation and dynamics studies suggest a strong possibility of lactyl-CoA as a potential substrate of HAT p300 and this may work as a potential mechanism for the lactate based lactylation process.

DISCUSSION

Cancer cells within tumor microenvironment achieve metabolic reprogramming by concerted contributions from cellular and non-cellular factors (1-4). Indeed, requirements of cancer cells are through various metabolic networking including glucose metabolism and distinctive metabolic products including lactate (5-11). In view of Warburg's effects in cancer cells, the production of lactate is suggested as a waste product. Currently, there is an emergence of understanding that cancer cells use metabolic waste lactate to fuel the growth and metastasis by supporting various intracellular metabolic and non-metabolic epigenetic regulation within the tumor microenvironment (12-18).

Epigenetic alterations such as methylation, acetylation and succinylation on chromatin materials are known to alter the transcriptional attributes of cancer and non-cancer cells (19-27). Furthermore, accumulating evidences have shown the existence of an axis between epigenomic changes and metabolic adaptations. (12-18 These post-translational modifications of histones need metabolites as cofactors or substrates such as acetyl CoA for acetylation of histone (14-18). In support of axis between metabolic regulation and epigenomic adaptations, lactate accumulation is reported to be associated with the lactylation process that modifies the lysine residue on histone as lysine-lactate (Kla) (16-18). At the same time, key insights on nature of substrate, biological abundance of substrate and associated enzymes are missing.

In a direction to unresolved question on the nature of substrate and enzymes in lactylation, there is a hint on the potential enzymatic role by HAT p300 (19-27). Among various classes of, HAT p300/CBP is a known paralog that serves as a transcriptional coactivator (19-27). HAT p300 is suggested to modulate transcription of genes that are linked to the development of various human diseases including cancer. HAT p300 is determined to comprise several domains including HAT domain (1285-1664) amino acid residues (24-27). In fact, HAT p300 catalytic domain is antagonized by various small molecules including A-485, I-CBP112, natural products and bi-substrate analogs (Lys-CoA) (24-27). However, binding

affinity and position by lactyl-CoA to HAT p300 is not known that may be potentially linked with the lactylation process. However, the active site on HAT p300 is reported in earlier works by showing key amino acid residues such as Phe1374, Leu1398, Ser1400, Arg1410, Thr1411, Tyr1414, His1415, Leu1418, Trp1436, Pro1440, Gln1455, Phe1458, Leu1463, Trp1466 and Tyr1467 (20-27). In fact, these amino acid residues interact with several known natural and synthetic substrates including acetyl-CoA, Lysyl-CoA, A-485 and I-CBP112. In case of present data, strong binding by lactyl-CoA to amino acid residues such as ASP1399, HIS1402, LYS1407, CYS1408, ARG1410, THR1411, TYR1414, TRP1436, ASP1454 and LYS1456, TRP1466, LYS1469 within the catalytic domain of p300 is revealed by molecular docking and dynamics simulation studies. It is interesting to note that two other substrates lactate and (R)-S-Lactoylglutathione did not any binding affinity to HAT p300 active site. Further, the binding affinity (-8.6 Kcal/Mol) and specificity is highly appreciable compared to well-known substrate such as acetyl-CoA and Lysyl-CoA. Besides docking affinity, molecular simulation data confirm the highly specific binding by lactyl-CoA to the active site amino acid residues of HAT p300. Therefore, our data suggest that lactyl-CoA generated within the tumor microenvironment is a potential candidate for lactylation by HAT p300.

This finding is in coherence with a recent finding that lactylation process modulates the transcriptional gene regulation in M1 macrophage and allows to change into M2 macrophage (12-18). In fact, findings from Zhang et al (2019)¹⁷ discovered the process of lactylation with experimental evidence at molecular and cellular levels. However, pertinent questions were not answered on the nature of enzyme and biological source and relevance of lactyl-CoA that may potentially act as a substrate for the enzyme p300. Our data is a first report and supports novel proposition on the biological possibilities of lactyl-CoA within the tumor microenvironment and mode of lactylation mediated by p300 enzyme.

LIMITATIONS

One major limitation is on the lack of data that show physiological abundance of lactyl-CoA in cancer cells using comprehensive metabolomics approaches (17-25). In our lab, we have not detected lactyl-CoA in cancer cells such as breast and colorectal cancer (34). Therefore, source of lactyl-CoA within the tumor microenvironment is debatable and may be speculated from non-cancer cells including microbiotas.

FUTURE PROPOSITIONS

It has been established that cancer cells generate up to 40 times more lactate than normal cells (5-11). Herein, authors propose a model that lactate is shuttled into the nearby

microbiotas that have the metabolic machinery to generate lactyl-CoA from lactate. Since, microbiotas are known to contain enzymatic machinery that may convert lactate to lactyl-CoA (35-36). In fact, the evidence is available that supports that some bacteria such as *Escherichia coli* have the enzymatic system to produce lactyl-CoA by the help of lactyl-coenzyme A (CoA) synthetase. To date, enzymatic systems are not reported in animal cells including normal cells, cancer cells and immune cells that can produce the lactyl-CoA from lactate. In contrary, some bacteria are known to convert lactate into propionic acid (37). Therefore, a strong proposition is warranted that the lactate mediated lactylation process may involve the nature of bacteria that may work as a pro-tumor. Hence, this proposition matches with a recent finding on the use of lactyl-CoA for lactylation to achieve polarization of macrophages M1 (anti-tumorigenic) to M2 (pro-tumorigenic) type. In a summarized view, a proposition model is given in Figure 6.

CONCLUSION

In conclusion, our findings based on molecular docking and simulation suggest that lactyl-CoA binds to HAT p300 enzyme as a potential route to achieving lactylation on the histone proteins to achieve desired epigenetic regulations. At the same time, binding position for lactyl-CoA significantly overlaps with the acetyl-CoA for HAT p300 enzyme. In this way, lactylation epigenetic marks may inhibit the extent acetylation marks on chromatin that drive certain cells such as macrophages towards polarization that may be accountable for the generation of pro-tumor M2 macrophage in the tumor microenvironment. In addition, authors make a proposition that the availability of lactyl-CoA within the tumor microenvironment may be linked to the nature of microbiotas that are equipped with an enzyme that may convert lactate into lactyl-CoA. Hence shuttling of lactate and lactyl-CoA are proposed between cancer cells, microbiotas and immune cells such as macrophages within the tumor microenvironment. In fact, the nature of data is based on molecular docking and simulation, but the findings are novel. The impact of these findings will have a significant contribution in solving the unanswered questions on the molecular mechanisms of lactylation in the context of the tumor microenvironment.

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Details of Figures and their legends:

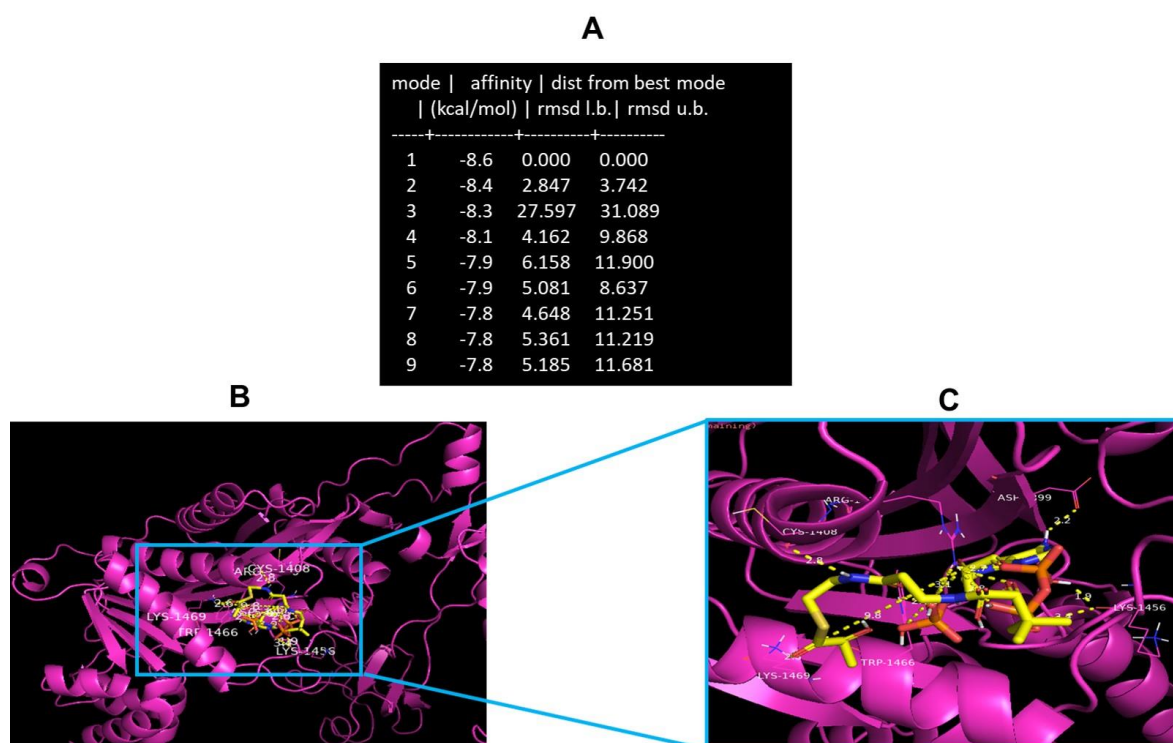


Figure 1. A lactate derived metabolic product lactyl-CoA (CHEBI ID:15529) shows strong and specific binding to histone acetyltransferase p300 (HAT p300) enzyme (PDB ID: 6GYR) with a docking energy value is at -8.6 Kcal/Mol.

(A). Molecular docking energy values generated by AutoDock Vina at different rmsd values. (B) A complete PyMol view on docked complex between lactyl-CoA (CHEBI ID:15529) and histone acetyltransferase p300 (HAT p300) enzyme (PDB ID: 6GYR) (C). A zoomed image of PyMol generated docked complex between lactyl-CoA (CHEBI ID:15529) and histone acetyltransferase p300 (HAT p300) enzyme (PDB ID: 6GYR) denoted with interacting amino acid residues and bond distance.

Protein-Ligand RMSD

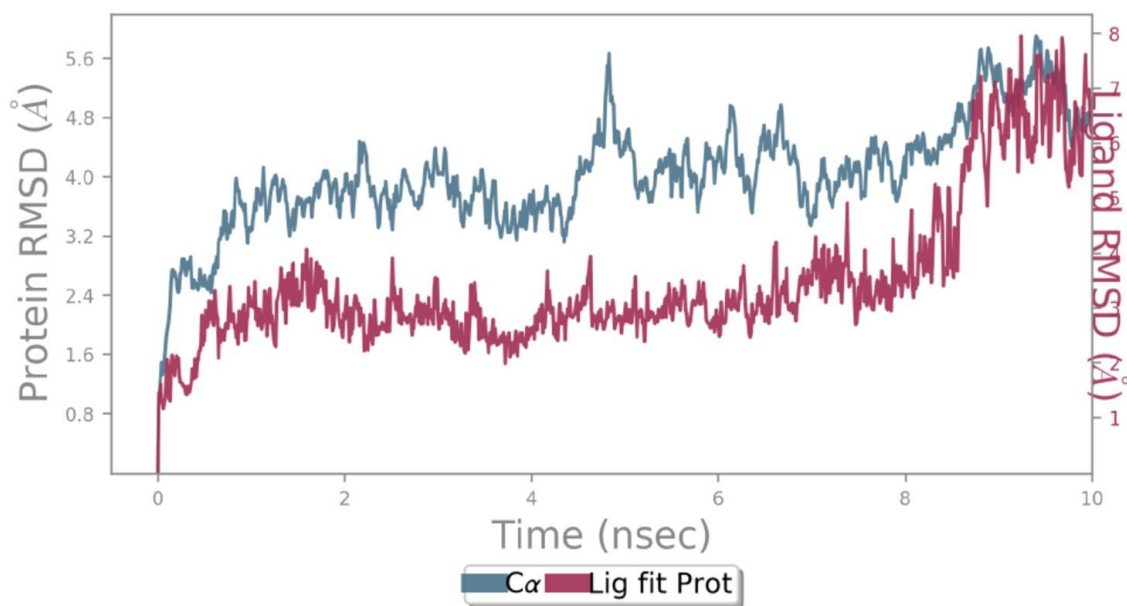


Figure 2. A 10 ns time frame protein-ligand Root Mean Square Deviation (RMSD) plot confirm the stable complex between lactyl-CoA (CHEBI ID:15529) and histone acetyltransferase p300 (HAT p300) enzyme (PDB ID: 6GYR).

In this figure, left-Y axis denotes the RMSD evolution of HAT p300 enzyme in view of structural conformation during simulation for duration 10 ns. The order of changes of RMSD values of protein is within the acceptable range of 1-3Å. Here, right-Y axis shows the RMSD value of lactyl-CoA (CHEBI ID:15529) as Lig-Fit-Pro and this value is not significantly larger than the RMSD value of p300 (HAT p300) enzyme (PDB ID: 6GYR). Hence, stable ligand-protein complex is indicated by this RMSD plot.

Protein-Ligand Contacts

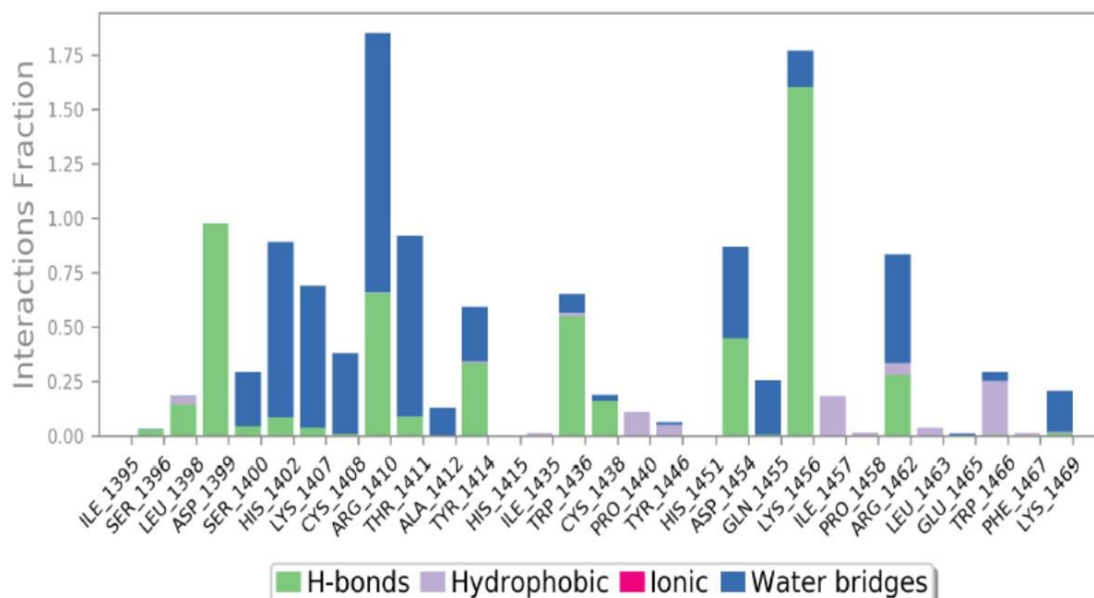


Figure 3. Protein HAT p300 and lactyl-CoA interaction plot indicates on the significant fraction of time by key active site amino acid residues establishes interaction with ligand are during 10 ns simulation. On the Y-axis, interaction fraction denotes the time for which key amino acids maintain distinct forms of interactions such as hydrogen bonds, hydrophobic, ionic and water bridges.

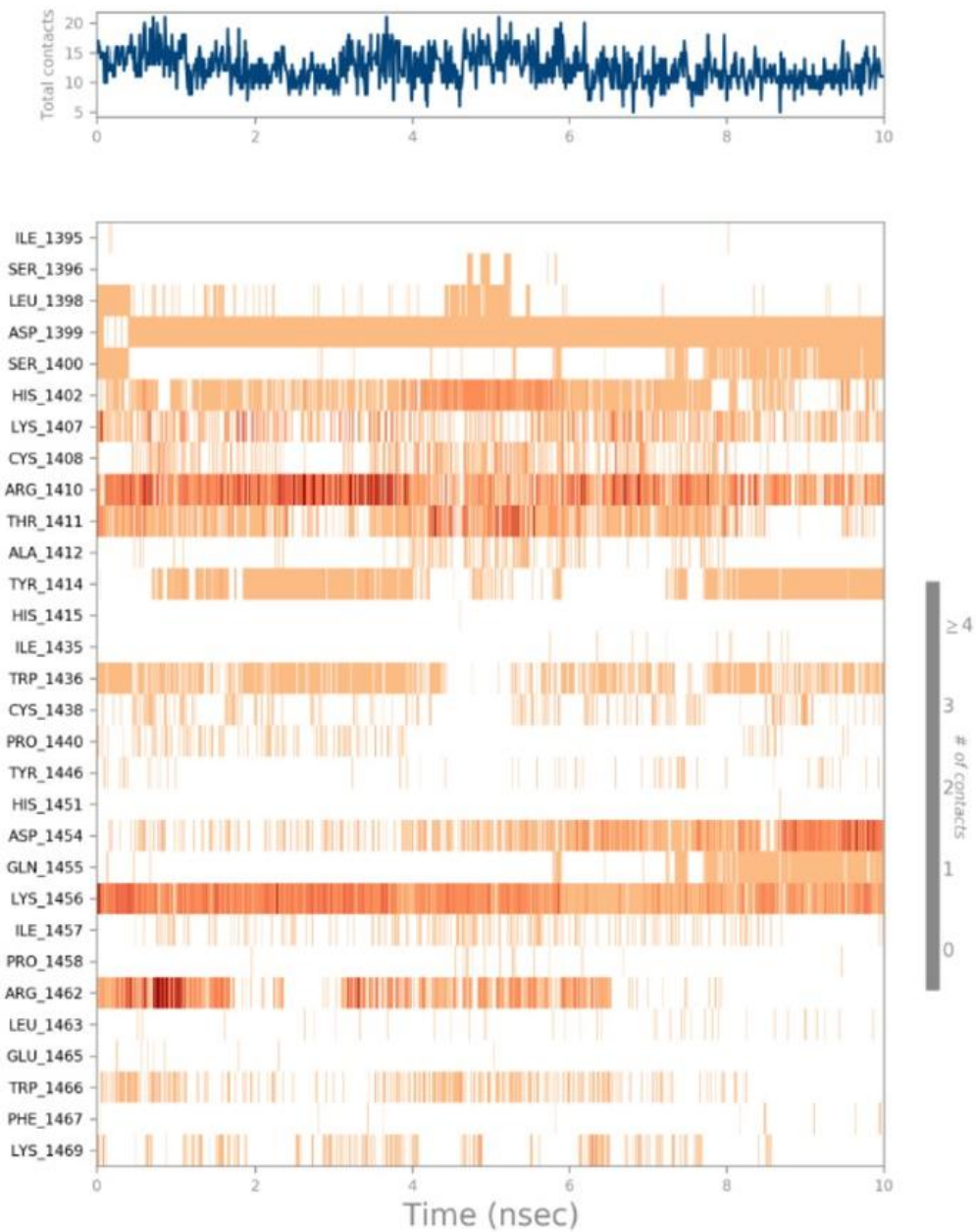


Figure 4. Protein HAT p300 and lactyl-CoA interaction plot (or contacts) shows the nature of amino acid residues that is a part of catalytic site of HAT p300 enzyme. The active site residual interaction with lactyl-CoA is shown in different color trajectories.

Ligand-Protein Contacts

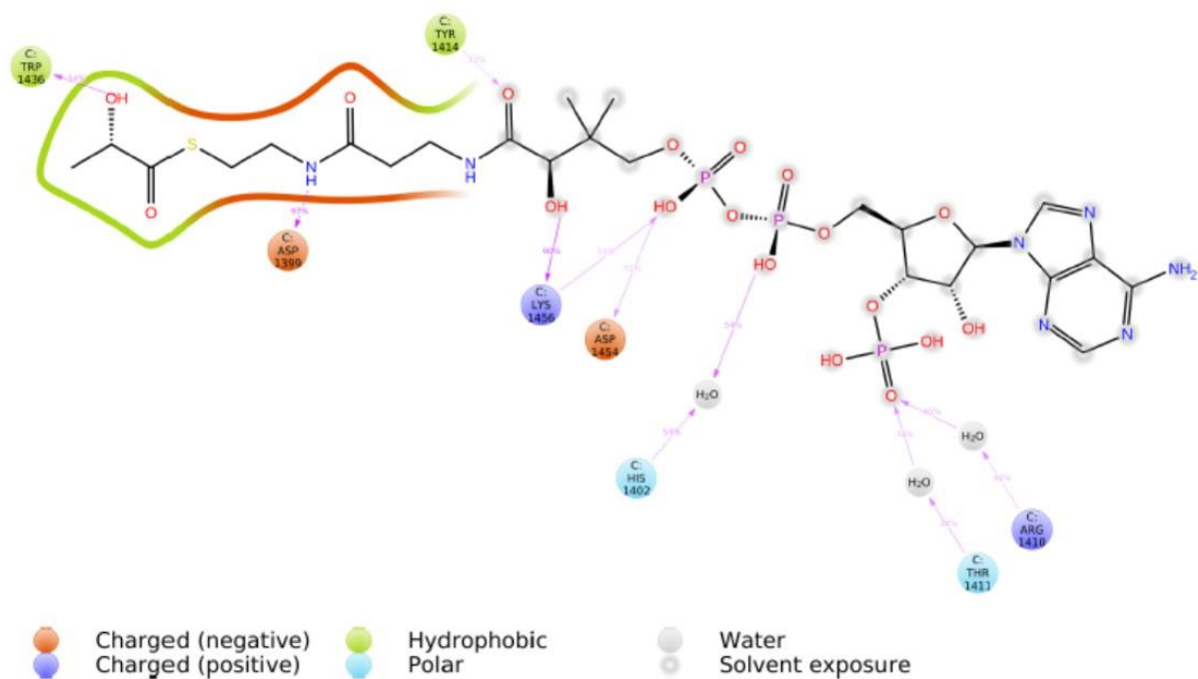


Figure 5. A schematic model on lactyl-CoA ligand atom interactions with HAT p300 residues indicate the stable binding within the active site of HAT p300 enzyme. Here, different color combinations represent the extent and nature of ligand to enzyme atomic interactions including ionic, hydrophobic, polar, water and solvent exposure.

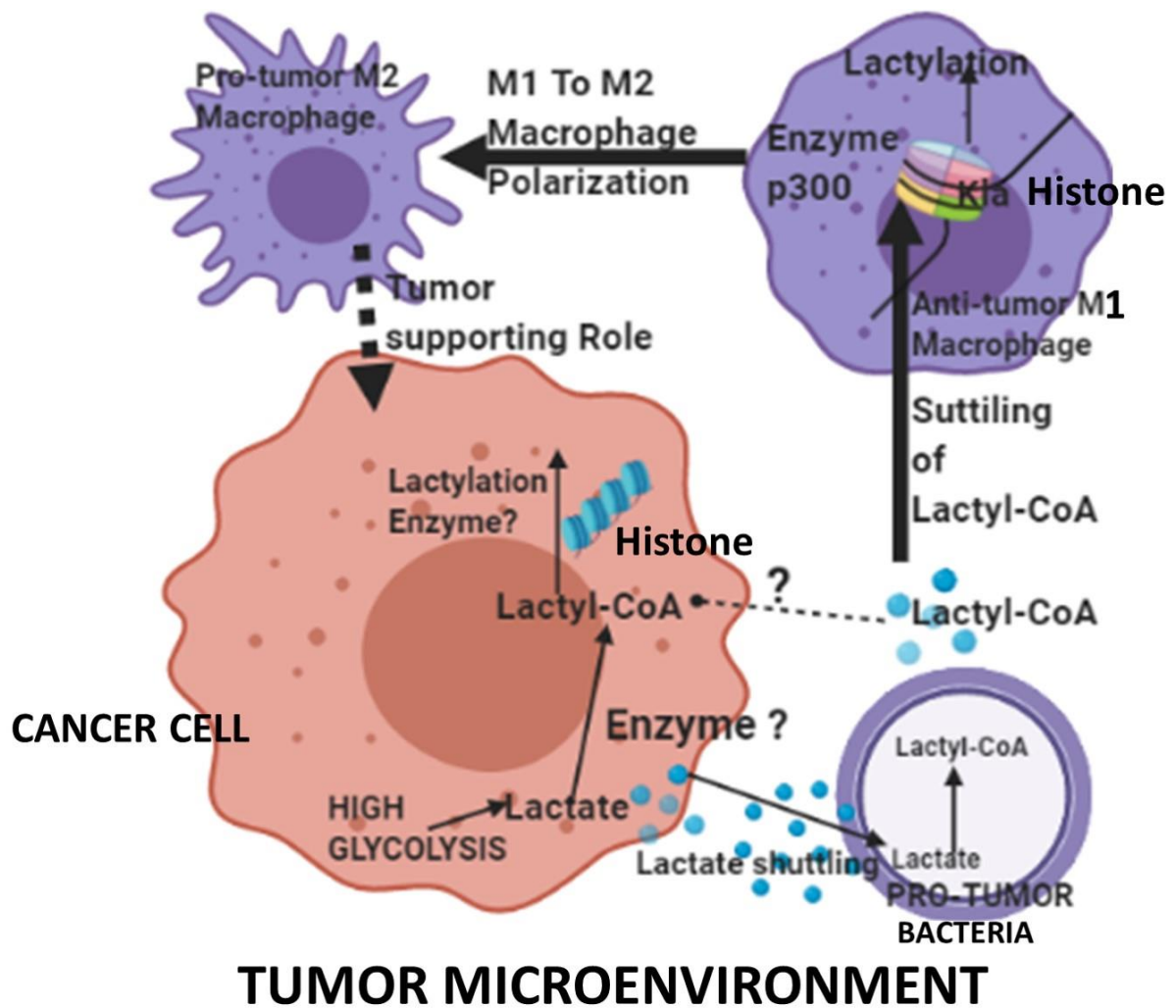


Figure 6. A proposed model on the role of lactyl-CoA in lactylation process during epigenetic changes. A possible pathway is speculated within the tumor microenvironment that hosts cancer cells and non-cancer cells including immune cells, stromal cells and microbiotas. In fact, this model is novel and logical that support the shuttling of lactate and lactyl-CoA within the tumor microenvironment. Finally, lactyl-CoA is used for lactylation process to modulate the epigenetic marks on chromatin and leading to polarization from M1 macrophage to M2 macrophage (Tumor supporting cells).