Frontiers in Health InformaticsISSN-Online: 2676-7104 www.healthinformaticsjournal.com
2024; Vol 13: Issue 3 Open Access

Proteomics Analysis of MSH2 Protein and Molecular Docking Approach for Colorectal Cancer Targeted therapy

Naveen Kumar Motukuri[1], Anjani Chaganti[2], Dr. Muni Kumar Dokka[3*]
Triveni Gudla[4], Tejovathi Bandike[5]
[1]Department of Life Sciences, School of Allied & Healthcare Sciences, Malla Reddy University, Hyderabad, Telangana, India.
[2]Department of Life Sciences, School of Allied & Healthcare Sciences, Malla Reddy University, Hyderabad, Telangana, India.
*[3]Associate Professor, Department of Biochemistry, School of Allied & Healthcare Sciences,Malla Reddy University, Hyderabad, Telangana, India.
[4]Assistant Professor, Department of Biochemistry, School of Allied & Healthcare Sciences,Malla Reddy University, Hyderabad, Telangana, India.
[5]Assistant Professor, Department of Biochemistry, School of Allied & Healthcare Sciences, Malla Reddy University, Hyderabad, Telangana, India.
*Corresponding Author: ***Dr. Muni Kumar Dokka***
E mail: drmunikumar@mallareddyuniversity.ac.in

Cite this paper as: Naveen Kumar Motukuri, Anjani Chaganti, Muni Kumar Dokka,Triveni Gudla, Tejovathi Bandike (2024) Proteomics Analysis of MSH2 Protein and Molecular Docking Approach for Colorectal Cancer Targeted therapy. *Frontiers in Health Informatics, 13* (3),8031-8041

ABSTRACT
Colorectal cancer (CRC) is the second leading cause of cancer-related deaths globally, with a diverse genetic profile. CRC is the outcome of a gradual accumulation of genetic and epigenetic changes, resulting in significant genomic instability. The present treatment procedures include surgery/polypectomy, chemotherapy, radiotherapy, combination therapy, and immunotherapy, while advanced methods include gene therapy, cellular therapy, and targeted immunotherapy which is in the emerging phase. Understanding the molecular mechanisms underlying CRC is crucial for developing effective diagnostic, prognostic and therapeutic strategies. Integrating Next Generation Sequencing (NGS) and proteomics data provides a comprehensive view of the molecular alterations in CRC. In the present study, the expression levels of MSH2/MSH6 genes and their proteomic analysis of MSH2 protein were carried that offers valuable insights into their role in CRC. Interactive views of the 3D structure of MSH2 was analysed, revealing sequence-structure relationships, and bound ligands, which are critical for understanding the functional implications of MSH2 alterations. Molecular docking studies highlighted potential therapeutic targets by performing interactions with potent ligands like Bevacizumab, a monoclonal antibody that targets the vascular endothelial growth factor (VEGF) and Tucatinib, a tyrosine kinase inhibitor drug. The docking results support the efficacy of targeted therapy of Bevacizumab and Tucatinib in CRC. These findings underscore the potential for early detection, genetic analysis, and computational approaches to drive forward colorectal cancer research and improve patient outcomes. Targeting MSH2, either through restoration or modulation, offers a promising therapeutic strategy. Further investigations and clinical trials are necessary to validate the efficacy of identified ligands and explore their therapeutic potential in treating colorectal cancer.

Keywords: *Colorectal Cancer, 3D Models Genetic Analysis, Docking, Computational Methods, Targeted Therapies, Bevacizumab and Tucatinib*

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer globally and is the second most common cause of cancer-related death according to GLOBOCAN 2022 data [1]. A diet high in fat, refined carbohydrates, animal protein, poor fiber content, alcoholism, obesity, long-term cigarette smoking, low physical activity, and age are risk factors for colon cancer. Adenocarcinomas derived from the epithelial cells of the colon mucosa account for more than 90% of colorectal carcinomas. Adenosquamous, Neuroendocrine, squamous cell, spindle cell, and undifferentiated carcinomas are other uncommon forms of colorectal cancers.

Microsatellite instability (MSI) and chromosomal instability (CIN) are the two separate routes by which CRC arises. Chromosomal instability accounts for 85% of CRC instances, whereas MSI accounts for 15% of cases. MSI refers to a change in the number of nucleotides in the microsatellite region. The heterodimers mismatch repair protein (MMRp) complex, which includes MSH2 in pairs with MSH6 (MutSα), should be able to detect and repair this change [2]. This complex facilitates the creation of the repair complex (MutLα) that reforms new DNA strands and plays a function in the identification of DNA strand damage. MSI may result from the inactivation of one or more MMRp. Even though MSI is only found in 15% of cases of CRC, it has a major clinical impact on how patients with CRC and MSI should be managed.

The instructions for producing a protein that is vital to DNA repair are provided by the MSH2 gene. When DNA is replicated in order to prepare for cell division, this protein aids in the correction of mistakes that are created. A two-protein complex known as a dimer is created when the MSH2 protein combines with either of the other two proteins, MSH6 or MSH3, which are each derived from a different gene. This complex locates mistakes that have occurred during DNA replication on the molecule. After binding to the MSH2 dimer, a different set of proteins called the MLH1-PMS2 dimer corrects the faults by copying a new sequence and deleting the mismatched DNA. The mismatch repair (MMR) genes are a group of genes that includes the MSH2 gene.

Constitutional mismatch repair deficit (CMMRD) syndrome has been linked to approximately ten variations, or mutations, in the MSH2 gene. People who have this illness are more likely to get colorectal cancer, which is the term for cancers of the colon (large intestine) and the rectum. Individuals with CMMRD syndrome inherit two MSH2 gene variants, one from each parent. Variants in the MSH2 gene cause a partial or total lack of MSH2 protein synthesis. When this protein is deficient, mismatch repair activity is eliminated and DNA replication mistakes cannot be properly repaired. As the aberrant cells divide, these mistakes mount up. Other genes involved in crucial biological functions, such as regulating cell division and growth (proliferation), are disrupted by the mistakes.

Colorectal cancer (CRC) is a genomic ailment, similar to other solid tumours. A range of genomic abnormalities, such as gene fusions, point mutations, rearrangements in the genome, and variations in the number of copies of a chromosome, can cause or worsen the disease. Next-generation sequencing (NGS), a recent advancement in sequencing technology, has made it easier to identify unique genetic mutations and analyse the whole genome of individual tumours [3]. Since the initial effort at cancer genome sequencing using NGS technology, numerous important human cancer types including gastrointestinal malignancies such as oesophageal, gastric, colorectal, and hepatocellular carcinomas have been successfully sequenced using NGS [4]. Over the past few decades, there has been a steady decline in the death rate from colorectal cancer (CRC), primarily as a result of improved diet, enhanced screening that removes precancerous colorectal polyps, and the introduction of targeted therapy [5].

The development of novel biologic medications that either target angiogenesis or epidermal growth factor signalling has been a major advancement, even though a variety of other factors have also contributed to improved clinical outcomes [6]. Bevacizumab, a monoclonal antibody that targets the vascular endothelial growth factor (VEGF), is one of these medicines. By inhibiting the formation of new blood vessels and causing the existing tumor vasculature to retreat by limiting the impact of VEGF, bevacizumab is thought to suppress tumor growth. Glioblastoma, cervical cancer, non-squamous non-small-cell lung cancer (NSCLC), recurrent epithelial ovarian, fallopian tube, or primary peritoneal cancer, as well as metastatic renal cell carcinoma, are among the tumors for which bevacizumab is approved of treatment [7].

The sequence-structure correlations, active sites, and bound molecules that were revealed by analyzing

interactive views of the 3D structures of homologous proteins are crucial for comprehending the functional consequences of MSH2 changes. Docking studies on MSH2 can help to identify small molecules or peptides that bind to the protein, either stabilizing it or inhibiting interactions that promote cancer progression.
In the present study, molecular docking approach was used for the MSH protein and ligands (Bevacizumab, an angiogenesis inhibitor used in targeted treatment for metastatic colorectal cancer and Tucatinib, a tyrosine kinase inhibitor drug). This approach can be used to screen a library of compounds for potential MSH2 modulators. A thorough understanding of the molecular changes in CRC is possible with the integration of proteomics and NGS data. The results of proteomics offer significant new insights into the role of these expressed proteins in colorectal cancer. The results from the present study demonstrate the potential to enhance patient outcomes and advance the field of colorectal cancer research through early diagnosis, genetic analysis, and computational approaches.

MATERIALS AND METHODS

The Protein Data Bank (PDB)

The Protein Data Bank (PDB) is a key repository for 3D structures of biological molecules like proteins and nucleic acids. The 3D structure of Human MutSalpha (MSH2/MSH6) bound to ADP was downloaded from PDB under the PDB ID: 2O8C [8].

Visualization

The visualization of 3D structure of Human MutSalpha (MSH2/MSH6) was performed through pymol and rasmol softwares. The visualization parameter included number of hydrogen bonds, peptide chains, C and N terminal points, attached ligands position and total number of residues. PyMOL is a well-known open-source program for comprehensive 3D molecular visualization and analysis, while RasMol offers quick, user-friendly molecular structure visualization and is widely used in education and research [9].

The Molecular Modelling Database (MMDB)

The Molecular Modeling Database (MMDB) and its data presentation, particularly concerning biologically significant complexes and molecular interactions, are intricately connected with NCBI's Entrez search and retrieval system. MMDB mirrors the content found in the Protein Data Bank, linking protein 3D structure data with sequence information, sequence categorization resources, and PubChem [10].

GeneCards

GeneCards is a searchable, integrative database that offers in-depth, user-friendly insights into all annotated and predicted human genes. The knowledge base seamlessly integrates gene-centric data from approximately 150 web sources, encompassing genomic, transcriptomic, proteomic, genetic, clinical, and functional information [11].

STRING

STRING is a biological database and an online resource of known and predicted protein–protein interactions. It has information from several sources including experimental data, algorithmic prediction techniques, and public text collections. These interactions include both direct and indirect interactions between the proteins, and the computer prediction makes use of data gathered from the organisms and main databases to visualize these interactions [12].

LIGPLOT

The LIGPLOT program autonomously produces schematic 2-D depictions of protein-ligand complexes utilizing standard Protein Data Bank file input. It provides a straightforward and enlightening illustration of the intermolecular interactions and their strengths, encompassing hydrogen bonds, hydrophobic interactions, and atom accessibilities [13].

PDBsum

PDBsum, functions as a platform that offers a multitude of visual interpretations for each record within the Protein Data Bank. It illustrates the structural characteristics of proteins, DNA, and ligands present in the record, while also illustrating the interactions among them [14].

CB dock 2

The molecular docking was performed and analysed by CB dock tool, a computational tool designed for protein-ligand docking, which predicts the fittest orientation of a ligand when it binds to a protein receptor [15].

RESULTS

The present study deals with the Proteomic Analysis of MSH2/MSH6 protein in complex with Human MutSalpha bound to ADP and O6-Methyl-Guanine T-Repair in Colorectal Cancer. The MSH2 protein, a tumor suppressor that aids in DNA repair, is encoded by the MSH2 gene. Numerous DNA repair mechanisms, such as homologous recombination, transcription-coupled repair, and base excision repair, rely on MSH2. Hereditary nonpolyposis colorectal cancer and other malignancies are linked to mutations in the MSH2 gene. The MSH6 protein, which is a member of the Mutator S family of proteins that aid in DNA repair, is encoded by the MSH6 gene. The MSH6 protein joins with the MSH2 protein to form a dimer, which identifies errors in DNA replication.

MSH2(MutS Homolog 2), tumor suppressor gene and more specifically a caretaker gene MSH2 is a protein coding gene and a component of the post-replicative DNA mismatch repair system (MMR). It forms two different heterodimers- MutS alpha (MSH2-MSH6 heterodimer) and MutS beta (MSH2-MSH3 heterodimer) which binds to DNA mismatches thereby initiating DNA repair. The MSH2 genomic locus has been cloned and shown to cover about 73 kb of genomic DNA and to contain 16 exons.

Figure-1 shows the mRNA expression in normal human tissues from GTEx, Illumina, BioGPS, and SAGE for MSH2 gene in Genecards. mRNA expression varied among adipose, artery, brain, colon, esophagus and heart with different intensities of expression.

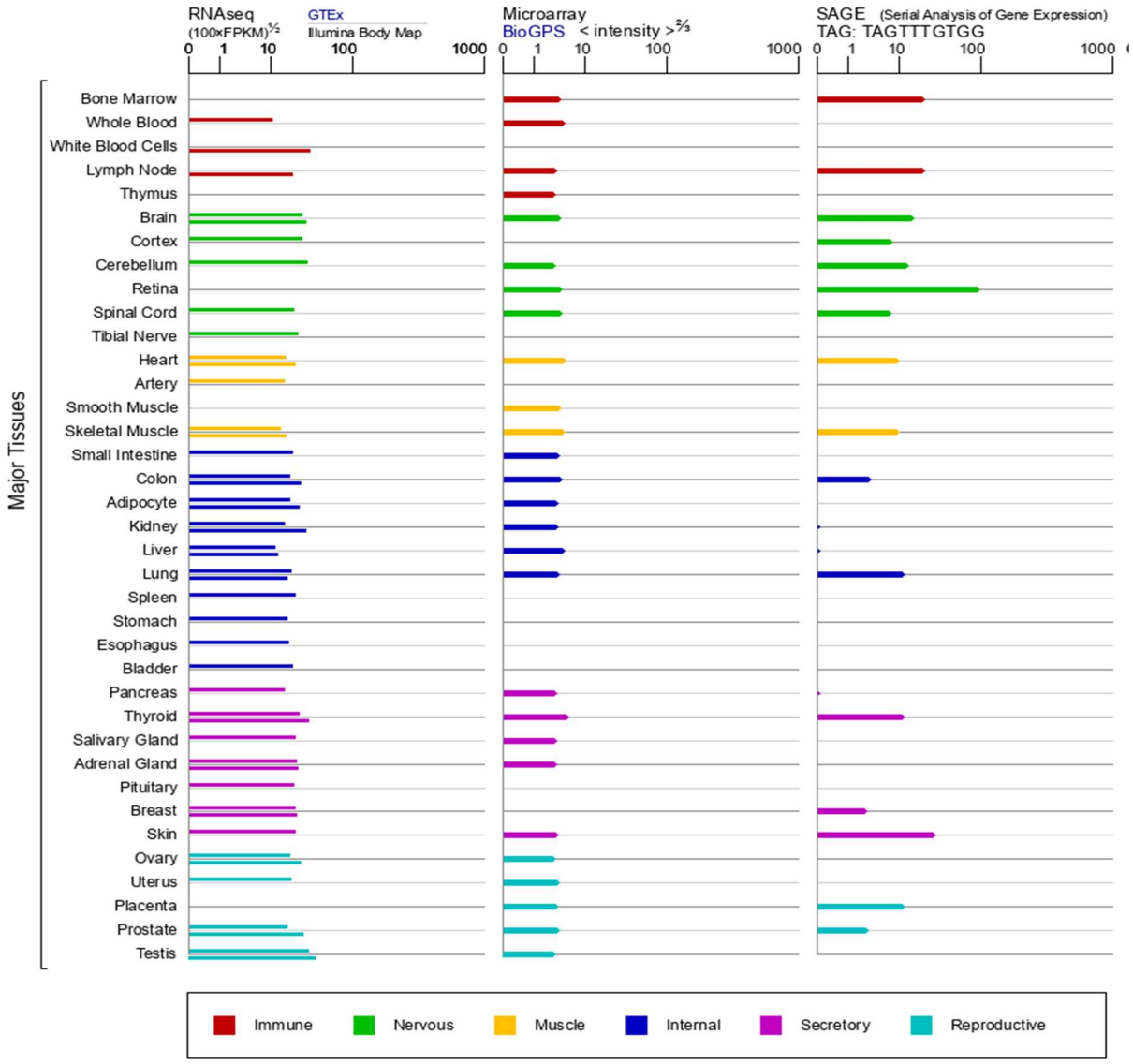
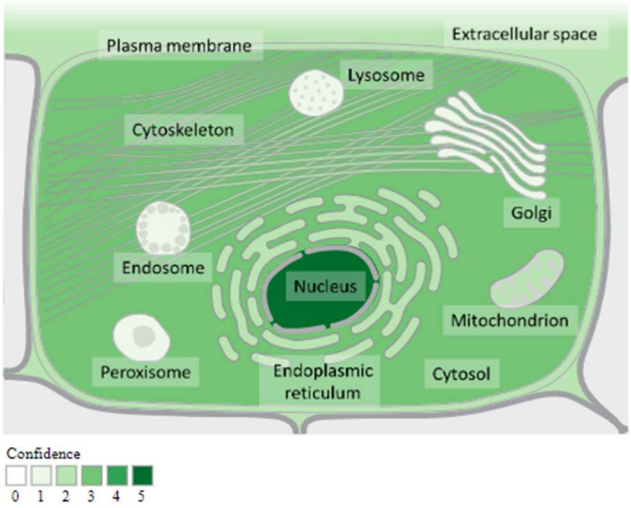


Figure:1 Genetic variant database analysis showing Expression of MSH2 gene in Genecards
Tissues and anatomical compartments are colored according to 6 categories - Immune (red), Nervous (green), Muscle (yellow), Internal (blue), Secretory (violet) and Reproductive (turquoise).

Compartments localization data is integrated from literature manual curation, high-throughput microscopy-based screens, predictions from primary sequence, and automatic text mining Unified confidence scores of the localization evidence are assigned based on evidence type and source, and visualized both in a table and in the schematic cell image. Figure-2 shows the subcellular locations of MSH2 gene from UniProtKB/Swiss-Prot. Confidence scale is color coded, ranging from light green (1) for low confidence to dark green (5) for high confidence. White (0) indicates an absence of localization evidence. It is clear that MSH2 is localized in larger extent when compared to other organelles basing on the high confidence score.



Compartment	Confidence
nucleus	5
cytosol	3
endoplasmic reticulum	2
mitochondrion	2
cytoskeleton	2
extracellular	2
plasma membrane	2
golgi apparatus	1
lysosome	1
endosome	1
peroxisome	1

Figure -2: Compartmentalisation of the MSH2 gene

Research findings indicate that gene expression is tightly controlled, leading to a series of unique co-expression patterns that coalesce into a network. Deciphering the molecular mechanisms behind the pathophysiology of diseases requires an awareness of these patterns. Figure-3 shows the gene network analysis showing the physical and co expression of MSH 2 gene.

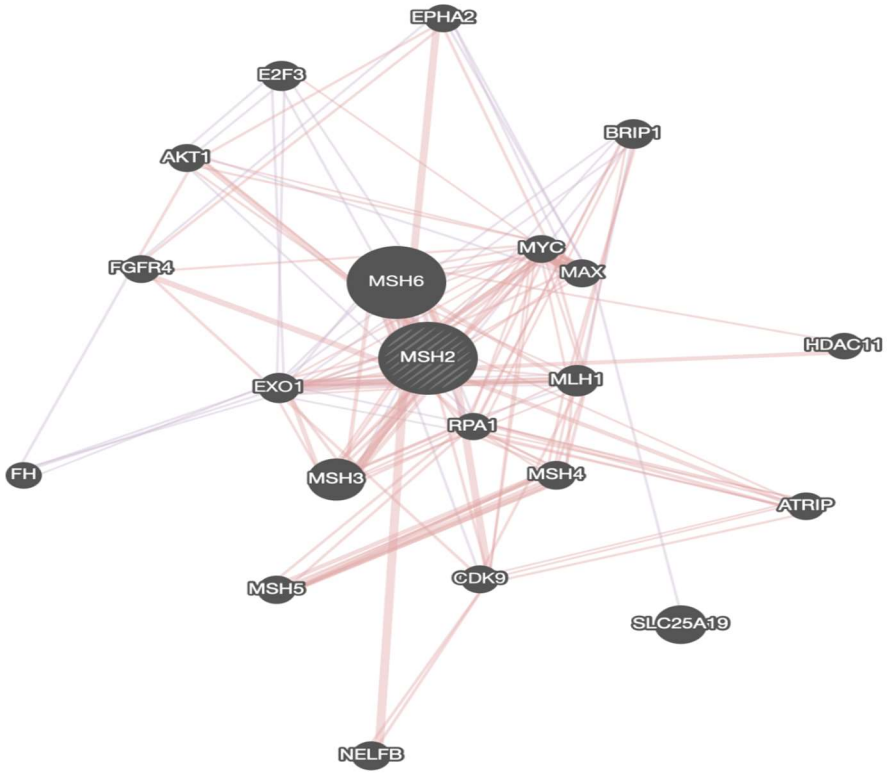


Figure 3: Gene network analysis showing the physical and co expression of MSH2 and MSH6

The 3D structure of Human MutSalpha (MSH2/MSH6) bound to ADP was downloaded from PDB under the PDB ID: 2O8C and visualization was performed through pymol and rasmol software. The visualization parameter included number of hydrogen bonds, peptide chains, C and N terminal points, attached ligands position and total number of residues.
Figure-4 depicts 3D structure of MSH2 protein represents the predicted local distance difference test for the protein.

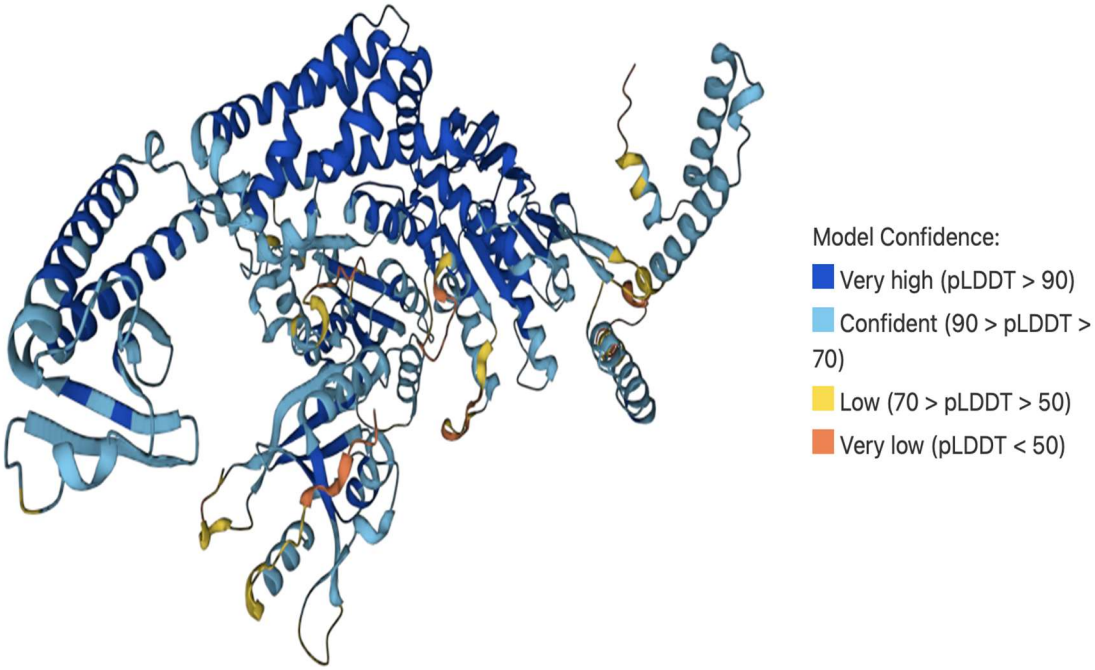


Figure:4 Three dimensional structure from PDB (representative) and AlphaFold (predicted) for MSH2 protein (different colours indicate ALPHA'S fold level confidence in its prediction).

Figure-5 represents a 3D molecular structure, 2O8C protein (human MutSalpha (MSH2/MSH6) bound to ADP), with distinct N-terminal (blue) and C-terminal (red) regions. The structure features a combination of alpha helices and beta sheets, connected by flexible loops, with colour variations indicating different functional domains. Yellow dashed lines suggest key interactions or binding sites, potentially highlighting functional or active regions within the protein. The identification of the N and C terminals is important for docking studies or mutational analysis, which will help in the mapping of the functional domain and a better understanding of the role of the protein in the biological process

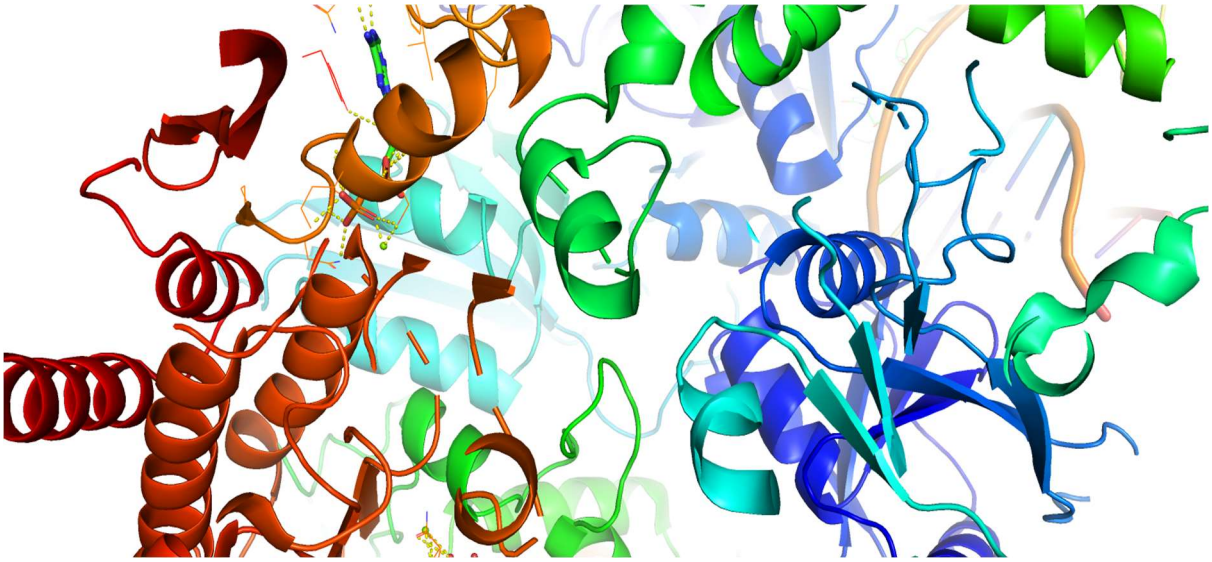


Figure-5: N terminal (blue) and C- terminal (red) view of 2O8C protein

Figure-6 depicts the 3D structural model of the protein MSH2, highlighting its predicted functional partners or interaction regions. The protein structure features prominent red α-helices and yellow β-sheets, which are typical secondary structure elements. Green loops connect these structured regions and may represent more flexible areas involved in binding or interaction with other molecules and dotted lines are hydrogen bonds. The accompanying 0.999 confidence score indicates a high level of certainty in these predictions, suggesting reliable interactions or functional associations between this protein and its potential partners.

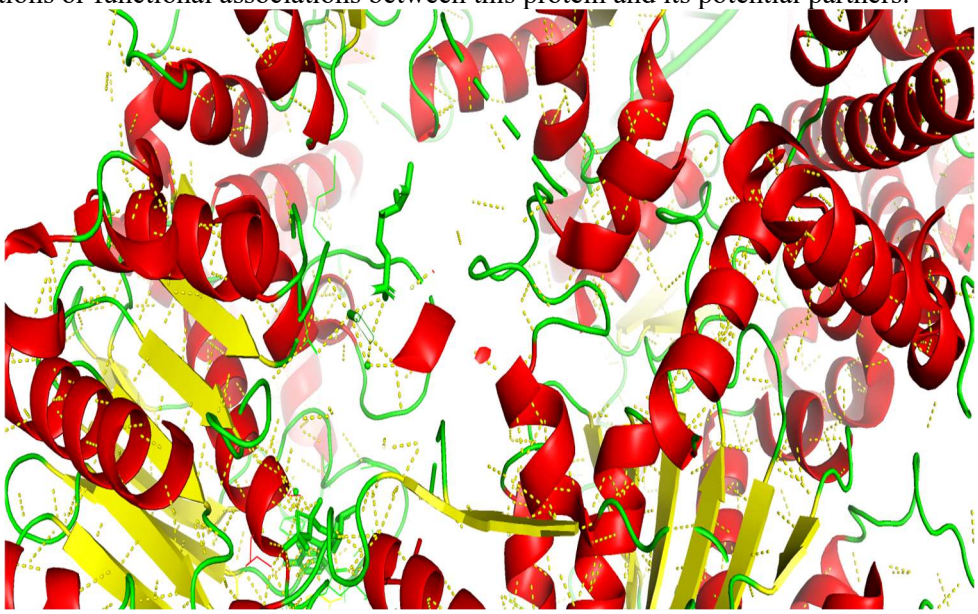


Figure 6: Visualising the polar contact in MSH2 protein using Pymol

Figure-7 illustrates the surface topology of MSH2, visualized using PyMOL. The color-coding (red, green, and yellow) reflects distinct structural or functional regions, such as hydrophobic and hydrophilic patches, potential active sites, or interaction domains. This representation provides a clear view of the protein's spatial organization, aiding in understanding the molecular architecture and key interaction sites.

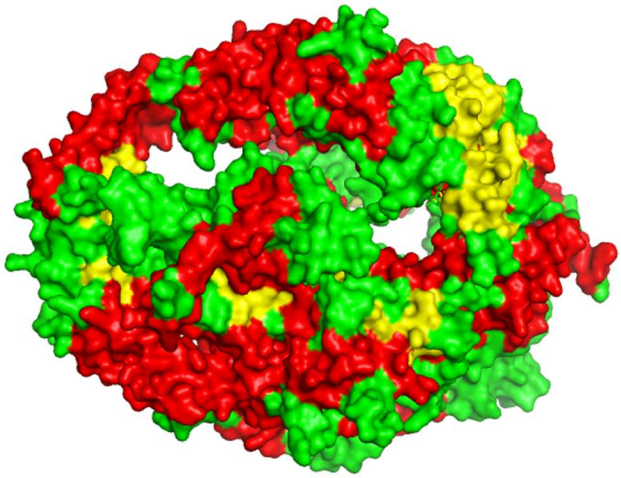


Figure 7: Illustration the surface topology of MSH2 visualized using PyMOL

LIGPLOT was used for automatic generation of 2D ligand-protein interaction diagrams. Figure-8 depicts the LIGPLOT of interactions involving MSH2 protein and the ligand ADP. In the figure, methionine, asparagine and serine are seen directly involving in the protein-ligand interaction

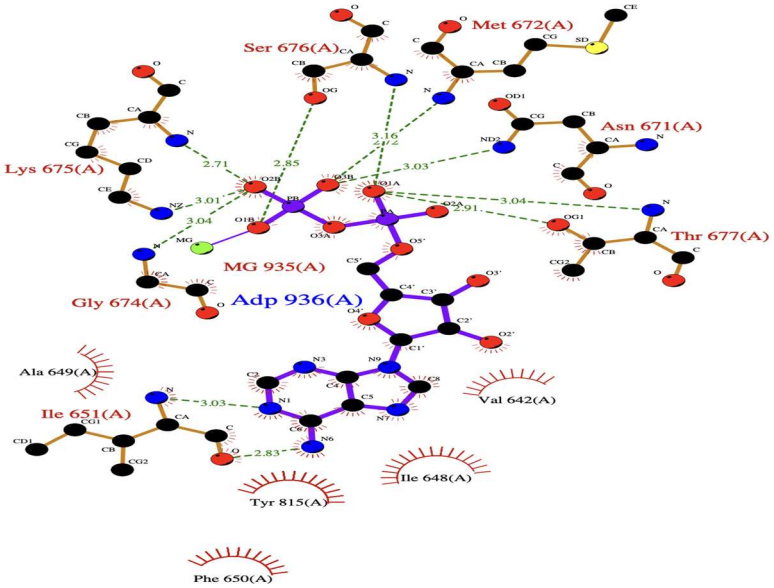


Figure 8: LIGPLOT of interactions involving MSH2 protein and ligand ADP

The green line represents the hydrogen bond between the atoms which are directly involved with amino acid residues in protein-ligand interaction.

PDBsum was used to generate a graphical and detailed summary of the 3D structure of MSH2 protein to visualize and understand its structural and functional aspects. Figure-9 shows the secondary structure prediction of Human protein sample (2O8C) in PDBsum. The figure illustrates the secondary structure of protein region, spanning from amino acid residues 184 to 254. It shows various structural elements, including α-helices (labeled H5-H9 and H1), β-strands (labeled B), and gamma (γ) turns, which contribute to the protein's overall fold and stability.

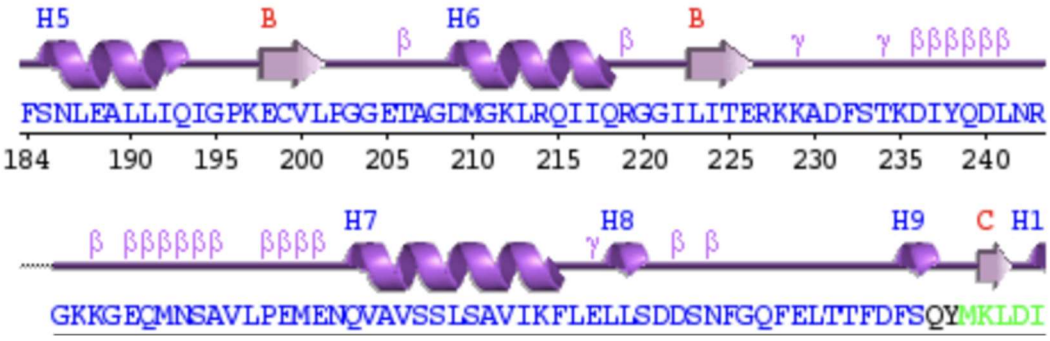


Figure-9: Secondary structure prediction of Human protein sample (2O8C) in PDBsum
The protein sequence is depicted in blue, with numbers indicating specific residue positions.

Molecular docking studies of Vascular Endothelial Growth Factor (VEGF), PDB ID: 2VPF, one of the target for CRC therapy were carried using CB-Dock2 with a humanized monoclonal antibody, Bevacizumab generally used to treat metastatic colorectal cancer (mCRC).
Figure-10 shows the protein ligand interaction of VEGF with Bevacizumab.
The docking analysis revealed five putative binding pockets in the target protein, as summarized in Table 1, out of which C1 pocket gives the best Vina (- 6.3 score) indicating the strongest binding affinity; it is coupled with the largest cavity volume of 1529 Å³, suggesting it is the most promising binding site. Pocket C2 has a slightly lower Vina score of - 5.9 and a cavity volume of 1105 Å, with center coordinates at (-17, 7, 25). Pockets C3, C4, and C5 have progressively higher but less favourable Vina scores of -5.4, -4.8 and -5.5, respectively, with smaller cavity volumes of 877 Å³, 678 Å³ and 606 Å³. The centroids of these three pockets are centrally located at (12, 29, 41), (-11, -13, 8), and (17, 45, 14). These findings highlight C1 as the key target for potential ligand binding, with the other pockets offering alternative sites for further exploration in drug design or molecular interaction studies. More the score is negative, more the binding energy used for docking. The docking results are showed in Figure 10.

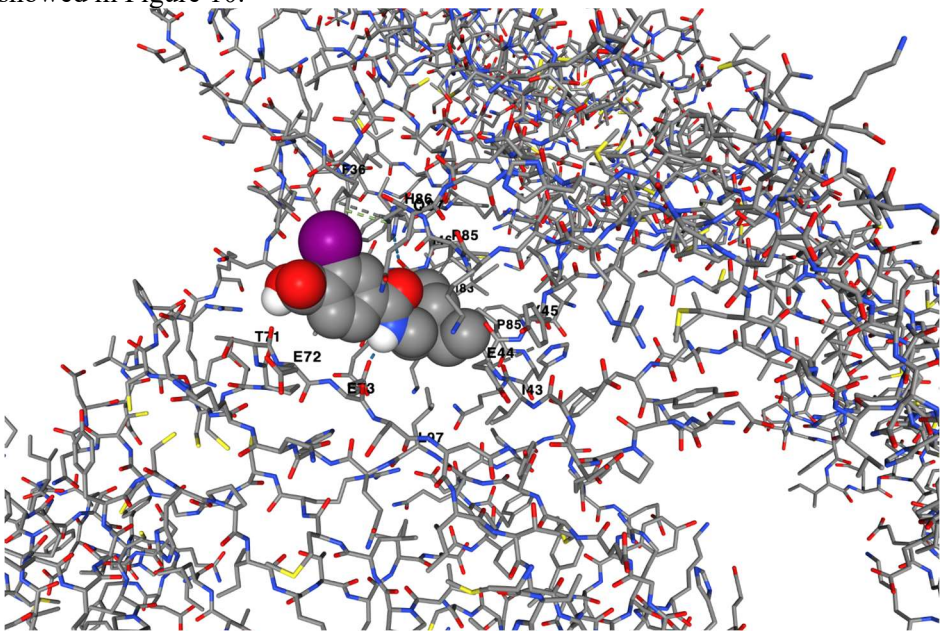


Figure-10: Protein ligand interaction of Vascular Endothelial Growth Factor (VEGF) with Bevacizumab.

Table -1: Illustration of VEGF - ligand (Bevacizumab) ligand scores

Cur Pocket ID	Vina score	Cavity volume ($Å^3$)	Center (x,y,z)	Docking size (x,y,z)
C1	**-6.3**	**1529**	**12, 2, 14**	**21, 21, 21**
C2	-5.9	1105	17, 7, 25	21, 28, 27
C3	-5.4	877	12, 29, 41	21, 21, 21
C4	-4.8	678	-11, -13, 8	21, 21, 21
C5	-5.5	606	17, 45, 14	21, 21, 21

Chain B: VAL33 GLU38 PRO40 ARG56 PRO70 THR71 GLU72 GLU73 SER74 LEU97 HIS99
Chain E: PHE36 PRO40 ILE43 GLU44 TYR45 ILE46 ARG82 ILE83 LYS84 PRO85 GLN87 GLY88 GLN89 HIS90
Chain H: ILE43 GLU44 TYR45 LYS84 PRO85 HIS86 GLN87

Likewise, HER2, a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases, which is a target for chemotherapy in the treatment of colorectal cancer was also docked with Tucatinib, a tyrosine kinase inhibitor drug that treats certain types of HER2-positive breast and colorectal cancer in adults. Tucatinib works by blocking the HER2 protein that signals cancer cells to multiply, which may help stop or slow the spread of cancer.
Figure-11 shows the protein ligand interaction of HER2 protein with Tucatinib.
The docking analysis revealed five putative binding pockets in the target protein, as summarized in the Table 2. Pocket C3 shows the lowest Vina score of -12.1, indicating the strongest binding affinity; it is coupled with the cavity volume of 921 Å³, suggesting it is the most promising binding site. It is centrally coordinated at (-83, -12, -86). Pocket C1 has a slightly lower Vina score of -9.9 than C3 and has a cavity volume of 1425 Å³, with center coordinates at (-37, -9, -110). Pockets C2, C4, and C5 have progressively higher but less favourable Vina scores of -8.6, -7.8, and -7.5, respectively, with smaller cavity volumes of 1216 Å³, 308 Å³ and 190 Å³. These findings highlight C3 as the key target for potential ligand binding, with the other pockets offering alternative sites for further exploration in drug design or molecular interaction studies.

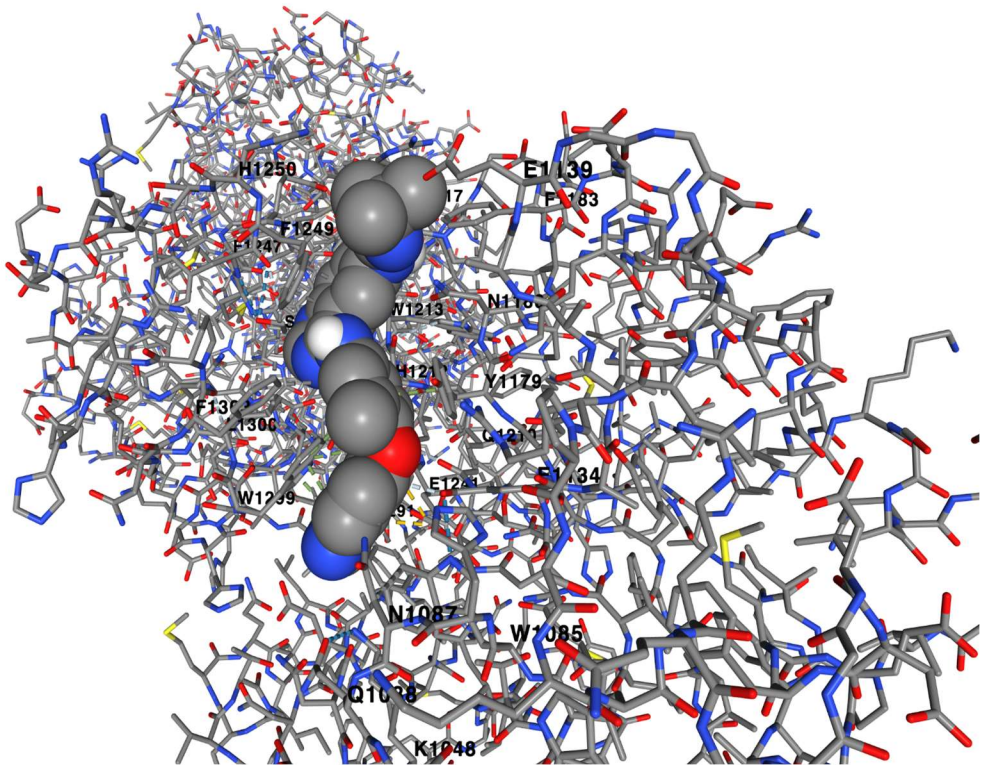


Figure-11: Protein ligand interaction of HER2 with Tucatinib

Table -2: Illustration of interaction of HER2 – Tucatinib ligand scores

CurPocket ID	Vina score	Cavity volume (Å^3)	Center (x,y,z)	Docking size (x,y,z)
C1	-11.3	1425	-37, -9, -110	28, 28, 28
C2	-8.8	1216	-64, -9, -104	28, 28, 28
C3	-12.1	921	-83, -12, -86	28, 28, 28
C4	-7.8	308	-94, -7, -112	28, 28, 28
C5	-7.5	190	-51, -21, -92	28, 28, 28

Chain B: GLU1044 ASN1045 LYS1048 HIS1081 TRP1085 HIS1086 ASN1087 GLN1088 ASN1133 GLU1134 GLU1139 TYR1179 ASN1180 PHE1183 GLN1210 HIS1212 TRP1213 SER1214 ARG1217 GLU1241 ASP1243 SER1245 PHE1247 GLU1248 PHE1249 HIS1250 TRP1291 TRP1299 LEU1300 ASN1302 PHE1303 PRO1304 HIS1306

The molecular docking studies reveal that Tucatinib and Bevacizumab to be potent and choice of drug in the treatment of colorectal cancer.

Further, STRING analysis was used to analyze protein-protein interactions and functional pathways in sequenced gene. Figure-12 shows the protein enrichment network analysis showing first cell of interaction. Network nodes represent proteins splice isoforms or post-translational modifications are collapsed, i.e. each node represents all the proteins produced by a single, protein-coding gene locus. Edges represent protein-protein associations meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding to each other.

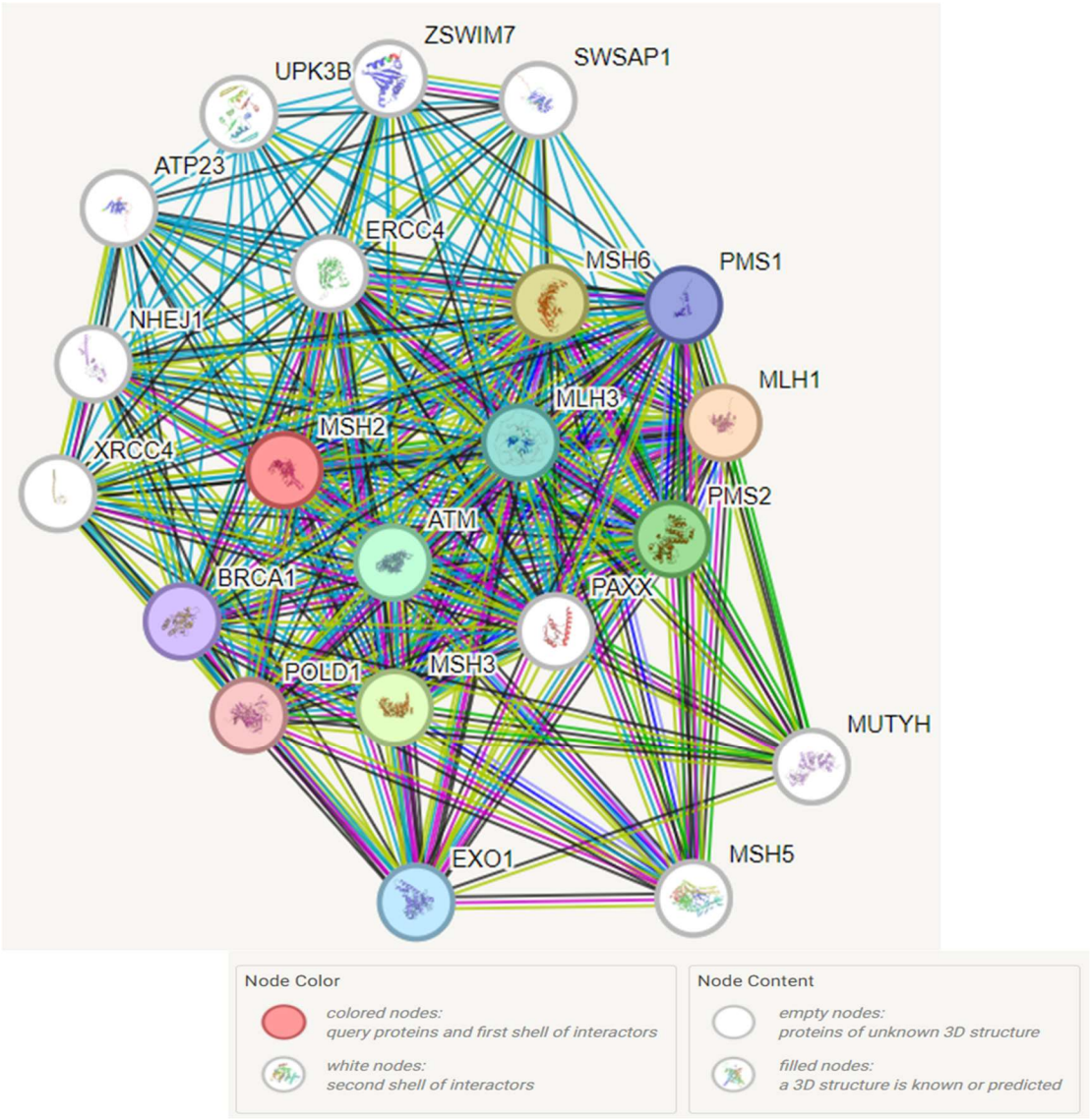


Figure-12: Protein Enrichment network analysis showing first and second shell of interactors

DISCUSSION

Colorectal cancer (CRC) is one of the most common and deadly cancers worldwide. For many years, the first options available to cancer patients were chemotherapy and surgery. But the prognosis for colorectal cancer has never been good, particularly for those who have metastatic lesions. A novel optional strategy that has successfully extended CRC patients' overall survival is targeted therapy. The study undertook a comprehensive computational and proteomic analysis of the MSH2 protein in colorectal cancer, utilizing bioinformatics tools. After the anti-EGFR (epidermal growth factor receptor) drug Cetuximab and the anti-angiogenesis drug bevacizumab proved successful, novel drugs that block several essential pathways and immunological checkpoints are appearing at a never-before-seen pace.

Targeted medications efficiently cure cancerous cells by directly inhibiting their migration, division, and proliferation. In addition to modifying the surrounding blood vessels and immune cells, targeted drugs can also alter the tumor microenvironment to stop tumor growth and boost the body's defenses against it. Among other tiny substances, monoclonal antibodies play a significant role in targeted therapies might penetrate into cells, mostly working within cells to inactivate selected enzymes, thereby interfering with tumor cell growth and even triggering apoptosis [16. 17].

Major genetic alterations involving the MSH2 gene were identified using human biological samples from the Protein Data Bank (PDB), offering crucial insights into the pathogenesis of colorectal cancer. Schematic 2D representations of protein-ligand complexes were generated using PYMOL, aiding in the visualization of interactions crucial for drug design. Localization of the MSH2 gene in human sample 208C provided critical insights into the cellular context of MSH2. Protein-protein interaction statistical analysis shed light on the complex molecular interactions involved in colorectal cancer, offering a comprehensive view of interaction networks. Key genetic variants of the MSH2 gene were identified, which could be pivotal in disease progression and treatment response.

Molecular docking studies were performed using CB-Dock2 to explore the binding interactions between Vascular Endothelial Growth Factor (VEGF) and the humanized monoclonal antibody Bevacizumab. VEGF is a critical regulator of angiogenesis, a process essential for tumor growth and metastasis, particularly in colorectal cancer (CRC). Bevacizumab, an anti-angiogenic agent, binds VEGF, thereby inhibiting its interaction with VEGF receptors (VEGFR) on endothelial cells and blocking the downstream signaling pathways that promote neovascularization and tumor progression. Docking results provided by CB-Dock2 show a strong binding affinity between VEGF and Bevacizumab, with the antibody effectively interacting with key epitopes on VEGF. The high docking score indicates robust interaction, consistent with the known mechanism of action of Bevacizumab, which neutralizes VEGF's biological activity by preventing its receptor binding. Docking results revealed five putative binding pockets in the target protein, out of which C1 pocket gives the best Vina (- 6.3 score) indicating the strongest binding affinity suggesting it is the most promising binding site. As of right now, the FDA has only approved bevacizumab as a VEGF-targeted first- and second-line treatment for colorectal cancer (CRC). However, new and innovative medicines are being licensed for the treatment of CRC in some cases. Hydrophobic interactions, and electrostatic forces were identified as major contributors to the stability of the VEGF-Bevacizumab complex. Previous studies have also demonstrated similar interaction patterns, confirming the importance of these forces in the inhibitory mechanism of Bevacizumab [18,19].

By inhibiting effectively reduces angiogenesis, which is vital for tumor survival and proliferation in CRC. Clinical studies have shown that significant reduction in tumor vascularization and progression, correlating with improved patient outcomes. The docking results obtained in this study reinforce the clinical relevance of Bevacizumab as an anti-VEGF therapeutic agent, showcasing the potential of molecular docking as a computational tool for evaluating drug-target interactions and predicting therapeutic efficacy. [20,21]. The utility of CB-Dock2 in this study highlights the value of advanced computational platforms for drug discovery and design [22]. This *in silico* approach not only supports the current understanding of Bevacizumab's role in CRC therapy but also provides a framework for exploring modifications that could enhance its binding affinity and clinical effectiveness.

Likewise, molecular docking studies were also conducted to evaluate the interaction between Tucatinib, a

selective tyrosine kinase inhibitor (TKI), medication used to treat some adult cases of HER2-positive breast and colorectal cancer, was docked with HER2, a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases and a target for chemotherapy in the treatment of colorectal cancer. Five potential binding pockets in the target protein were found by docking study; of them, Pocket C3 has the highest binding affinity and the lowest Vina score of -12.1, indicating that it is the most promising binding site.
HER2 is a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases, which are key regulators of cellular growth and differentiation. Aberrant activation of HER2 has been implicated in the pathogenesis of various cancers, particularly HER2-positive breast and colorectal cancers, making it an essential therapeutic target in these malignancies.

The results from the docking simulations indicate that Tucatinib exhibits a high binding affinity for HER2, suggesting a strong interaction that is likely to inhibit the receptor's tyrosine kinase activity. Tucatinib's mechanism of action primarily involves competitive inhibition of the ATP-binding site, thereby obstructing HER2's phosphorylation and subsequent downstream signaling pathways that promote tumor growth and survival.
The therapeutic implications of Tucatinib in HER2-positive colorectal cancer are significant. HER2 overexpression in colorectal cancer is associated with a more aggressive disease phenotype and poor prognosis, particularly in cases resistant to standard therapies. By selectively targeting HER2, Tucatinib presents an innovative approach to overcoming resistance to conventional chemotherapeutic agents, offering a viable treatment option for patients with advanced disease. Moreover, the selective nature of Tucatinib may result in a favorable safety profile compared to non-selective TKIs, minimizing off-target effects and enhancing patient tolerability [23-26].
In addition to its clinical applications, the use of molecular docking in this study emphasizes the value of computational approaches in drug discovery and development. The insights gained from docking studies can inform further experimental validation and optimization of Tucatinib, potentially leading to the identification of novel therapeutic strategies for HER2-positive cancers.
The molecular docking studies conducted significantly advance our understanding of colorectal cancer at a molecular level. These findings underscore the potential for early detection, genetic analysis, and computational approaches to drive forward colorectal cancer research and improve patient outcomes. Further investigations and clinical trials are necessary to validate the efficacy of identified ligands and explore their therapeutic potential in treating colorectal cancer.

CONCLUSION

In conclusion, the molecular docking studies conducted on Vascular Endothelial Growth Factor (VEGF) with Bevacizumab and Human Epidermal Growth Factor Receptor 2 (HER2) with Tucatinib provide critical insights into the mechanisms underlying targeted therapies for colorectal cancer. The strong binding interactions observed between Bevacizumab and VEGF reaffirm the efficacy of this anti-VEGF antibody in inhibiting angiogenesis, a fundamental process in tumor growth and metastasis. Similarly, the docking analysis of Tucatinib with HER2 highlights the potential of this selective tyrosine kinase inhibitor to disrupt HER2 signaling pathways. Together, these studies emphasize the significance of targeted therapies in the management of colorectal cancer, particularly in patients with tumors that exhibit overexpression of HER2 and high levels of VEGF. Ultimately, the combination of Bevacizumab and Tucatinib represents a promising strategy for enhancing treatment efficacy in colorectal cancer, paving the way for more personalized and effective therapeutic regimens.

ACKNOWLEDGMENT

The authors thank School of Allied and Healthcare Sciences, Malla Reddy University, Hyderabad, for providing necessary facilities.

Declaration of Conflicts of Interests

he author declares that there is no conflict of interest.

Declarations

The author declare that all works are original and this manuscript has not been published in any other journal.

REFERENCES

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024; 74(3):229-263.
2. Sandeep NS, Suzanne EH, Kristin AE. Defective mismatch repair, microsatellite mutation bias, and variability in clinical cancer phenotypes. Cancer Res. 2010; 70: 431–435.
3. Tran B, Dancey JE, Kamel-Reid S, McPherson JD, Bedard PL, et al. Cancer genomics: technology, discovery, and translation. J Clin Oncol, 2012; 30: 647–660.
4. Kim TM, Lee SH, Chung YJ. Clinical applications of next-generation sequencing in colorectal cancers. World J Gastroenterol. 2013; 19(40):6784-6793.
5. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer. 2015;136(5): E359–86
6. Kasi PM, Hubbard JM, Grothey A. Selection of biologics for patients with metastatic colorectal cancer: the role of predictive markers. Expert Rev Gastroenterol Hepatol. 2015; 9:273–276.
7. Rosen LS, Jacobs IA, Burkes RL. Bevacizumab in Colorectal Cancer: Current Role in Treatment and the Potential of Biosimilars. Target Oncol. 2017; 12(5):599-610.
8. Warren, J.J., Pohlhaus, T.J., Changela, A., Iyer, R.R., Modrich, P.L., Beese, L.S.Structure of the Human MutSalpha DNA Lesion Recognition Complex. Mol Cell. 2007; 26: 579-592.
9. Shuguang Yuan, H.C. Stephen Chan and Zhenquan Hu. (2017). Using PyMOL as a platform for computational drug design. WIREs Comput Mol Sci. 2017; 7: e1298, 1-10.
10. Thomas Madej, Kenneth J. Addess, Jessica H. Fong, Lewis Y. Geer, Renata C. Geer, Christopher J. Lanczycki, Chunlei Liu, Shennan Lu, Aron Marchler-Bauer, Anna R. Panchenko, Jie Chen, Paul A. Thiessen, Yanli Wang, Dachuan Zhang, Stephen H. Bryant, MMDB: 3D structures and macromolecular interactions, Nucleic Acids Research, 2012; 40(D1): D461–D464.
11. Stelzer G, Rosen N, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, Stein TI, Nudel R, Lieder I, Mazor Y, Kaplan S, Dahary D, Warshawsky D, Guan-Golan Y, Kohn A, Rappaport N, Safran M, Lancet D. The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. Curr Protoc Bioinformatics. 2016; 54:1.30.1-1.30.33.
12. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, Gable AL, Fang T, Doncheva NT, Pyysalo S, Bork P, Jensen LJ, von Mering C. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023; 51(D1):D638-D646.
13. Wallace AC, Laskowski RA, Thornton JM. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Eng. 1995; 8(2):127-134.
14. de Beer TA, Berka K, Thornton JM, Laskowski RA. PDBsum additions. Nucleic Acids Res. 2014; 42(Database issue): D292-296.
15. Yang Liu, Xiaocong Yang, Jianhong Gan, Shunag Chen, Zhi-Xiong Xiao, Yang Cao. CB-Dock2: improved protein-ligand blind docking by integrating cavity detection, docking and homologous template fitting. Nucleic acids Research. 2002; 50(W1), W159-W164.
16. Oh, D. Y. & Bang, Y. J. HER2-targeted therapies—a role beyond breast cancer. Nat. Rev. Clin. Oncol. 2020; 17:33–48.
17. Ferguson, F. M., Gray, N. S. Kinase inhibitors: the road ahead. Nat. Rev. Drug Discov. 2018; 17: 353–377 (2018).
18. Ferrara, N., Hillan, K. J., Gerber, H. P., & Novotny, W. Discovery and development of Bevacizumab, an anti-VEGF antibody for treating cancer. Nature Reviews Drug Discovery. 2004; 3(5): 391-400.

19. Karaman, S., Leppänen, V. M. Alitalo K. Vascular endothelial growth factor signaling in development and disease. Development. 2018; 145(14): dev151019.
20. Hurwitz, H., Fehrenbacher, L., Novotny, W., Cartwright, T., Hainsworth, J., Heim, W., Kabbinavar, F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. New England Journal of Medicine. 2004; 350(23):2335-2342.
21. García-Foncillas, J., Díaz-Rubio, E., Rivera, F., Massutí, B., Valladares-Ayerbes, M., Salud, A., Viéitez, J. M. Management of Bevacizumab-related toxicities in metastatic colorectal cancer: expert panel recommendations. Clinical & Translational Oncology. 2010; 12(4): 271-279.
22. Morris, G. M., Huey, R., & Olson, A. J. Using AutoDock for ligand-receptor docking. Current Protocols in Bioinformatics. 2008; 24(1): 8-14.
23. Cortés, J., Kim, S. B., Chung, W. P., Park, Y. H., Im, S. A. Tucatinib in combination with trastuzumab and capecitabine for HER2-positive metastatic breast cancer: results from the HER2CLIMB trial. Journal of Clinical Oncology. 2020; 38(16):1635-1645.
24. Vanneste, B. G., De Jong, J. The role of HER2 in colorectal cancer. Current Opinion in Gastroenterology. 2018; 34(5): 331-337.
25. Raghav, K., & Overman, M. J. The emerging role of targeted therapies in HER2-positive colorectal cancer. Journal of Gastrointestinal Oncology. 2019; 10(2): 352-363.
26. Blackwell, K. L., Burstein, H. J., Storniolo, A. M. First-in-human study of tucatinib, an oral HER2-selective tyrosine kinase inhibitor, in patients with advanced solid tumors. Clinical Cancer Research. 2015; 21(21):5375-5382.