Article

Interaction profiling of cow milk metabolites against human Renin-Angiotensin System (RAS) proteins

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Abstract
To maintain healthy human physiology and promote growth and development, it is imperative to consume milk, which provides essential nutrients like vitamins and minerals. However, cow milk compounds contain different types of molecules, which may elicit varied responses within individuals. Milk metabolites are studied to impact several human biological processes that result in altered physiology. The Renin-Angiotensin System (RAS) is responsible for regulating blood pressure and maintaining a proper balance of fluids and electrolytes. However, impaired regulation of RAS may cause medical conditions such as heart failure, kidney disease, or hypertension. RAS is one of the studied systems, whose proteins reportedly interacted with and were affected by milk metabolites. The study attempts to find milk metabolites with high affinity towards RAS-proteins, and results from circumstances of interaction between them. Molecular docking between milk metabolites and RAS-proteins' and an interaction network was utilized to achieve the objective. In total 206 milk metabolites and 13 Ras proteins are considered for the study. Network analysis depends on the docking score, which helps us understand the interaction between milk molecules. Based on free energy analysis study indicates that out of 206, 35 milk metabolites showed free energy < -8 Kcal/mol, which indicates high binding affinity between these metabolites with 12 RAS-proteins. Four RAS proteins, i.e., ANPEP, CTSA, MRGPRD, and ACE, were found to have significantly interacted with more than 15 milk metabolites. Based on binding affinity, we can predict whether the specific metabolites with effective binding scores modulate the function of specific RAS proteins.

Keywords milk metabolites; molecular docking, Renin-Angiotensin System; dairy products, biological network, human physiology.

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1 Introduction

Milk is widely acknowledged as the only food that encompasses almost all the essential nutrients required for human growth. The impact of dairy products on human physiology and other infirmities is complex, with both protective and potentially harmful effects (Zhang et al., 2021). The studies, regarding whether dairy products

can prevent or increase the risk of changes in human physiology or infirmities are attempt in last one and half decades, but most of them are inconclusive (Rozenberg et al., 2016). Metabolomics is concerned with the comprehensive analysis and characterization of molecules, which are a diverse group of small molecules with molecular weight range of 50 – 1500 Daltons. This field of study encompasses the identification, quantification, and elucidation of the chemical structures and functions of molecules within biological systems (Dubey 2020). Over the years, there has been an increasing interest in the molecules present in milk, as they have been found to have important roles in human physiology, including immune system development, growth, and inflammation. Understanding the role of milk metabolites in human physiology is crucial for the growth and development of the human body (Belkaid et al., 2014). The cow milk metabolites and their interactions with proteins, and other small molecules found to play crucial role in human physiology (Sun et al., 2019). From time to time, there have been sporadic reports suggesting a potential causal relationship between the consumption of milk or milk products and specific changes in human physiology and longevity (Larsson et al., 2015). In general, dairy products has established health benefits, and it plays a crucial role in maintaining strong bones and teeth, warding off osteoporosis, and serving as a key factor in reducing the risk of cardiovascular disease, hypertension, type II diabetes, and metabolic syndrome (Larsson et al., 2015). Reported studies hypothesized that consuming milk and dairy products might affect one's vulnerability to certain cancers and other illness (Zhang et al., 2021). Dairy products are a complex food group, and their composition varies from source that making it challenging to assess the association between dairy products and disease-risk (Wang et al., 2023).

Renin-Angiotensin System (RAS) is one of the crucial systems which control number of biological process (Fig. 1), i.e., systemic vascular resistance, fluid and electrolyte balance, and blood pressure (Wang et al., 2022). The system comprises a cascade of enzymes and peptides that work together to maintain the balance of the body's fluids and electrolytes (Fountain et al., 2023). Blood pressure, fluid and electrolyte balance, and systemic vascular resistance are all controlled by the RAS and hormone systems. Consumption of dairy products controls blood pressure, and there is mounting evidence that dairy peptides lower blood pressure via ACE inhibitory pathways (Kanugula et al., 2023). The RAS's participation in inflammation and tissue damage raises the possibility that some of its constituents may indirectly affect inflammation and sensation. A crucial member of the RAS, angiotensin II, has been shown to possess pro-inflammatory actions that can help it accumulate and persist. Recent studies have suggested that milk-derived molecules may have effects on the RAS pathway (Samtiya et al., 2022). The consumption of low-fat dairy products, calcium, and vitamin D in one's diet may have a beneficial effect on preventing hypertension and related cardiovascular complications. This information highlights the potential roles of low fat dairy products and other nutrients in the primary prevention of hypertension and its associated health issues (Wang et al., 2022).

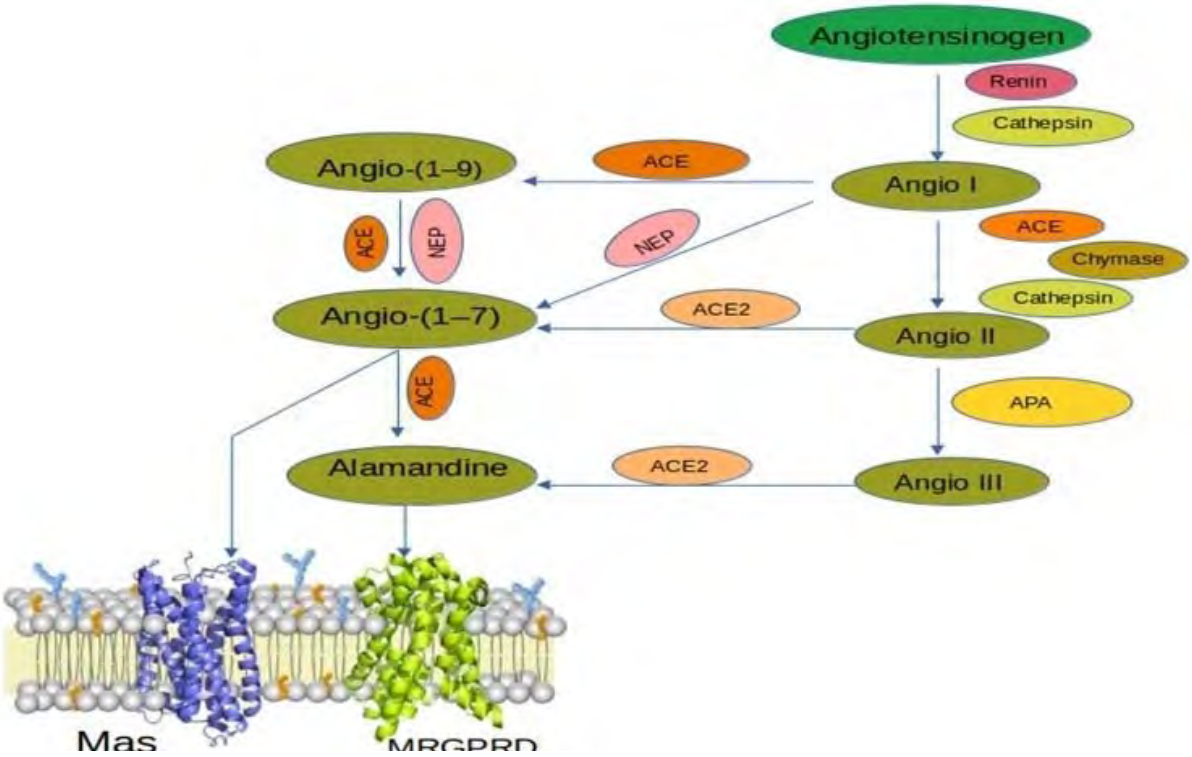

Fig. 1 The inhibitory effects of Anigo II, ACE (angiotensin-converting enzyme), ACE2, and neprilysin (NEP) are used to activate the RAS pathway. Angio I, Angio II, and Angio III are assisted in activation by CTSA (Lysosomal protective protein), APA (Aminopeptidase N) REN (Renin), CMA1 (Chymase) and CTSG (Cathepsin G); Angio (1-7); receptor Mas, MRGPRD (Mas-related G-protein coupled receptor member D).

This study aims to deduce milk metabolites that interact with human RAS proteins, thus crucial for human physiology. Study attempts to find out potential molecular interactions between cow milk metabolites and proteins involved in RAS pathways that have the potential to affect human physiology. For this purpose, we investigate the binding affinities through docking between milk metabolites and human proteins and determine the extent of their interactions. Further, molecular interaction networks have been designed, and network centrality has been utilized to establish the importance of predicted molecular interactions.

2 Materials and Methodology

2.1 Screening of milk metabolites

Milk metabolites refer to the different chemical compounds that are produced during the metabolic processes of lactating animals and are found in milk. These milk metabolites are collected from MCDB (https://mcdb.ca/metabolites). A total of 206 documented milk compounds (Table S1) were considered to evaluate their potential to interact with human proteins. All these milk metabolites have different physicochemical properties that make them unique. For the study, the 3D structure of all the compounds was retrieved.

2.2 Selection of human RAS-proteins

To check the binding affinity of RAS proteins with milk metabolites, RAS proteins were listed from the KEGG pathway and literature survey (Table 1). To perform structure-based affinity analysis, 3D structures of these proteins were retrieved from the PDB (https://www.rcsb.org/).

Table 1 Details of RAS pathway proteins considered for the study.

S. No.	Protein name	PDB ID	Molecular weight (kDa)	References
1	ACE	7Z6Z	151.91	(Gribouval et al., 2005)
2	NEP	1DMT	80.89	(Pare et al., 2013)
3	ACE2	1R42	76.77	(Zisman et al., 2003, Krishna et al., 2024)
4	CTSA	1IVY	104.26	(Liu et al., 2018)
5	NTS	5LUZ	164.29	(Januzzi et al., 2016, Skidgel et al., 1984)
6	PRCP	3N2Z	51.98	(Wu et al., 2020)
7	REN	4AMT	43.58	(Nguyen et al., 2002)
8	CMA1	4K60	25.49	(Wang et al., 2020)
9	CTSG	1AU8	25.96	(De Garavilla et al., 2005)
10	MRGPRD	7Y12	170.51	(Etelvino et al., 2014)
11	ANPEP	4FYQ	106.77	(Kim et al., 2022)
12	PREP	3DDU	81.46	(Tian et al., 2014)
13	RGL1	7SCW	32.85	(Gaudet et al., 2011)

2.3 Protein and Ligand preparation

Retrieve the 3D protein structure in .pdb format from the Protein Data Bank, and Chimera is used to visualize the structure to check the quality of structure, number of chains, ligands, and ions that are available in the structure. After that, the MGL tool was used for protein preparation, i.e., removing water molecules, adding hydrogen, repairing the missing and miscellaneous ions present in proteins, and adding charges.

Milk metabolite structures were converted from SDF to PDBQT file format using Open Babel. Prepare the ligand for docking by optimizing their geometry, tautomerism, ionization state, and conformation using ligand preparation software, Autodock suit (Forli et al., 2016).

2.4 Docking

Molecular-Docking allows predicting the different binding and interaction modes and reporting the interaction in terms of binding affinity between a molecule (a ligand) and a protein target. For this study, l was used Autodock_vina_1_1_2 to check the interaction between milk metabolites and proteins (Trott et al., 2010). Two

hundred six milk metabolites were docked against 13 RAS proteins. The docking calculation provides ΔG values and affinity values in Kcal/mol, along with the RMSD of the interacted mode.

2.5 Interaction analysis and visualization

Docking scores are used to determine the binding energy and affinity of a small molecule with a target protein. On the basis of docking scores, the best binding mode (high affinity) has been selected and considered for further analysis. Atom-level interaction visualization has been performed through discovery studio (https://discover.3ds.com/discovery-studio-visualizer-download), which helps to observe the type of interaction between molecules and bonds (Pawar et al., 2021).

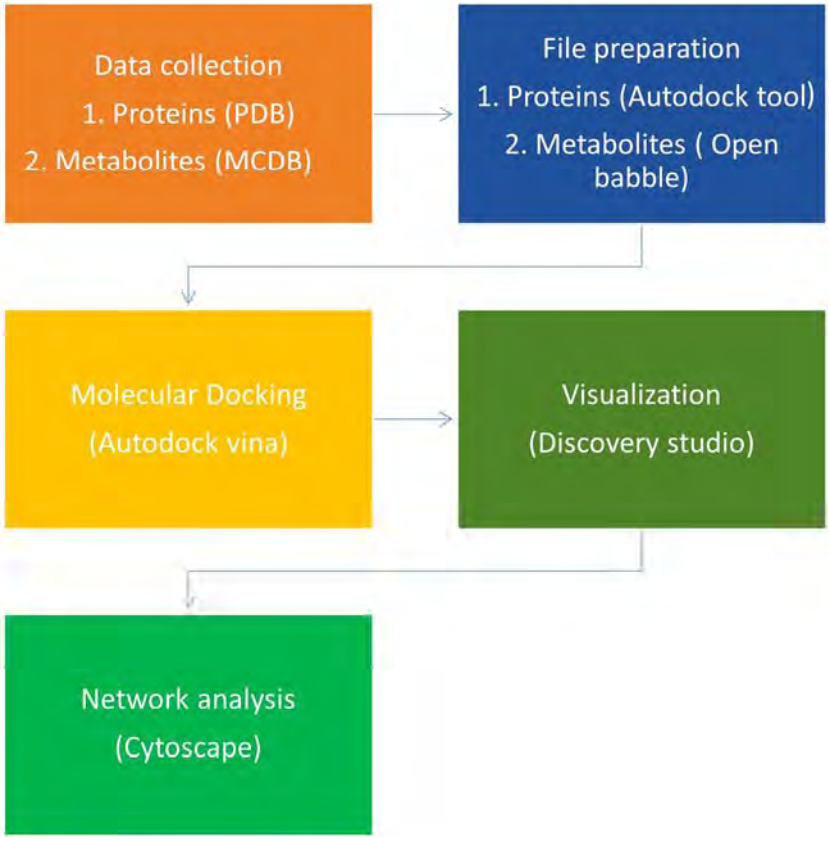

Fig. 2 Schematic representation of the considered methodology.

2.6 Molecular interaction network analysis

To gain insights about the interaction patterns between metabolites and proteins, interaction network based observations has been attempt. To create a network, Cytoscape (https://cytoscape.org/) (Zhang, 2016, 2018) was used to conduct a comprehensive interaction network analysis of RAS proteins and milk metabolites. Network, focusing on interactions characterized by high binding strength, this helps us to understand how common metabolites interaction with different proteins. A detailed examination of the interconnections and interactions within the network was undertaken (Fig. 2).

3 Results and Discussion

Milk metabolites are very important compounds that are responsible for various properties and biological activities of the milk. The research focused on determining how interactions between RAS proteins and milk

metabolites affect RAS. Structure based molecular interaction analysis has been done through a docking approach between 206 milk metabolites and 13 human RAS proteins.

3.1 Docking analysis between RAS proteins and Milk metabolites:

We considered 13 human proteins (Table 1), as targets and evaluated their interaction in terms of binding affinity against milk compounds. Molecular docking results are expressed in terms of free energy (binding affinity) and RMSD.

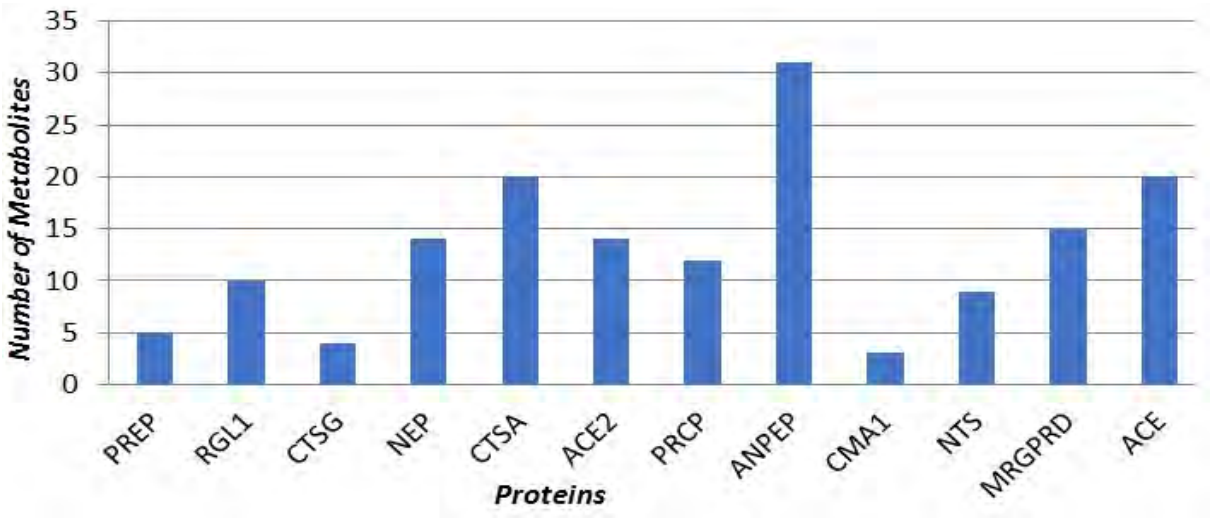

Fig. 3 All proteins interact with the selected milk metabolites that have binding affinities is ≤ -8 Kcal/mol, although only 35 of the 206 milk metabolites show good scores with all proteins.

Resulted, free energy analysis indicates that out of 206, only 35 milk metabolites (Table S2) showed free energy < -8 Kcal/mol, which indicates high binding affinity with 12 RAS-proteins. It has been found that none of the milk metabolites display significant affinity with REN protein (PDBID: 4AMT). As represented in fig. 3, four RAS proteins, i.e., ANPEP, CTSA, MRGPRD and ACE found to be significantly interacted with more than 15 milk metabolites.

In total 35 molecules are found to interact with 12 RAS proteins, each of them possessing distinct physicochemical properties. Molecular docking-based findings revealed that several milk metabolites, such as BMDB0012305, BMDB0002174, BMDB0063639, BMDB0005781, BMDB0001095, and BMDB0063640, had a high propensity for binding to RAS proteins. BMDB0012305 had the highest binding affinity -11 kcal/mol of these compounds for the RAS protein MRGPRD (Fig. 4).

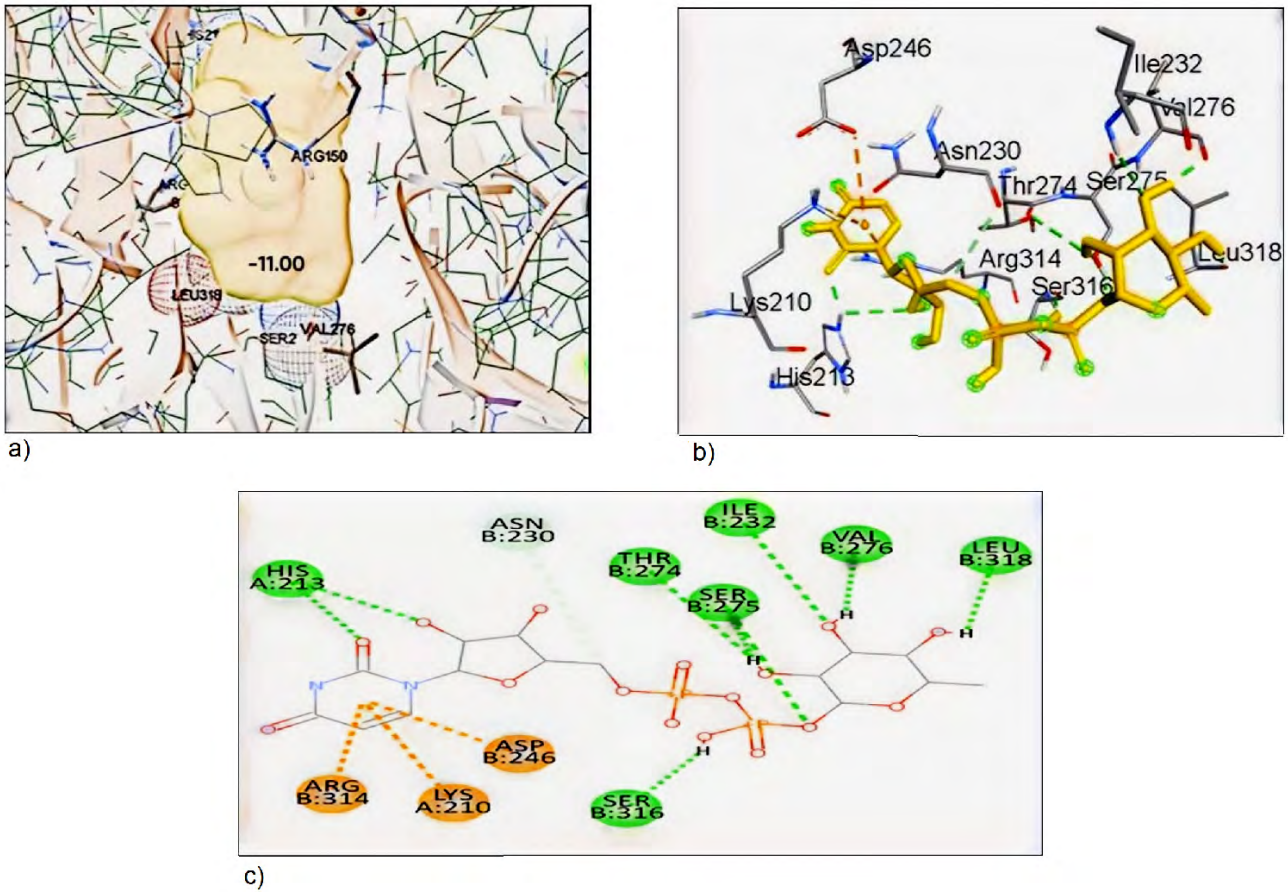

Fig. 4 (a) Binding site visualized by the autodock tool, in which ligands BMDB0012305 bind in a MRGPRD cavity with binding affinity of -11 kcal/mol; and (b) Diagrammatic representation of best docked site for each MRGPRD with milk metabolites, [dash green line (Hydrogen bond), dash purple line (Hydrophobic bond), dash orange line (Electrostatic); (c) 2-D representation of interaction, conventional hydrogen bond (green color), pi-cation and pi-anion (orange color) and (light green color).

Additionally, the docking study demonstrated that the majority of the RAS protein binding sites have been identified on the surface of the protein molecules. The identification of these molecules, which encompass a class of small molecules intricately involved in diverse biochemical processes, and the molecular mechanisms underlying the interaction between proteins and their molecules. Physical and chemical characteristics cause the receptor, which may be a biological molecule or system with which it interacts, to have a pharmacological reaction. The possibility of high aquatic toxicities may be calculated using physicochemical data. The physicochemical properties of milk metabolites and proteins impact how they interact with one another. Most of the interacted milk metabolites have significant physiochemical properties, which helps them to maintain a considerable ADME (absorption, distribution, metabolism and excretion) features.

3.3 Interaction network analysis between milk metabolites and proteins

Docking results indicates that all 12 proteins and 35 metabolites have considerable binding affinity with multiples. To observe the interaction patterns between metabolites and proteins, interaction network has been constructed. Overall topology of the network resembles the scale free network features which is a signature pattern of all kind of biological network (Fig. 5).

The conducted network analysis has provided valuable insights into the complex interactions between milk metabolites and proteins, shedding light on their intricate. The network that was constructed displayed a topology that adhered to the scale-free property, characterized by a distribution of node connections that

followed a power-law distribution (Shannon et al., 2003). The ANPEP protein exhibits a significant level of interaction with various milk metabolites, specifically interacting with a total of 32 distinct milk metabolites. In comparison, the CTSA protein demonstrates interaction with 21 metabolites, while the ACE protein interacts with 20 and the MRGPRD protein exhibits interaction with 15 metabolites. The CTSG is connected to 4 and CMA1 proteins with a limited set of 5 milk metabolites. At the same time, metabolite BMDB0012305 interacted with 10 RAS proteins among all of them.

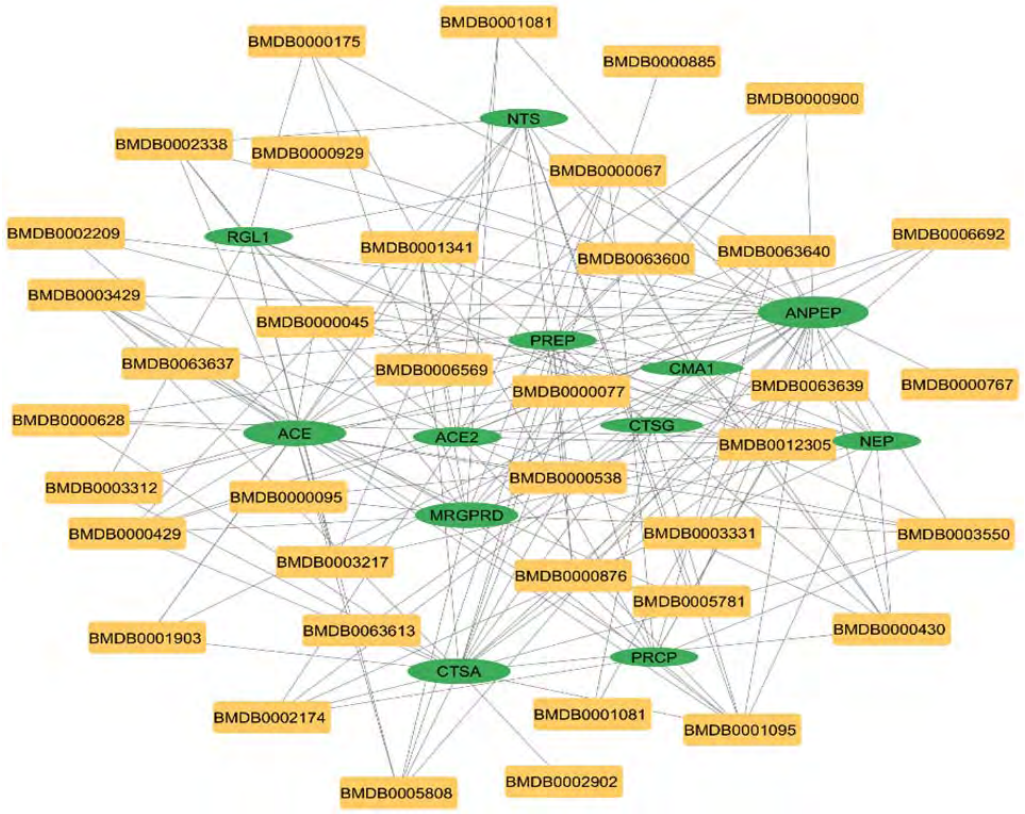

Fig. 5 Milk metabolites (orange) and proteins (green) interaction, with ANPEP having the uppermost interaction and CMA1 and CTSG having very less interaction.

The analysis of the milk metabolites and proteins was done to figure out how the many metabolites and proteins work together in a complex network. Through rigorous examination, it was observed that certain milk metabolites and the ANPEP protein exhibited a significantly higher degree of connectivity within the network. These findings highlight the central roles played by these identified hubs in the complex processes governing milk composition and physiological properties. Through observation we found that RAS proteins showed interaction with milk metabolites such as BMDB0002174, BMDB0001095, BMDB0063640, and BMDB0000900. The regulatory system, referred to as the RAS, plays a crucial role in the regulation of cardiovascular function, maintenance of fluid and electrolyte balance, and control of blood pressure (Mirabito et al., 2019). The dysregulation of the RAS has been identified as a potential factor in the pathogenesis of various diseases, such as hypertension, cardiovascular disease, and renal disease (Almutlaq et al., 2021). Numerous studies have provided evidence supporting the notion that milk and dairy products possess anti-hypertensive properties, thereby potentially reducing the likelihood of developing cardiovascular disease (Fontecha et al., 2019 & Tsuboi et al., 2022). The potential mechanisms underlying the effects of BMDB0002174 might be modulating RAS activity.

Milk metabolites might have the potential to modulate the function of ACE and ACE2 inhibitors, which in turn could help regulate blood pressure and fluid balance (Tsuboi et al., 2011). These interactions could change how these receptors activate signaling pathways, which might lead to more benefits for some RAS-related conditions. Overall, the study provides a novel insight into the potential use of milk metabolites might be modulate the activity of the RAS pathway.

There may be a potential link between the anti-hypertensive effects of RAS proteins and their interactions with milk metabolites, which play a crucial role in regulating their activity. It is important to note that this research study is subject to certain limitations. The docking analysis was conducted to explore the potential interactions between RAS proteins and milk metabolites. However, it is important to note that these findings are purely theoretical in nature. To validate and strengthen these results, further experimental confirmation is required. Additional investigation is necessary to explore the potential interactions between RAS proteins and various milk metabolites, as our current analysis was limited to a limited number of milk metabolites.

4 Conclusion

There is a likelihood of an association between milk metabolites and the RAS proteins. The RAS pathway is a well-known signaling mechanism that is crucial mainly for growth and proliferation, along with that it also associated with the progression and development of various types of cancer. To observe the role of cow milk metabolites on RAS pathway, docking has been performed between RAS proteins and cow milk metabolites. Through results it has been observed that 35 milk metabolites share significant affinity towards 12 RAS proteins. Through interaction network it has been observed that the affinity between RAS proteins and milk metabolites is non- specific, each of the 35 metabolites shows affinity towards multiple RAS-proteins, i.e., BMDB0012305 shows affinity with 10 RAS proteins. Study about the mentioned protein clearly indicates that all these 12 RAS proteins play a significant role in multiple biological processes. Affinity indicates that milk metabolites are tend to bind with these proteins and probably modulates their activities which bring abnormality in related process, mainly cell proliferation and growth and resulted into malignancy of the cell.

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