IN-SILICO STUDY BASED EVIDENCE OF MICROBE DRIVEN MODULATION OF CANCER PROGNOSIS

A DISSERTATION

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CANDIDATE'S DECLARATION

I, Ramsha Hashmi, Roll No. 2K19/BIO/03 student of M.Tech Bioinformatics, hereby declare that the project Dissertation titled "**In-silico study based evidence of microbe driven modulation of cancer prognosis**" which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any Degree, Diploma Associateship, Fellowship or other similar title or recognition.

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Date: 28/06/2021

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CERTIFICATE

I hereby certify that the Project Dissertation titled "**In-silico study based evidence of microbe driven modulation of cancer prognosis**" which is submitted by Ramsha Hashmi, Roll No. 2K19/BIO/03 Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is a record of the project work carried out by the students under my supervision. To the best of my knowledge this work has not been submitted in part or full for any Degree or Diploma to this University or elsewhere.

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I perceive this opportunity as a big milestone in my career development. I will strive to use gained skills and knowledge in the best possible way and will continue to work on their improvement. Hope to continue cooperation with all of you in the future.

Sincerely,

RAMSHA HASHMI

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ABSTRACT

Cancer has been a major cause of disease burden on humanity for eons with one-fifth of all instances of cancer being connected with microbial prevalence and dysbiosis. There has been an inrush of instances asserting the dual character of human microbiota in maintaining homeostasis and its association with various ailments, including cancer. The diverse microbiota inhabiting the gut constitute a complicated symbiotic relationship conferring benefits to the organisms' body in numerous ways, such as aiding metabolism, immunity, and nutrition. The microbiome has been seen to be affected by behavior, central nervous system & cardiovascular physiology, dysbiosis, innate & adaptive immunity, and diet & environment. Disturbances in regulatory pathways mainly responsible for guarding homeostasis as well as microbial dysbiosis lead to disease development. Pathogens that are specifically associated with cancer do not work in solitude, rather, it's an association of microbes that have a cumulative impact on immune function and genome stability. Accumulation of certain bacteria promotes persistent inflammation, genetic alterations in principal inflammation-modulating genes which in turn elevate dysbiosis and thus cancer.

Tumor antigens that are present on the cancer cell surface like MUC16 and mesothelin show high-affinity binding towards each other and have been attributed to increasing the metastasis and migration capabilities of cancerous cells. In numerous instances of this morbidity certain bacteria have been found to be closely associated in solid tumors as well as in surrounding normal tissues. The new age of bioinformatics has revolutionized the science of omics and vaccinology by aiding high-speed in-silico protein structure determination & epitope identification using fast and precise

tools. In the present study, we have used the immunoinformatics strategy to identify and model a microbial peptide in one such cancer-associated microbe which shows close homology with tumor antigen CA125 which is aberrantly overexpressed in ovarian cancer cells among various other diseases. The present study is based on a methodology which utilizes various bioinformatics tools which are online as well as offline. The major techniques employed in the project are homology modelling, protein-protein docking, and epitope prediction aided by visualization tools. Our results exhibit a novel epitope-containing microbial peptide present in a cancer-associated bacteria that shows binding to mesothelin through molecular docking studies, which could possibly hinder its extensive binding to CA125 and therefore putatively alter the disease prognosis.

Keywords- Cancer, Dysbiosis, Microbes, Inflammation, Tumor antigens, MUC16, Mesothelin, CA125, Cancer-associated microbe, immunoinformatics, Homology Modelling, Protein-protein Docking.

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CHAPTER 1. INTRODUCTION

Our body is home to a large diversity of microbes that play various crucial roles in aiding the host physiology through nutrition and metabolism, CNS physiology and cognitive functioning, behavior, innate and adaptive immunity, and thus also aiding in our combat against cancer. Besides their contributory role in host homeostasis, various pathogens and commensals have been found to cause inflammation-induced cancer [6,7,8]. Gut microbes can cause inflammation, cause tumors to further oppose chemotherapeutic drugs, produce DNA-damaging toxins and carcinogenic metabolites, and modulate the body's anticancer immune responses. The immune system of our body recognizes and responds to microbial antigens and metabolites through modulation of the immune response. Various complex diseases have been found to be associated with microbial dysbiosis. This is majorly due to the effects of microbes on immunity, metabolism, inflammation, cellular proliferation, regulation of cancer progression through genetic variability, initiation, susceptibility to host immune activation, response to therapy, and comorbidity [9]. The microbiota performs a significant part in developing the organism's immune system.

Tumors have been found to be intracellularly inhabited by bacteria. Identification of tumor microbiome relies on sequencing technique which amplifies selective parts of the gene (16S rRNA) to characterize the taxonomy of obscure communities of bacteria. Bacterial presence is linked to the detection of lipopolysaccharides (LPS) and lipoteichoic acid (LTA) comprising microbe-associated molecular patterns (MAMPs), pathogen-associated molecular patterns (PAMPs), or danger-associated molecular patterns (DAMPs).

Tumor cells produce antigenic substance commonly known as a tumor antigens which stimulates the immune system of the host and are used as an important biomarker in cancer diagnosis and aiding therapy. One such tumor antigen is Mucin 16 also known as CA125 which has been widely used as a biomarker in ovarian cancer due to its aberrant overexpression. Its expression is confined to the apical membranes of exposed epithelial cells with its functions of maintaining protection of the epithelium. MUC16 binding to mesothelin with high affinity presented the first evidence of MUC16s direct role in cancer metastasis. Mesothelin is a protein present on the mesothelial lining of the peritoneal cavity. This interaction promotes the attachment of cancer cells to the mesothelium thus driving peritoneal metastasis of ovarian and pancreatic cancer cells. MUC16 and its ligands have been applied as potential targets for therapeutic targets in various human malignancies of the ovary, breast, pancreas, and lung due to their aberrant overexpression employing monoclonal antibodies and immunotherapy.

MMP7 upregulation is yet another mechanism driving intensified metastatic competence through MUC16–mesothelin interaction. Some of the novel targeting approaches exercise disruption of MUC16-Mesothelin interaction using HN125 immunoadhesin and sensitizing MUC16 expressing cells to Meso-TR3 chimera to induce apoptosis.

Cancer remains of the most concerning diseases to humans owing almost more than one-fifth of all cancer cases to infectious agents [5]. An estimate of 19.3 million new cancer cases with nearly 10.0 million deaths from cancer occurred worldwide in 2020 [6]. Tumors have been found to possess their own microbiome which is specific to the tumor type. The intra-tumoral bacteria are largely seen to inhabit intracellularly within cancerous as well as the immune cells with breast tumors having the highest microbial diversity and prevalence [7]. Among the examples of carcinogenesis initiated by the microbiome in the gut are Type IV carcinogenic secretions by Helicobacter pylori [8], adherence and intrusion to epithelial cells by F. nucleatum are succeeded by oncogenic and inflammatory effects, [9]

and inflammation provoked by E. coli can modify the composition of the microbiota and promote carcinogenesis [10].

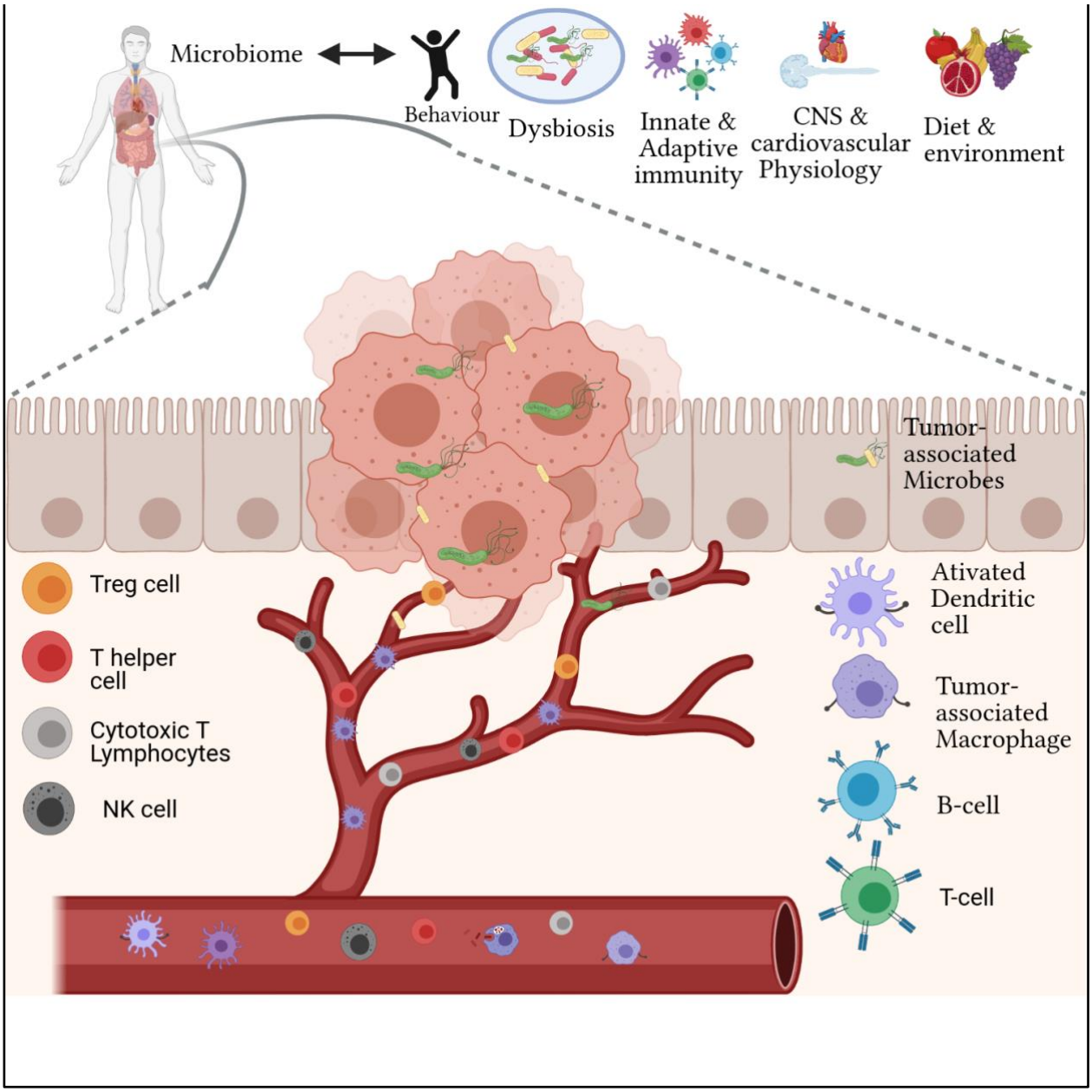

Fig.1 Role of Microbes in tumor immunology

1.1 Role of MUC16 in Epithelial-Mesenchymal Transition (EMT)

MUC16 expression is highly regulated and is found on the epithelial lining of various organs such as the tracheal surface, ocular surface, mesothelium lining of the abdominal cavity, female reproductive tract, and cervical epithelium. MUC16 is densely glycosylated with O-linked and N-linked oligosaccharides having implications in cell-matrix interactions, cell-cell contact growth regulation, and epithelial cell differentiation. The overexpression of MUC16 has been seen by many tumors of epithelial origin thereby suggesting their important purpose in tumorigenesis. Mesothelin which is a glycosylphosphatidylinositol-anchored glycoprotein binds with high affinity to the mucin MUC16 and likely promotes the peritoneal epithelial-mesenchymal transition metastasis of ovarian tumors due to the resulting alterations in cell-cell adhesion and signaling [11]. Mucins with high molecular weight like MUC16 have been seen to play a significant role in modifying signals and cellular transformation in EMT [12]. Certain structural changes in MUC16 structure like alterations in the glycosylation profile appear to be associated with malignant transformation & migration of epithelial ovarian cancer and solid tumor growth. Cancer cells commonly exhibit shorter capped and early biosynthetic intermediates of the antigens N-acetylgalactosamine carbohydrates; which are usually extended and branched in normal cells, namely STn and ST antigens [13]. The abnormal expression of truncated O-glycans is a hallmark of epithelial cancers and their presence display increased migration and decreased metastasis and invasiveness in different ovarian cancer stages. MUC16 is bound to E-cadherin/β-catenin junctional complexes extracellularly and intracellularly respectively which play an important role in EMT [14][13]. The overexpression of MUC16 promotes p120-catenin translocation to the cytoplasm, & activates RhoA/Cdc42 to temper the proliferation and migration capabilities of EOC cells [15]. A recent study

shows the soluble proteolytic fragments referred to as CA125 stimulates the SGK3/FOXO3 pathways and decreases DKK1 expression thus increasing ovarian cancer cell migration [16].

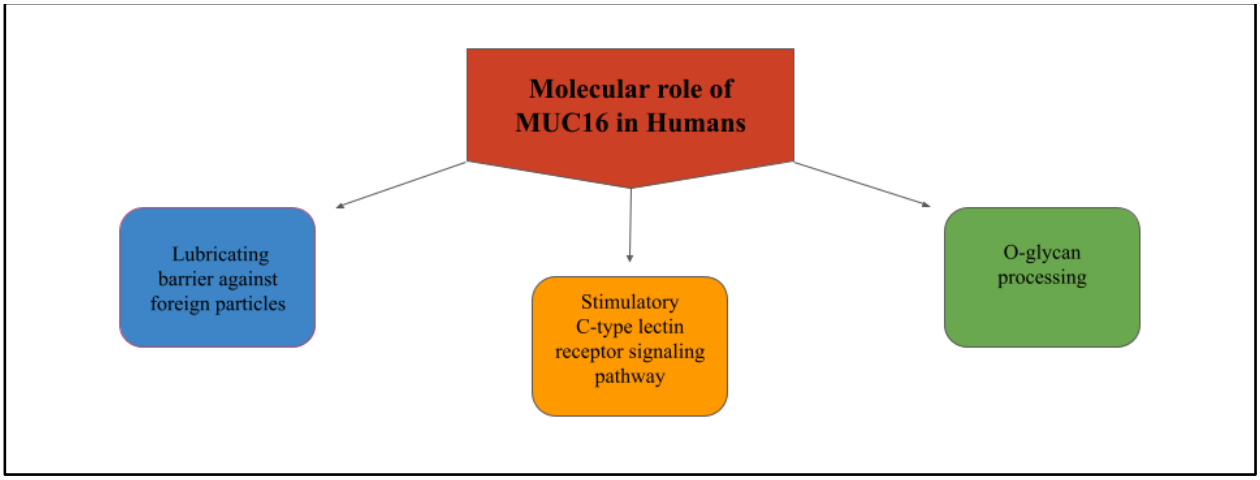

Fig.2 Molecular role of MUC16

1.2 Role of Mesothelin in EMT

It has been recently demonstrated that CA125 interacts with the Mesothelin (MSLN) pathway along with SGK3/FOXO3 & DKK1 to accelerate the migration of cells and that mesothelin targeting holds potential for utilization in ovarian cancer therapy. Knockouts of MSLN have been found to reverse EMT, significantly reduce tumor formation, metastasis, cell growth, and adhesion in lung epithelial and mesothelial cells whereas conversely, MSLN overexpression was linked with EMT in non-cancerous cells in-vivo [17].

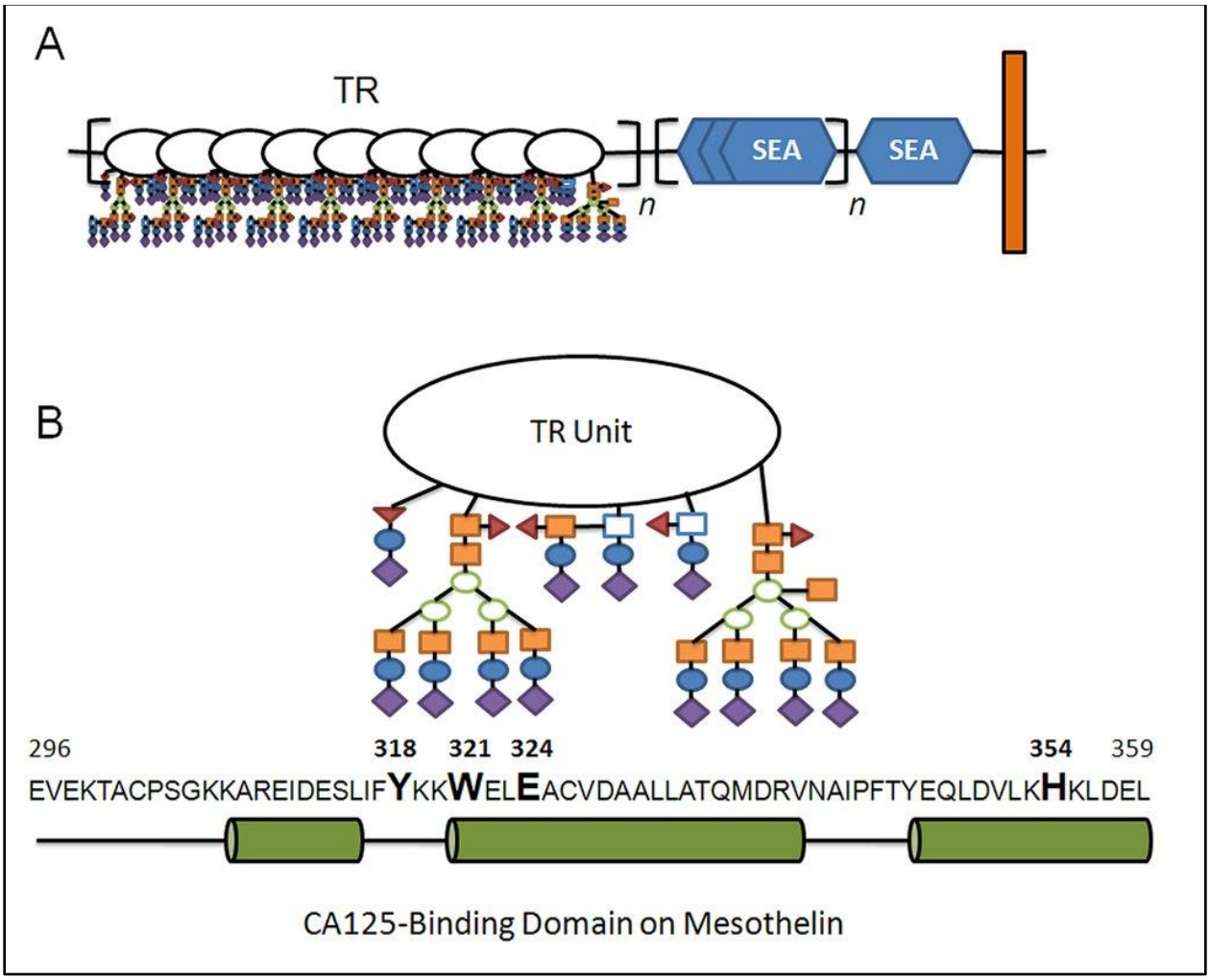

Fig.3 Interaction of MUC16 (CA125) and mesothelin [93].

Their study also demonstrated that MSLN expression regulates multiple EMT genes with the upregulation of 8 genes related to epithelial differentiation.

Acinetobacter baumannii association has been detected in breast cancer patients [18][19] and also in higher numbers in males with bladder cancer [20]. The presented study suggests the alleged role of Acinetobacter baumannii in driving the modulation of cancer prognosis by binding to mesothelin and hindering the migration, cell-cell adhesion, and metastasis property.

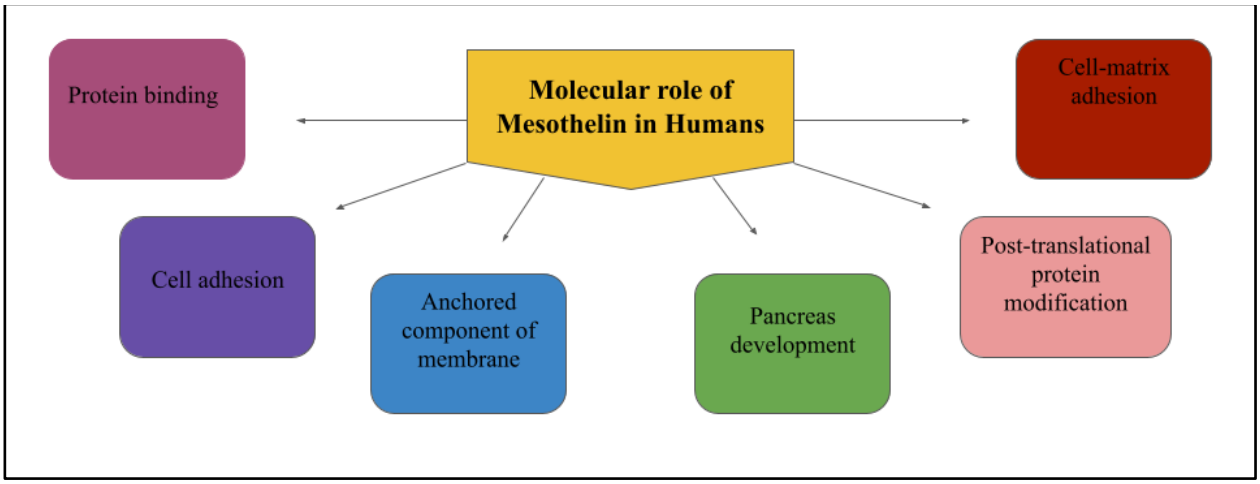

Fig.4 Molecular role of Mesothelin

CHAPTER 2. REVIEW OF LITERATURE

2.1 Introduction

The Microcosmos of organisms inhabiting our body affects various host physiological mechanisms and even modulates responsiveness to chemotherapy and immunotherapies. Even though the explicit mechanisms of these events are vaguely understood, a lot of effort is being made in this area to have a better stance on the role of microbes in nutrition and metabolism, disease physiology, CNS physiology and cognitive functioning, behavior, innate and adaptive immunity, and thus also aiding in our combat against cancer. The unique set of diverse species colonizing each individual provides us a distinct microbial fingerprint. Microbial specificity & balance are seen to be linked with various pathologies like cancer. Virchow first described tumor infiltration by leukocytes, which leads to inflammation [21]. This leukocyte infiltration was initially thought to indicate immune surveillance for tumor and antitumor responses of the immune system. It's apparent that microbes pose both pro-tumor and antitumor influences, yet the underlying intricacies are beginning to be realized. Gut microbes can cause inflammation, cause tumors to further oppose chemotherapeutic drugs, produce DNA-damaging toxins and carcinogenic metabolites, and modulate the body's anticancer immune responses. Our bodies consist of a thriving ecosystem of commensal bacteria, viruses, archaea, fungi, that command diverse physiological features along with aiding enzymatic ability. The term microbiota defines the ecological community of microorganisms in a particular environment. On the other hand, the microbiome focuses on all microorganisms' genomes in a specific environment [22]. The metagenome encodes for diverse metabolic processes and products, influencing the body's overall physiological and pathological state. Exploring the metagenome and the human microbiota became possible with the advent of advancements

in microscopy, culturing techniques, molecular biology technologies, and the high-throughput sequencing approaches that produce huge amounts of sensitive and informative DNA, RNA, protein, and metabolites' data of these microorganisms—telling about their basis of functioning in a complex environment. The human cells' ratio to that of bacterial cells in our bodies is closer to 1:1 with approximately 3.0 x 1013 human and 3.8 x 1013 bacterial cells. Human and microbial cells can operate as sensors for biological, physical, chemical, and environmental signals by detecting homeostatic alterations and remodeling the composition or role thereof through reciprocal communications. [23,24]. Microbiota shapes our immune system through stimulation and toleration of the commensals, which has largely evolved by means of the symbiotic relationship with these highly diverse and evolving microbes. [25]. Besides their contributory role in host homeostasis, various pathogens and commensals have been found to cause inflammation-induced cancer [26,27,28]. The immune system of our body recognizes and responds to microbial antigens and metabolites through modulation of the immune response. Various complex diseases have been found to be associated with microbial dysbiosis. This is majorly due to the effects of microbes on immunity, metabolism, inflammation, cellular proliferation, regulation of cancer progression through genetic variability, initiation, susceptibility to host immune activation, response to therapy, and comorbidity [29]. Among the examples of carcinogenesis initiated by the microbiome in the gut are Type IV carcinogenic secretions by Helicobacter pylori [30], adherence and intrusion to epithelial cells by F. nucleatum are succeeded by oncogenic and inflammatory effects, [31] and inflammation provoked by E. coli can modify the composition of the microbiota and promote carcinogenesis [32]. The microbiota performs a significant part in developing the organism's immune system.

2.2 Earliest pieces of evidence

The application of microbes in cancer therapeutics records back to the early nineties when Dr. William Coley originated a primordial bacterial microbe mixture becoming the father of immunotherapy and treating various types of cancer [33,34]. Virchow postulated 150 years ago that H.pylori and hepatitis C virus further advance cancer through inflammation and epithelial injury. [35]. Marshall and his mentor Robin Warren linked the bacterium to peptic ulcers, persistent inflammation, and stomach ailments including cancer, and acquired the Nobel Prize under Physiology or Medicine for their findings [36].

2.3 Identification & Screening methods

Sequencing and amplifying selective parts of the gene (16S rRNA) is the traditional method for characterizing the taxonomy of obscure communities of bacteria. 16S rRNA is a ubiquitously present 1.5 kb long gene which is a prokaryotic integral part of the small ribosomal subunit with a hypervariable region that aids in bacterial taxonomic classification [37]. Metagenomic shotgun sequencing, a less biased technique without a PCR amplification step, is also applied for microbe identification, although infrequent, to generate short-length reads describing the whole genomic content present in an environmental sample [38]. Detection of *F. nucleatum*, in colon adenomas and colon cancer at primary, distant metastasis sites, and even within tumors, has depended upon PCR amplification of nucleic acid. Microbes from patient-derived xenograft models and colon and liver cancer patients have been cultured [39].

2.4 Evidence of microbes in altering tumor progression

Our body contains as many microbes as human cells [23]. An association has been observed between the changes in microbial composition and in cases of altered physiological states like in the tumor microenvironment, with the cause and effect attributions between the two still being inconspicuous. Certain types of cancers are seen to be closely linked with specific bacterial microbes. A plethora of reports is now coming up stating some or the other bacterial associated with cancer. One of the biggest microcosms in humans resides in the gut with more than 1013 bacteria residing in the colon. *H. pylori* has been listed by IARC as a Class I carcinogen, which upon infection ultimately leads to gastric cancer [40] along with its substantial contribution to global cancer mortality [41]. It's worthy to note that the promotion of gastric cancer is an outcome associated with the union of various other microbes. Mice associated with only *H. pylori* have shown to develop fewer tumors compared to the pathogen-free hypergastrinemic transgenic mouse model [42]. Along with tumor-promoting effects of *H. pylori* in certain cases, its infection has been associated with lowered risk of esophageal adenocarcinoma in humans [43], signifying that the bacterial microbiota has altering effects in carcinogenesis on specific organs. Among other bacterial species known to promote carcinogenesis include Salmonella infection triggers pathogen-specific adaptive immune responses which cause MALT lymphomas and gallbladder cancer [35]. *H. pylori* reactive B cells and T helper cells show clonal expansion, and upon removal of *H. pylori*, regression thereof has been seen in Gastric MALT lymphoma. Likewise, infections with *Campylobacter jejuni*, *Chlamydia psittaci*, and *Borrelia burgdorferi* are linked with some lymphomas which generally relapse after treatment with antibiotics [35]. An extensive catalog describing the disease-microbe-related published information in a standardized way is available online called Disbiome [44]. There is insufficient information about the microbiota's role in initiating carcinogenesis at various other

organs having an abundant microbiome, like skin, lungs, female genital tract, and oral cavity. All the upcoming efforts and knowledge can prove to be a valuable asset toward our better understanding and gaining new insights into the disguised mechanisms.

2.5 Cancer modulation by the microbiota.

Shifts in the microbial community composition are termed “Dysbiosis”. Sometimes these changes may lead to a diseased condition. On various occasions, it has been observed that common dysbiosis of the gut microbiota contributes to colorectal carcinogenesis. Research done in Japan presented metagenomic and metabolomic studies on fecal samples revealing - phenotypes of the microbiota specific to the colorectal cancer (CRC) stage [45]. Another study showed that apigenin- a plant flavonoid modulated the microbiota of the gut thereby showing tumor inhibitory effects, furthermore on the depletion of these microbes, Apigenin was incompetent in decreasing the number/size of tumors [46]. A correlation between repeated exposure to antibiotics and tumor development has been portrayed in a study [47]. The liver is a prime example of indirect influences of microbes promoting cancer where intestinal dysbiosis aggravates liver cancer through the production of inflammation-causing microbe-associated molecular patterns (MAMPs) & microbial metabolites that arrive through the portal vein to the liver [48]. It is widely seen that MAMPs like LPS along with TLR4 cause inflammation and thus cancer. A variety of stances are taken by researchers explaining the dysbiotic mechanisms affecting tumorigenesis however overall & absolute insights are needed, and investigations are ongoing to adequately learn how carcinogenesis is affected by microbes.

2.6 Specific carcinogenic bacterial pathogens.

Pathogens that are specifically associated with cancer do not work in solitude, rather, it's an association of microbes that have a cumulative impact on immune function and genome stability. Bacteria like *E. coli* and *B. fragilis* operate cooperatively in tumor growth. These two microbes show colonic predominance in bodies genetically inclined to bowel cancer displaying patchy bacterial biofilms [49]. The microbiota associated with the tumor diverges from that of the normal mucosa furthermore, studies from preclinical models imply that polyp formation can be induced from the CRC patient's stool transplants which can alter the local immune environment and induce pro-carcinogenic signals in mice as contrasted with that from healthy controls [50]. Certain bacteria can excite an inflammatory condition which can elevate carcinogenesis via inhibiting actuation of the immune response [51] or through the initiation of toxins as generated by *Bacteroides fragilis*, [52,53]. enhanced production of ROS- reactive oxygen species [54], & modifications in tumor-immune microenvironment modulating signaling pathways as seen by *Fusobacterium nucleatum* within human and mouse tumor models [55]. Accumulation of certain bacteria promotes persistent inflammation, genetic alterations in principal inflammation-modulating genes which in turn elevate dysbiosis and thus cancer. Also, the production of genotoxic metabolites by certain bacteria in mice can induce carcinogenesis, for eg. E. coli generating colibactin or cytolethal distending toxin by Campylobacter jejuni. Finally, activation of the β-catenin–Wnt pathway by the FadA adhesion (FadAc) complex of F. nucleatum can result in oncogenic transcriptional changes in human colon cancer cell lines [59]. Reports show that Fusobacteria utilizes a fusobacterium lectin, Fap2 to recognize a polysaccharide (Gal-GalNAc) on the host cancerous cells to localize the tumors [56].

2.7 Tumor-Immune microenvironment & Microbes.

Cancer cells create a tumor microenvironment (TME) with the neighboring non-transformed cells, cancer-associated microbes, and the immune components. The composition of TME is an important determinant of the stage of cancer progression. The physiological state of the tumor microenvironment (TME) is intimately related to each stage of tumorigenesis i.e initiation, progression, and metastasis. The essential components of the TME include neuroendocrine cells, immune and inflammatory cells, myofibroblasts, and fibroblasts, blood and lymphatic vascular networks, adipose cells, and ECM [60]. Within the TME, tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), lymphocyte-derived cells like tumor-infiltrating lymphocytes (TILs), tumor-associated neutrophils (TANs), a network of cytokines, inflammatory mediators, matrix remodeling enzymes, and growth factors spur communication [57]. The immune system of our body consists of the two lines of defence innate arm of immune cells; constituting granulocytes (neutrophils, basophils, eosinophils, and mast cells), natural killer (NK) cells, phagocytes (macrophages and dendritic cells, DCs), and the complement system, and the adaptive arm of immune cells, comprising T-cells and B-cells which responds to extraneous invaders such as bacteria, parasites, and viruses, through the identification of non-self molecular patterns like “microbe-associated molecular patterns” (MAMP) “pathogen-associated molecular patterns” (PAMPs) or “damage-associated molecular patterns” (DAMPs), most of which are foreign or displaced carbohydrate chains that also aid in preventing autoimmunity [58]. A persistent state of inflammation is promoted by cancer with elevated ROS levels, growth factors, cytokines, and chemokines [59]. Extraneous pathogens and Senescent, dead, or dying cells are eliminated by patrolling leukocytes in the tissues to eradicate inflammation. In the situation of cell-mediated immunity, epitope-specific interaction between T-cell receptors and antigen-presenting cells (APCs) upon recognizing

antigen acts as “signal 1” in priming the naïve T-cell, which is authorized by the display of major histocompatibility (MHC)-antigen complex on their cell surface. After the establishment of the TCR engagement, signaling happens through the CD3 protein. Immunoreceptor tyrosine-based activation motifs (ITAMs) phosphorylation causes naïve T-cell activation following the reception of signal 1. The activation of naïve T-cells that haven’t previously encountered an antigen requires a response and additional signals from the APCs, only signal 1 is not sufficient [60]. Our body is equipped with a mechanism known as immune tolerance, which under normal physiological conditions prevents the immune system from attacking the gut inhabiting self-antigens (central tolerance), and antigens from the liver microbiome as well as food (peripheral tolerance), also for preventing the immune system from rejecting of the fetus [61]. Hyporesponsiveness of lymphocytes to antigens or subsequent inadequate CD28 co-stimulation leads to diminished proliferation and IL-2 production [62]. It has been observed that various commensals differ in their capacity to communicate with the immune components and modulate innate and adaptive immune signaling. Commensals like segmented filamentous bacteria have been reported to help in Th17 cell differentiation, polarize T cell responses, IgA production, provide barrier protection [63]. *Bacteroides fragilis* and *Clostridium* clusters are reported to cause Treg differentiation and functionalization [64]. Th1-type responses are seen to be induced from the capsule component of *B.fragilis* -Polysaccharide A (PSA), influencing the maturation and homeostasis of the immune system [65]. Frequencies concerning CD103+ dendritic cells, Treg, macrophages, pDC, and mononuclear phagocytes along with cytokine level has been observed at various immunological sites of the body upon mono-colonization of 52 different human commensals in germ-free mice [66].

2.8 Influence of microbiota on local and systemic immunity.

Microbes can directly affect the tumor site through intratumoral associations and also affect the tumor site indirectly through long-distance systemic effects. Microbial products travel through the circulation and contact different tissues and influence the progression of cancer there. For instance, microbes in the gut metabolize the bile acid released by the liver to mediate antitumor immunity in liver cancer [67]. The inner mucus layer of the intestine lacks bacteria and profoundly responses to microbial and immune-mediated signals where the epithelium of the intestine serves as a barrier, partitioning the lumen of the intestinal from the interior of the body. The epithelial cells constitute specialized secretory Goblet cells that release mucins which is the major mucus layer forming component above the epithelial cell layer furthermore, genetic insults in mucin drive colitis, symbolizing the necessary purpose of mucus in sustaining the physical barrier [28]. Early malignancy is aborted by killing virus-infected cells or the ones expressing tumor-associated antigens (TAAs) [69]. Another example shows that the risk of breast cancer is altered by the estrogen metabolized by the gut microbes [68,69]. The bacteria present in the tumor microenvironment are mostly intracellular which are specific to the tumor type and inhabit both immune and cancer cells [71]. A study analyzes the potential influence of viral antigens on anticancer immunosurveillance [72]. Perforin and granzyme A expression is correlated with high CTL expression in a variety of cancers [72]. Some endogenous retroviruses (ERVs) have been shown to drive stomach adenocarcinomas in immune-deficient mice moreover local infiltration of CTLs also reactivated ERV suggesting it to be a constituent of a class of TAAs [72,73]. A combination of the presence of TAAs i.e antigenicity and the extent of immune excitation i.e adjuvanticity determines the degree of susceptibility to immunosurveillance and immunogenicity of the cancer cells [74]. Thus, local or systemic alterations may tamper with the optimum state of the immune system and the microbiome, raising various clinical concerns.

2.9 Immune modulation and Tumorigenesis.

Among the numerous components of the immune system, some have positive while others have a negative impact on tumorigenesis. One recent study shows the integral role of complement C5aR1 in modulating the immune system and thereby abrogating colorectal tumorigenesis by the means of recruiting MDSCs through C5a/C5aR1 signaling into the inflamed colorectum thus undermining CD8+ T cells and the carcinogenic messengers causing colorectal tumorigenesis [75]. Fusobacterium nucleatum has been seen to increase tumor progression and multiplication by recruiting tumor-infiltrating myeloid cells [55]. It has been seen that the immune cells having repair-related roles like angiogenesis and tissue repair are tumor-promoting like M2 macrophages or Th-2 cell response, while those playing part in tissue damage have a tumor-suppressive role like M1 macrophages or Th-1 cell. Incidence of keratinocyte cancer or other barrier surface cancers imperiled to the microbiota are seen to be elevated in immunosuppressed recipients of organ transplants, which may be due to defective tumor immunosurveillance or variations in the microbiota composition at these sites [29].

2.10 Influence of inflammatory signaling on carcinogenesis.

The earliest shreds of evidence showing that inflammatory tumorigenesis can be induced by microbiota came from a study that showed that the TLR-signaling adaptor protein MYD88 contributes to cancer progression [76] furthermore suggesting that inflammation and cancer development regulation is necessitated by innate microbial sensing in the intestine. pro-inflammatory cytokines like IL-1, IL-6, TNF released by tumor-infiltrating myeloid cells also add to carcinogenesis by activating NF-κB and

STAT3 signaling in cancer cells leading to cell cycle progression and suppression of apoptosis [77]. Additionally, NF-κB and STAT3 signaling also provoke epithelial-mesenchymal transition (EMT) by undermining E-cadherin expression which is an epithelial differentiation marker [78].

2.11 Bacterial tumor-targeting mechanisms.

Various anaerobic bacteria like Bifidobacterium, Clostridium, Escherichia coli, Listeria, and Salmonella species, display natural tumor-targeting and killing behavior [84]. Bacteria tend to localize the tumor microenvironment after systemic administration via injection thereby causing different modifications in tumor-infiltrating immune cells, chemokines, and cytokines which have been seen to facilitate tumor regression. Connexin 43 (Cx43) can be upregulated via Salmonella-released toxins that can lead to the generation of gap junctions between the dendritic cells (DCs) and tumor, allowing for tumor antigen cross-presentation to the DCs. Extensive release of IL-1β, a proinflammatory cytokine, upon encountering bacterial components or tumor antigens thereby activating CD8+ T cells. Bacterial flagellin (a bacterial flagellum protein subunit) further stimulates the activated CD8+ T cells' antitumor response via TLR5 activation. Primary and metastatic tumor cells are efficiently killed by granzyme and perforin released by activated CD8+ T cells [84]. The activated CD8+ T cells' antitumor response is subsequently ameliorated as TLR5 and Flagellin signaling decreases the abundance of CD4+ CD25+ regulatory T (Treg) cells. The S. Typhimurium flagellin stimulates the release of interferon-γ from NK cells, an essential cytokine for overall immunity. MDSCs infected by Listeria produce excess IL-12 further magnifying the NK and CD8+ T cell responses. Both Clostridium and S. Typhimurium infection spurs significant accumulation of neutrophils. Intensified immune response and apoptosis are seen due to heightened secretion of TNF-related apoptosis-inducing ligand (TRAIL) and TNF-α by neutrophils.

Cancer by Salmonella shows elevated TNF-α and IL-1β secretion into the TME, and the inflammasome of macrophage is actuated by the contact with the components of bacteria like flagellin and LPS [79].

CHAPTER 3

3.1 METHODOLOGY

1. **Protein sequence retrieval and BLAST (Basic Local Alignment Search Tool)-** The protein sequence of tumor-associated antigen CA-125 were retrieved in FASTA format from the publicly available database at NCBI (http://www.ncbi.nlm.nih.gov). The sequence residues 12067-13939 with 12X approximate tandem repeats were subjected to the microbial protein BLAST with the intention of retrieving any hits consisting of cancer-associated microbial proteins. The BLAST was run with default parameters of an e-value threshold of 0.05.

2. **Protein Localization prediction-** The protein was subjected to subcellular localization prediction softwares to recognize surface, outer-membrane, or secreted proteins using the following localization prediction Softwares: Cello (http://cello.life.nctu.edu.tw/) [80], PSORTb (http://www.psort.org/psortb/index.html) [81], and Pslpred (http://www.imtech.res.in/raghava/submit.html) [82]. FASTA format of the protein sequences was provided as input, the organism type was chosen as 'Bacteria', Gram strain was selected as 'Negative' and the output format was selected as normal in the required field. These softwares utilize support vector machines (SVMs) classifiers which are trained to recognize various locations based on amino acid composition or proteins' physico-chemical properties.

3. **Homology Modelling**- Homology Modelling of the microbial protein was done using Modeller employing a multi template strategy to obtain improved quality and accurate models. First, the structure related target sequences were searched using the standard protein BLAST while choosing the search set database as Protein Data Bank (pdb). Multiple sequence alignment was performed using the default options of TCoffee Espresso

(http://tcoffee.crg.cat/apps/tcoffee/do:expressoo) [83] and ClustalW (https://embnet.vital-it.ch/software/ClustalW.html) [84].

4. **Model Refinement**- The loop regions generated in the structure were modelled using Loop modelling strategy in the Modeller. The structure was further refined using GalaxyWEB server (http://galaxy.seoklab.org/index.html) [85] to obtain accurate structural conformation. The generated model structures were visualized in PyMol software.

5. **Model Evaluation** was done using ERRAT (https://servicesn.mbi.ucla.edu/ERRAT/) [86] which is used to assess the "overall quality factor" for nonbonded atomic interactions, with higher scores indicating higher quality and PROCHECK (https://servicesn.mbi.ucla.edu/PROCHECK/) [87] which checks the geometry of each residue as well as the overall structure geometry.

6. **Protein allergenicity prediction**- The allergenicity of the protein was predicted using Algpred (https://webs.iiitd.edu.in/raghava/algpred/submission.html) [88] and ANTIGENpro (http://scratch.proteomics.ics.uci.edu/) [89] was used to predict the protein antigenicity and solubility.

7. **B-cell and T-cell epitopes prediction**- B cell epitope was predicted using ABCpred (https://webs.iiitd.edu.in/raghava/abcpred/ABC_submission.html) [90] using the target protein sequence in FASTA format as the input with a window length of 20 and the default threshold value of 0.5. IEDB server http://tools.iedb.org/main/ [91] was used for the prediction and analysis of T cell immune epitopes.

8. **Protein-protein docking-** ClusPro 2.0 server (https://cluspro.bu.edu) [92] was employed to carry out Protein-protein docking of the MUC16 & mesothelin and microbial peptide & mesothelin.

3.2 RESULTS

1. The MUC16 protein sequence residues from 12067-13939, consisting of 12X approximate tandem repeats, were subjected to the microbial protein BLAST due to indulgence of this portion with other ligands due to its presence on the outer membrane of the cell. The sequence shows homology with a 60 residues long SEA domain protein of Acinetobacter baumannii with an expect value of 4e-26, 84.21% identity, and 32% query cover.

2. The microbial proteins' structure was not available in the online repositories and therefore needed to be modelled in order to proceed with the binding studies. The modelled structure was subjected to loop modelling to fold the loop region in proper conformation further refinement was done using GalaxyWEB server to obtain accurate structural conformation as shown in Fig. 5**. a,b**.

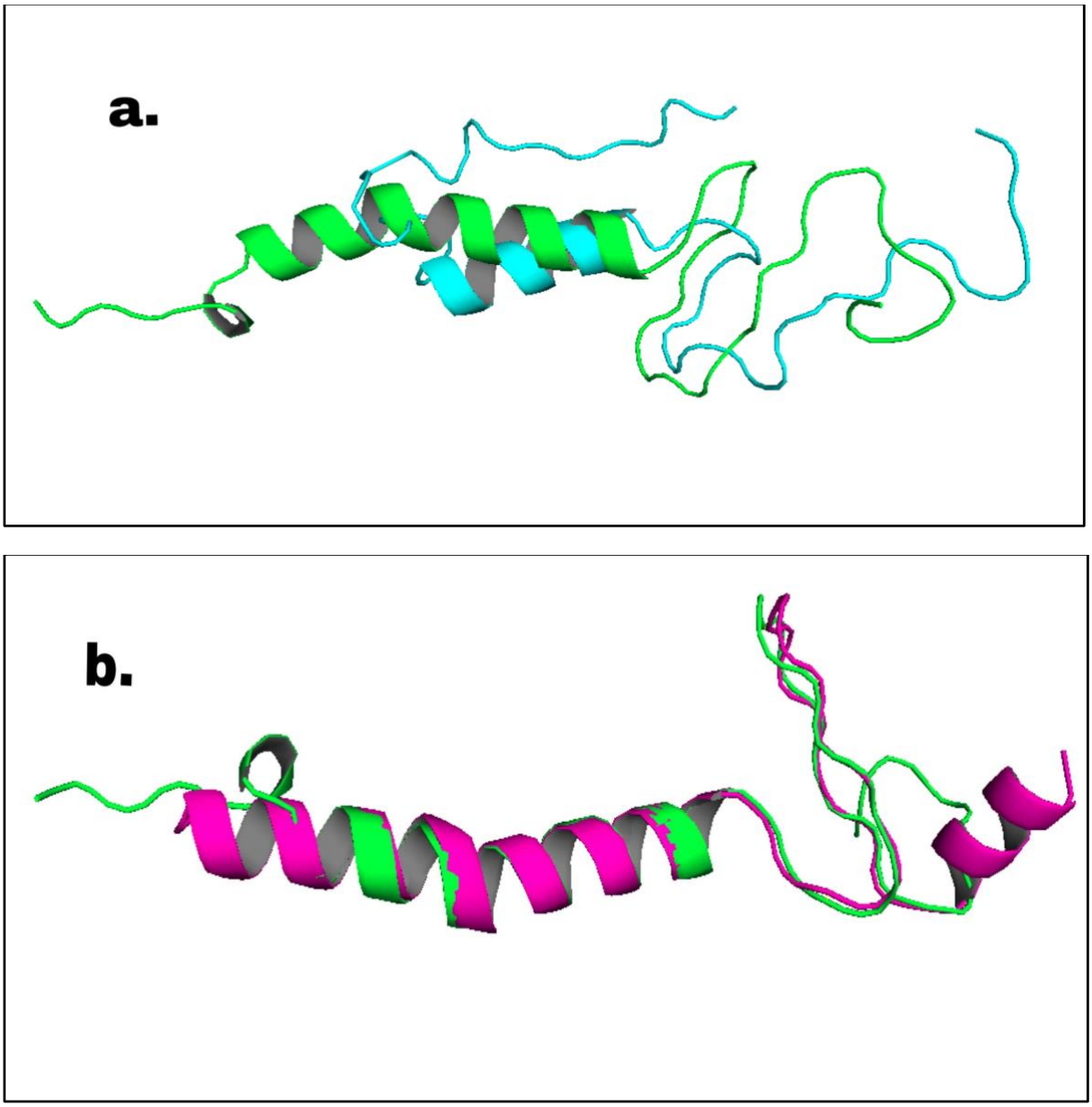

Fig.5 **a**, The structure before (cyan) and after (green) Loop modelling is shown. **b,** before(green) and after (magenta) Galaxy refinement.

3. The modelled protein structure was further validated using ERRAT showing 100% overall quality (Fig6 a). PROCHECK displayed the percentage of residues in the most favored region to be 92%,

where a good quality model would be expected to have over 90% residues in the most favored region (Fig6 b).

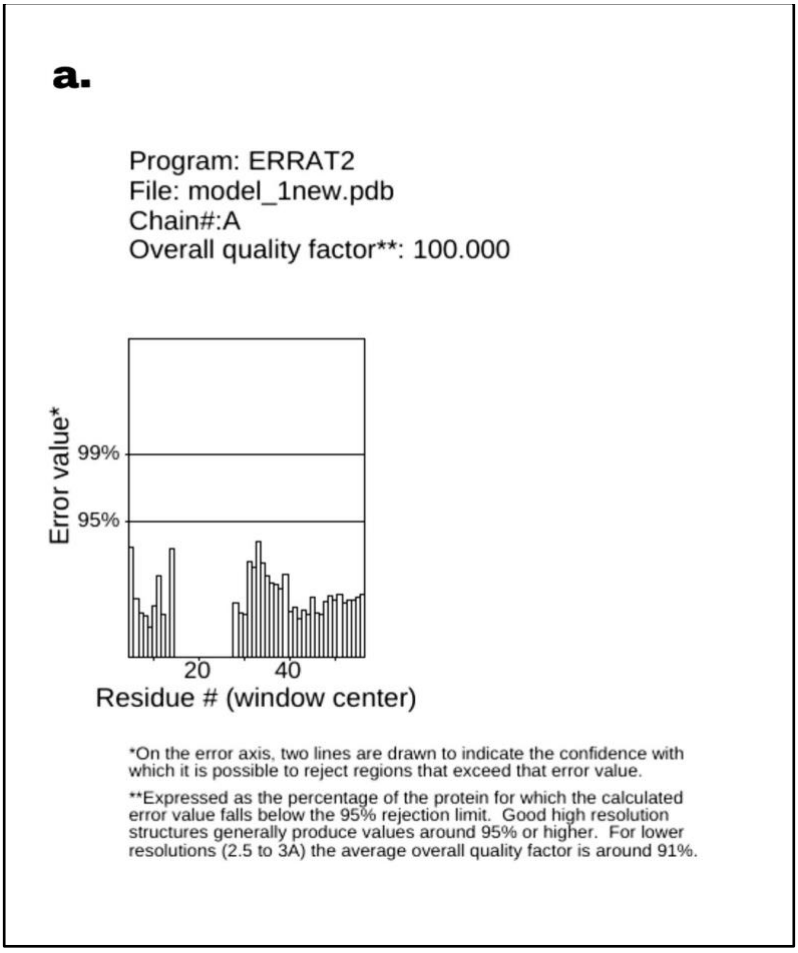

Fig.6 **a**, ERRAT results showing an overall quality factor of 100 for the *Acinetobacter baumannii* SEA-domain containing protein.

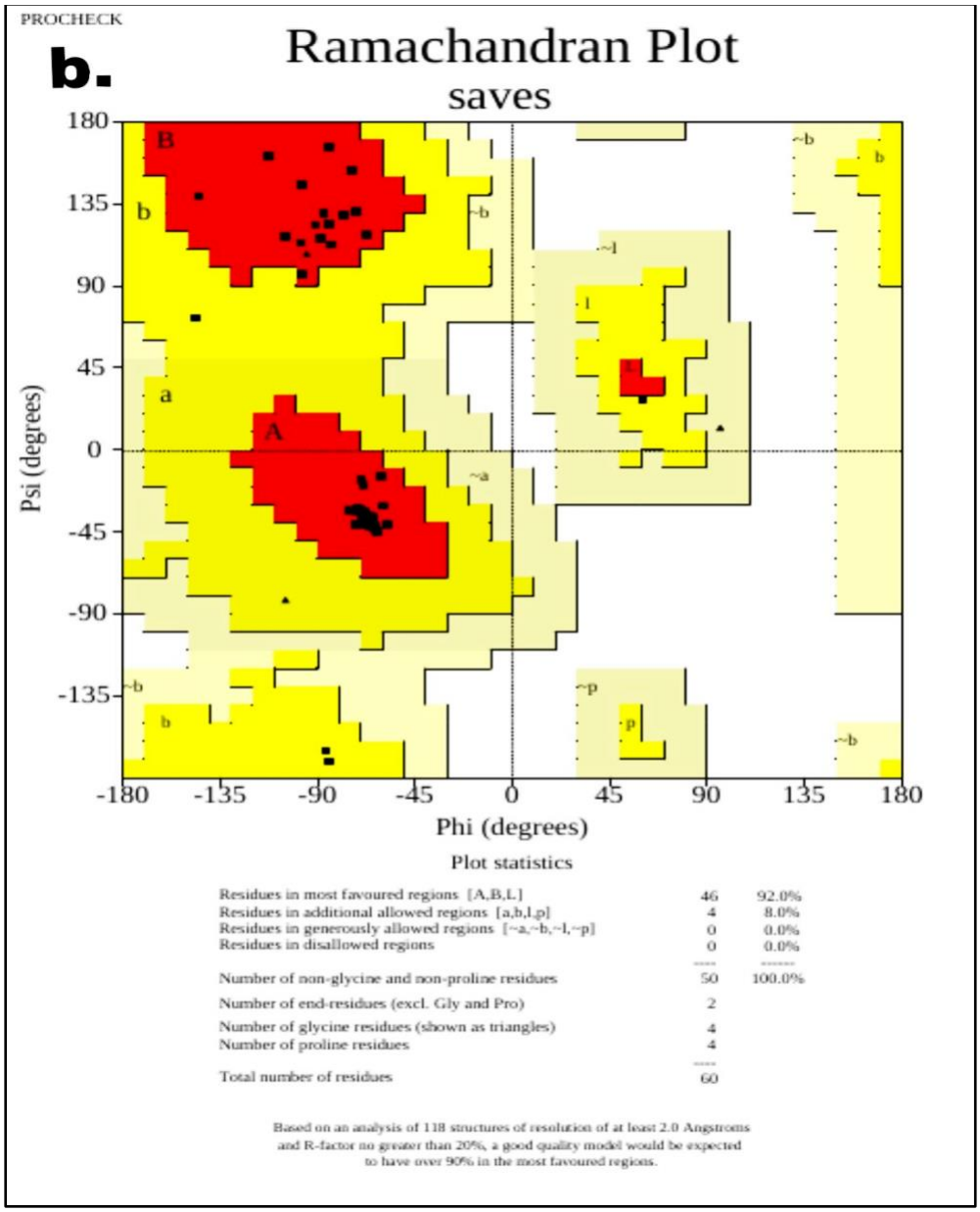

Fig.6 **b**, PROCHECK result displaying the Ramachandran plot having 92% residues in the most favoured region for the *Acinetobacter baumannii* SEA-domain containing protein.

4. Protein-protein docking between the modelled microbial protein and the mesothelin structure obtained from PDB was done using ClusPro 2.0 server. Out of the 12 generated models the best model was selected with the highes number of interacting members and minimum energy.

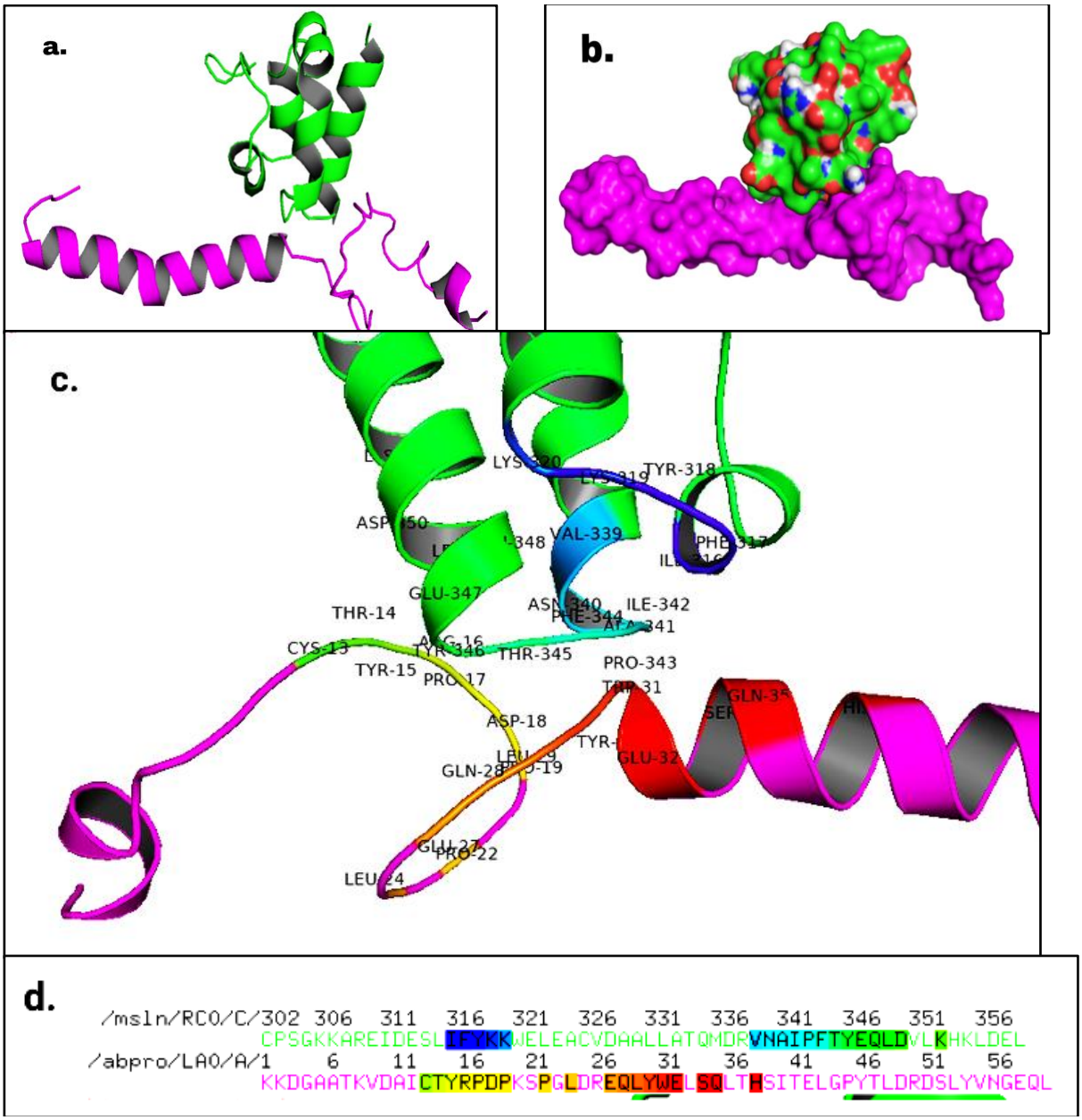

Fig.7 **a, b** Docking of Mesothelin and SEA-domain containing protein in *Acinetobacter baumannii*; **c, d** highlighting the residues participating in binding shown as labeled residues in the image and color-coded in the sequence below.

Cluster	Members	Representative	Weighted scores

0	212	Center	-624.0
		Lowest Energy	-703.8
1	145	Center	-586.2
		Lowest Energy	-642.4
2	116	Center	-599.7
		Lowest Energy	-689.2
3	108	Center	-581.9
		Lowest Energy	-669.9
4	81	Center	-582.3
		Lowest Energy	-714.0
5	81	Center	-606.5
		Lowest Energy	-643.1
6	59	Center	-564.4
		Lowest Energy	-663.3
7	55	Center	-591.9
		Lowest Energy	-681.5
8	47	Center	-651.1
		Lowest Energy	-651.1
9	28	Center	-566.0
		Lowest Energy	-639.5
10	25	Center	-640.4
		Lowest Energy	-640.4
11	13	Center	-626.4
		Lowest Energy	-626.4
12	7	Center	-565.1
		Lowest Energy	-589.2

Table 1. ClusPro protein-protein docking scores

5. Allergenicity of the protein was assessed using Algpred to be as “non-allergen”. ANTIGENpro predicted the protein antigenicity to be 0.548 and solubility to be 0.838.

6. B-cell and T-cell epitopes prediction was done using the ABCpred and IEDB respectively. The portion of microbial protein showing homology to MUC16 was used as input to find out the epitope region showing reactivity to B-cells and T-cells. Top 5 epitopes for each are displayed below.

Rank	Sequence	Start position	Score
1	WELSQLTHSITELGPYTLDR	31	0.89
2	ICTYRPDPKSPGLDREQLYW	12	0.84
3	SITELGPYTLDRDSLYVNGE	39	0.76
4	KSPGLDREQLYWELSQLTHS	20	0.74

Table 2. B cell epitopes

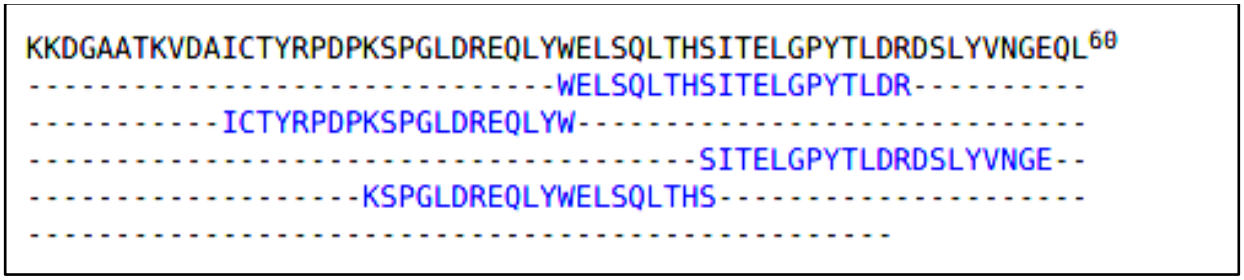

Fig. 8 Overlap view of B cell epitope regions

Allele	#	Start	End	Length	Peptide	Score	Percentile rank
HLA-B*07:02	1	16	24	9	RPDPKSPGL	0.983117	0.01
HLA-A*01:01	1	46	54	9	YTLDRDSLY	0.939352	0.02
HLA-B*07:02	1	21	29	9	SPGLDREQL	0.816152	0.08
HLA-A*02:03	1	28	36	9	QLYWELSQL	0.804165	0.05
HLA-A*26:01	1	46	54	9	YTLDRDSLY	0.764816	0.05

Table 3. MHC I Cytotoxic-T cell epitope

Allele	#	Start	End	Length	Peptide	Percentile rank
HLA-DQA1*01:01/DQB1*05:01	1	23	37	15	GLDREQLYWELSQLT	0.33
HLA-DQA1*01:01/DQB1*05:01	1	24	38	15	LDREQLYWELSQLTH	0.33
HLA-DQA1*01:01/DQB1*05:01	1	25	39	15	DREQLYWELSQLTHS	0.38

HLA-DQA1*01:01/DQB1*05:01	1	26	40	15	REQLYWELSQLTHSI	0.48
HLA-DRB3*01:01	1	42	56	15	ELGPYTLDRDSLYVN	0.61

Table 4. MHC II T-helper cell epitope

3.3 DISCUSSION

We have inched a bit closer in understanding how the immune system is activated by the microbial presence helping in the generation of an antitumor response and how some of them also trigger the immune system in eliciting a pro-tumor condition. However, all that we know is just the tip of the iceberg and a lot yet remains to be unraveled such as the specific immune pathways triggered by distinctive cancerous microbes with respect to the natural flora found at different tumor sites in the body, whether tumorigenesis is a result of dysbiosis or vice-versa, the influence of microbiota on different stages of carcinogenesis, pinpointing specific dysbiosis-triggering factors and how we can control it through natural means. Finding concrete connections with particular microbes and their cancer-causing potential while determining the principal virulence factors can significantly revolutionize epitope-based vaccine therapeutics for cancer, especially preventing drug resistance which accounts for a large percentage of failures in cancer therapies. More attention should be drawn towards the putative role of normal microflora in fine-tuning the immune system and regulating its potential in preventing tumorigenesis and tumor metastasis, thus resulting in more successful applications in developing combinatorial cancer therapies. The role of nutrigenomics and modulation of normal microflora as an active combinatorial strategy in cancer therapeutics also needs to be explored further.

3.4 CONCLUSION

By the means of this study, the possible role and association of Acinetobacter baumannii with cancer can be deduced by concluding that Acinetobacter baumannii can interfere with mesothelin and alter the cancer prognosis by putatively causing decreased migration of cells, reversing EMT, reducing tumor formation, metastasis, cell growth, and limiting adhesion in lung epithelial; which is otherwise seen to be opposed with mesothelin overexpression. As displayed in the results the mesothelin binding site to MUC16 is showing binding with the acinetobacter hypothetical protein it can be suggested that the presence of Acinetobacter baumannii bacteria possibly limits the ability of mesothelin to interact with MUC16 and thereby abrogate cancer. As a matter of future prospects, it should be taken into extra attention that further affirming immunohistochemical studies need to be performed to validate these findings.

3.5 REFERENCES

1. Moore, Patrick S, and Yuan Chang. “Why do viruses cause cancer? Highlights of the first century of human tumour virology.” Nature reviews. Cancer vol. 10,12 (2010): 878-89. doi:10.1038/nrc2961

2. Polk, D Brent, and Richard M Peek Jr. “Helicobacter pylori: gastric cancer and beyond.” Nature reviews. Cancer vol. 10,6 (2010): 403-14. doi:10.1038/nrc2857

3. Samaras, Vassilis et al. “Chronic bacterial and parasitic infections and cancer: a review.” Journal of infection in developing countries vol. 4,5 267-81. 3 Jun. 2010, doi:10.3855/jidc.819

4. Dzutsev, Amiran, et al. “Microbes and Cancer.” Annual Review of Immunology, vol. 35, no. 1, 2017, pp. 199–228.

5. de Martel, C., Ferlay, J., Franceschi, S., Vignat, J., Bray, F., Forman, D., & Plummer, M. (2012). Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. The Lancet. Oncology, 13(6), 607–615. https://doi.org/10.1016/S1470-2045(12)70137-7

6. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: a cancer journal for clinicians, 71(3), 209–249. https://doi.org/10.3322/caac.21660

7. Nejman, Deborah et al. “The human tumor microbiome is composed of tumor type-specific intracellular bacteria.” Science (New York, N.Y.) vol. 368,6494 (2020): 973-980. doi:10.1126/science.aay9189

8. Hatakeyama, Masanori. “Oncogenic mechanisms of the Helicobacter pylori CagA protein.” Nature reviews. Cancer vol. 4,9 (2004): 688-94. doi:10.1038/nrc1433

9. Rubinstein, Mara Roxana et al. “Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin.” Cell host & microbe vol. 14,2 (2013): 195-206. doi:10.1016/j.chom.2013.07.012

10. Arthur, Janelle C et al. “Intestinal inflammation targets cancer-inducing activity of the microbiota.” Science (New York, N.Y.) vol. 338,6103 (2012): 120-3. doi:10.1126/science.1224820

11. Patankar, M. S., Gubbels, J. A., Felder, M., & Connor, J. P. (2012). The immunomodulating roles of glycoproteins in epithelial ovarian cancer. Frontiers in bioscience (Elite edition), 4, 631–650. https://doi.org/10.2741/405

12. Ponnusamy, M. P., Seshacharyulu, P., Lakshmanan, I., Vaz, A. P., Chugh, S., & Batra, S. K. (2013). Emerging role of mucins in epithelial to mesenchymal transition. Current cancer drug targets, 13(9), 945–956. https://doi.org/10.2174/15680096113136660100

13. Giamougiannis, P., Martin-Hirsch, P. L., & Martin, F. L. (2021). The evolving role of MUC16 (CA125) in the transformation of ovarian cells and the progression of neoplasia. Carcinogenesis, 42(3), 327–343. https://doi.org/10.1093/carcin/bgab010

14. Comamala, M., Pinard, M., Thériault, C. et al. Downregulation of cell surface CA125/MUC16 induces epithelial-to-mesenchymal transition and restores EGFR signalling in NIH:OVCAR3 ovarian carcinoma cells. Br J Cancer 104, 989–999 (2011). https://doi.org/10.1038/bjc.2011.34

15. Chen, X., Li, X., Wang, X. et al. MUC16 impacts tumor proliferation and migration through cytoplasmic translocation of P120-catenin in epithelial ovarian cancer cells: an original research. BMC Cancer 19, 171 (2019). https://doi.org/10.1186/s12885-019-5371-4

16. Huo, Q., Xu, C., Shao, Y., Yu, Q., Huang, L., Liu, Y., & Bao, H. (2021). Free CA125 promotes ovarian cancer cell migration and tumor metastasis by binding Mesothelin to reduce DKK1

expression and activate the SGK3/FOXO3 pathway. International journal of biological sciences, 17(2), 574–588. https://doi.org/10.7150/ijbs.52097

17. He, X., Wang, L., Riedel, H., Wang, K., Yang, Y., Dinu, C. Z., & Rojanasakul, Y. (2017). Mesothelin promotes epithelial-to-mesenchymal transition and tumorigenicity of human lung cancer and mesothelioma cells. Molecular cancer, 16(1), 63. https://doi.org/10.1186/s12943-017-0633-8

18. Thompson, K. J., Ingle, J. N., Tang, X., Chia, N., Jeraldo, P. R., Walther-Antonio, M. R., Kandimalla, K. K., Johnson, S., Yao, J. Z., Harrington, S. C., Suman, V. J., Wang, L., Weinshilboum, R. L., Boughey, J. C., Kocher, J. P., Nelson, H., Goetz, M. P., & Kalari, K. R. (2017). A comprehensive analysis of breast cancer microbiota and host gene expression. PloS one, 12(11), e0188873. https://doi.org/10.1371/journal.pone.0188873

19. Parida, S., & Sharma, D. (2019). The Microbiome-Estrogen Connection and Breast Cancer Risk. Cells, 8(12), 1642. https://doi.org/10.3390/cells8121642

20. Wu, P., Zhang, G., Zhao, J., Chen, J., Chen, Y., Huang, W., Zhong, J., & Zeng, J. (2018). Profiling the Urinary Microbiota in Male Patients With Bladder Cancer in China. Frontiers in cellular and infection microbiology, 8, 167. https://doi.org/10.3389/fcimb.2018.00167

21. Virchow, R. “An Address on the Value of Pathological Experiments.” British medical journal vol. 2,1075 (1881): 198-203. doi:10.1136/bmj.2.1075.198

22. Flandroy, Lucette et al. “The impact of human activities and lifestyles on the interlinked microbiota and health of humans and of ecosystems.” The Science of the total environment vol. 627 (2018): 1018-1038. doi:10.1016/j.scitotenv.2018.01.288
23. Sender, Ron et al. “Revised Estimates for the Number of Human and Bacteria Cells in the Body.” PLoS biology vol. 14,8 e1002533. 19 Aug. 2016, doi:10.1371/journal.pbio.1002533
24. Candela, Marco et al. “Intestinal microbiota is a plastic factor responding to environmental changes.” Trends in microbiology vol. 20,8 (2012): 385-91. doi:10.1016/j.tim.2012.05.003
25. Belkaid, Yasmine, and Timothy W Hand. “Role of the microbiota in immunity and inflammation.” Cell vol. 157,1 (2014): 121-41. doi:10.1016/j.cell.2014.03.011
26. Moore, Patrick S, and Yuan Chang. “Why do viruses cause cancer? Highlights of the first century of human tumour virology.” Nature reviews. Cancer vol. 10,12 (2010): 878-89. doi:10.1038/nrc2961
27. Polk, D Brent, and Richard M Peek Jr. “Helicobacter pylori: gastric cancer and beyond.” Nature reviews. Cancer vol. 10,6 (2010): 403-14. doi:10.1038/nrc2857
28. Samaras, Vassilis et al. “Chronic bacterial and parasitic infections and cancer: a review.” Journal of infection in developing countries vol. 4,5 267-81. 3 Jun. 2010, doi:10.3855/jidc.819
29. Dzutsev, Amiran, et al. “Microbes and Cancer.” Annual Review of Immunology, vol. 35, no. 1, 2017, pp. 199–228.
30. Hatakeyama, Masanori. “Oncogenic mechanisms of the Helicobacter pylori CagA protein.” Nature reviews. Cancer vol. 4,9 (2004): 688-94. doi:10.1038/nrc1433
31. Rubinstein, Mara Roxana et al. “Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling via its FadA adhesin.” Cell host & microbe vol. 14,2 (2013): 195-206. doi:10.1016/j.chom.2013.07.012

32. Arthur, Janelle C et al. “Intestinal inflammation targets cancer-inducing activity of the microbiota.” Science (New York, N.Y.) vol. 338,6103 (2012): 120-3. doi:10.1126/science.1224820

33. Łukasiewicz, Klaudia, and Marek Fol. “Microorganisms in the Treatment of Cancer: Advantages and Limitations.” Journal of immunology research vol. 2018 2397808. 27 Feb. 2018, doi:10.1155/2018/2397808

34. J. Bickels, Y. Kollender, O. Merinsky, and I. Meller, “Coley’s toxin: historical perspective,” Israel Medical Association Journal, vol. 4, no. 6, pp. 471-472, 2002.

35. Schwabe, Robert F, and Christian Jobin. “The microbiome and cancer.” Nature reviews. Cancer vol. 13,11 (2013): 800-12. doi:10.1038/nrc3610

36. Marshall, B J, and J R Warren. “Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration.” Lancet (London, England) vol. 1,8390 (1984): 1311-5. doi:10.1016/s0140-6736(84)91816-6

37. Woese, C R, and G E Fox. “Phylogenetic structure of the prokaryotic domain: the primary kingdoms.” Proceedings of the National Academy of Sciences of the United States of America vol. 74,11 (1977): 5088-90. doi:10.1073/pnas.74.11.5088

38. Fessler, Jessica et al. “Exploring the emerging role of the microbiome in cancer immunotherapy.” Journal for immunotherapy of cancer vol. 7,1 108. 17 Apr. 2019, doi:10.1186/s40425-019-0574-4

39. Helmink, Beth A, et al. “The microbiome, cancer, and cancer therapy.” Nature medicine vol. 25,3 (2019): 377-388. doi:10.1038/s41591-019-0377-7

40. Asaka, Masahiro, et al. “Gastric Cancer.” Helicobacter pylori: Physiology and Genetics, edited by Harry LT Mobley et. al., ASM Press, 2001.

41. Fox, James G, and Timothy C Wang. “Inflammation, atrophy, and gastric cancer.” The Journal of clinical investigation vol. 117,1 (2007): 60-9. doi:10.1172/JCI30111

42. Lofgren, Jennifer L et al. “Lack of commensal flora in Helicobacter pylori-infected INS-GAS mice reduces gastritis and delays intraepithelial neoplasia.” Gastroenterology vol. 140,1 (2011): 210-20. doi:10.1053/j.gastro.2010.09.048

43. Islami, Farhad, and Farin Kamangar. “Helicobacter pylori and esophageal cancer risk: a meta-analysis.” Cancer prevention research (Philadelphia, Pa.) vol. 1,5 (2008): 329-38. doi:10.1158/1940-6207.CAPR-08-0109

44. Janssens, Yorick, et al. “Disbiome database: linking the microbiome to disease.” BMC microbiology vol. 18,1 50. 4 Jun. 2018, doi:10.1186/s12866-018-1197-5

45. Mizutani, Sayaka et al. “Significance of the gut microbiome in multistep colorectal carcinogenesis.” Cancer science vol. 111,3 (2020): 766-773. doi:10.1111/cas.14298

46. Shichang, Wan, et al. "Inhibitory Effects of Apigenin on Tumor Carcinogenesis by Altering the Gut Microbiota", Mediators of Inflammation, vol. 2020, Article ID 7141970, 9 pages, 2020. doi:10.1155/2020/7141970

47. Boursi, Ben et al. “Recurrent antibiotic exposure may promote cancer formation--Another step in understanding the role of the human microbiota?.” European journal of cancer (Oxford, England: 1990) vol. 51,17 (2015): 2655-64. doi:10.1016/j.ejca.2015.08.015

48. Etienne-Mesmin, Lucie et al. “Hepatocyte Toll-Like Receptor 5 Promotes Bacterial Clearance and Protects Mice Against High-Fat Diet-Induced Liver Disease.” Cellular and molecular gastroenterology and hepatology vol. 2,5 584-604. 5 May. 2016, doi:10.1016/j.jcmgh.2016.04.007

49. Dolgin, Elie. “Fighting cancer with microbes.” Nature vol. 577,7792 (2020): S16-S18. doi:10.1038/d41586-020-00199-x

50. Wong, Sunny H et al. “Gavage of Fecal Samples From Patients With Colorectal Cancer Promotes Intestinal Carcinogenesis in Germ-Free and Conventional Mice.” Gastroenterology vol. 153,6 (2017): 1621-1633.e6. doi:10.1053/j.gastro.2017.08.022

51. Gur, Chamutal et al. “Binding of the Fap2 protein of Fusobacterium nucleatum to human inhibitory receptor TIGIT protects tumors from immune cell attack.” Immunity vol. 42,2 (2015): 344-355. doi:10.1016/j.immuni.2015.01.010

52. Wu, Shaoguang et al. “A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses.” Nature medicine vol. 15,9 (2009): 1016-22. doi:10.1038/nm.2015

53. Boleij, Annemarie et al. “The Bacteroides fragilis toxin gene is prevalent in the colon mucosa of colorectal cancer patients.” Clinical infectious diseases: an official publication of the Infectious Diseases Society of America vol. 60,2 (2015): 208-15. doi:10.1093/cid/ciu787

54. Mangerich, Aswin et al. “Infection-induced colitis in mice causes dynamic and tissue-specific changes in stress response and DNA damage leading to colon cancer.” Proceedings of the National Academy of Sciences of the United States of America vol. 109,27 (2012): E1820-9. doi:10.1073/pnas.1207829109

55. Kostic, Aleksandar D et al. “Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment.” Cell host & microbe vol. 14,2 (2013): 207-15. doi:10.1016/j.chom.2013.07.007

56. Abed, Jawad et al. “Fap2 Mediates Fusobacterium nucleatum Colorectal Adenocarcinoma Enrichment by Binding to Tumor-Expressed Gal-GalNAc.” Cell host & microbe vol. 20,2 (2016): 215-25. doi:10.1016/j.chom.2016.07.006
57. Wang, Maonan et al. “Role of tumor microenvironment in tumorigenesis.” Journal of Cancer vol. 8,5 761-773. 25 Feb. 2017, doi:10.7150/jca.17648
58. Baghban, Roghayyeh et al. “Tumor microenvironment complexity and therapeutic implications at a glance.” Cell communication and signaling: CCS vol. 18,1 59. 7 Apr. 2020, doi:10.1186/s12964-020-0530-4
59. Macpherson, Anne M et al. “Epithelial Ovarian Cancer and the Immune System: Biology, Interactions, Challenges and Potential Advances for Immunotherapy.” Journal of clinical medicine vol. 9,9 2967. 14 Sep. 2020, doi:10.3390/jcm9092967
60. Driessens, Gregory et al. “Costimulatory and coinhibitory receptors in anti-tumor immunity.” Immunological reviews vol. 229,1 (2009): 126-44. doi:10.1111/j.1600-065X.2009.00771.x
61. Barnet, Megan B et al. “Understanding Immune Tolerance of Cancer: Re-Purposing Insights from Fetal Allografts and Microbes.” BioEssays: news and reviews in molecular, cellular and developmental biology vol. 40,8 (2018): e1800050. doi:10.1002/bies.201800050
62. Crespo, Joel et al. “T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment.” Current opinion in immunology vol. 25,2 (2013): 214-21. doi:10.1016/j.coi.2012.12.003
63. Flannigan, Kyle L, and Timothy L Denning. “Segmented filamentous bacteria-induced immune responses: a balancing act between host protection and autoimmunity.” Immunology, vol. 154,4 537–546. 17 May. 2018, doi:10.1111/imm.12950

64. Cheng, Hongyu et al. “The Th17/Treg Cell Balance: A Gut Microbiota-Modulated Story.” Microorganisms vol. 7,12 583. 20 Nov. 2019, doi:10.3390/microorganisms7120583

65. Troy, Erin B, and Dennis L Kasper. “Beneficial effects of Bacteroides fragilis polysaccharides on the immune system.” Frontiers in bioscience (Landmark edition) vol. 15 25-34. 1 Jan. 2010, doi:10.2741/3603

66. Geva-Zatorsky, Naama et al. “Mining the Human Gut Microbiota for Immunomodulatory Organisms.” Cell vol. 168,5 (2017): 928-943.e11. doi:10.1016/j.cell.2017.01.022

67. Ma, Chi et al. “Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells.” Science (New York, N.Y.) vol. 360,6391 (2018): eaan5931. doi:10.1126/science.aan5931

68. Kwa, Maryann et al. “The Intestinal Microbiome and Estrogen Receptor-Positive Female Breast Cancer.” Journal of the National Cancer Institute vol. 108,8 djw029. 22 Apr. 2016, doi:10.1093/jnci/djw029

69. Baker, James M et al. “Estrogen-gut microbiome axis: Physiological and clinical implications.” Maturitas vol. 103 (2017): 45-53. doi:10.1016/j.maturitas.2017.06.025

70. Vesely, Matthew D, et al. “Natural innate and adaptive immunity to cancer.” Annual review of immunology vol. 29 (2011): 235-71. doi:10.1146/annurev-immunol-031210-101324

71. Nejman, Deborah et al. “The human tumor microbiome is composed of tumor type-specific intracellular bacteria.” Science (New York, N.Y.) vol. 368,6494 (2020): 973-980. doi:10.1126/science.aay9189

72. Rooney, Michael S et al. “Molecular and genetic properties of tumors associated with local immune cytolytic activity.” Cell vol. 160,1-2 (2015): 48-61. doi:10.1016/j.cell.2014.12.033

73. Young, George R et al. “Resurrection of endogenous retroviruses in antibody-deficient mice.” Nature vol. 491,7426 (2012): 774-8. doi:10.1038/nature11599

74. Kroemer, Guido et al. “Natural and therapy-induced immunosurveillance in breast cancer.” Nature medicine vol. 21,10 (2015): 1128-38. doi:10.1038/nm.3944

75. Ding, Peipei, et al. “C5aR1 is a master regulator in Colorectal Tumorigenesis via Immune modulation.” Theranostics vol. 10,19 8619-8632. 9 Jul. 2020, doi:10.7150/thno.45058

76. Rakoff-Nahoum, Seth, and Ruslan Medzhitov. “Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88.” Science (New York, N.Y.) vol. 317,5834 (2007): 124-7. doi:10.1126/science.1140488

77. Yu, Hua et al. “STATs in cancer inflammation and immunity: a leading role for STAT3.” Nature reviews. Cancer vol. 9,11 (2009): 798-809. doi:10.1038/nrc2734

78. Sullivan, N J et al. “Interleukin-6 induces an epithelial-mesenchymal transition phenotype in human breast cancer cells.” Oncogene vol. 28,33 (2009): 2940-7. doi:10.1038/onc.2009.180

79. Duong, Mai Thi-Quynh et al. “Bacteria-cancer interactions: bacteria-based cancer therapy.” Experimental & molecular medicine vol. 51,12 1-15. 11 Dec. 2019, doi:10.1038/s12276-019-0297-0

80. Yu, C. S., Lin, C. J., & Hwang, J. K. (2004). Predicting subcellular localization of proteins for Gram-negative bacteria by support vector machines based on n-peptide compositions. Protein science : a publication of the Protein Society, 13(5), 1402–1406. https://doi.org/10.1110/ps.03479604

81. Yu, N. Y., Wagner, J. R., Laird, M. R., Melli, G., Rey, S., Lo, R., Dao, P., Sahinalp, S. C., Ester, M., Foster, L. J., & Brinkman, F. S. (2010). PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. Bioinformatics (Oxford, England), 26(13), 1608–1615. https://doi.org/10.1093/bioinformatics/btq249

82. Bhasin, M., Garg, A., & Raghava, G. P. (2005). PSLpred: prediction of subcellular localization of bacterial proteins. Bioinformatics (Oxford, England), 21(10), 2522–2524. https://doi.org/10.1093/bioinformatics/bti309

83. Thompson S.M. (2009) An Introduction to Multiple Sequence Alignment — and the T-Coffee Shop. Beyond Just Aligning Sequences: How Good can you Make your Alignment, and so What?. In: Krawetz S. (eds) Bioinformatics for Systems Biology. Humana Press. https://doi.org/10.1007/978-1-59745-440-7_15

84. M.A. Larkin, G. Blackshields, N.P. Brown, R. Chenna, P.A. McGettigan, H. McWilliam, F. Valentin, I.M. Wallace, A. Wilm, R. Lopez, J.D. Thompson, T.J. Gibson, D.G. Higgins, Clustal W and Clustal X version 2.0, Bioinformatics, Volume 23, Issue 21, 1 November 2007, Pages 2947–2948, https://doi.org/10.1093/bioinformatics/btm404

85. Junsu Ko, Hahnbeom Park, Lim Heo, Chaok Seok, GalaxyWEB server for protein structure prediction and refinement, Nucleic Acids Research, Volume 40, Issue W1, 1 July 2012, Pages W294–W297, https://doi.org/10.1093/nar/gks493

86. Colovos, C., & Yeates, T. O. (1993). Verification of protein structures: patterns of nonbonded atomic interactions. Protein science : a publication of the Protein Society, 2(9), 1511–1519. https://doi.org/10.1002/pro.5560020916

87. Laskowski, R.A., MacArthur, M.W., Moss, D.S. and Thornton, J.M. (1993), PROCHECK: a program to check the stereochemical quality of protein structures. J. Appl. Cryst., 26: 283-291. https://doi.org/10.1107/S0021889892009944

88. Saha, S., & Raghava, G. P. (2006). AlgPred: prediction of allergenic proteins and mapping of IgE epitopes. Nucleic acids research, 34(Web Server issue), W202–W209. https://doi.org/10.1093/nar/gkl343

89. Christophe N. Magnan, Michael Zeller, Matthew A. Kayala, Adam Vigil, Arlo Randall, Philip L. Felgner, Pierre Baldi, High-throughput prediction of protein antigenicity using protein microarray data, Bioinformatics, Volume 26, Issue 23, 1 December 2010, Pages 2936–2943, https://doi.org/10.1093/bioinformatics/btq551

90. Saha, S., & Raghava, G. P. (2006). Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins, 65(1), 40–48. https://doi.org/10.1002/prot.21078

91. Saha, S., & Raghava, G. P. (2006). Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. *Proteins*, *65*(1), 40–48. https://doi.org/10.1002/prot.21078

92. Kozakov, D., Hall, D. R., Xia, B., Porter, K. A., Padhorny, D., Yueh, C., Beglov, D., & Vajda, S. (2017). The ClusPro web server for protein-protein docking. *Nature protocols*, *12*(2), 255–278. https://doi.org/10.1038/nprot.2016.169

93. Kaneko O, Gong L, Zhang J, Hansen JK, Hassan R, Lee B, Ho M (February 2009). "A binding domain on mesothelin for CA125/MUC16". The Journal of Biological Chemistry. 284 (6): 3739–49. doi:10.1074/jbc.M806776200.