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In-silico analysis of Lysenin as potential anti-cancer agents targeting sphingomyelin in colon cancer

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Abstract

Colorectal cancer is a malignant neoplasm disease and is defined as a malignancy that occurs in the large intestine. In the world colon cancer ranks fourth meanwhile in Indonesia, colon cancer placed in the third rank after another cancer. The aim of this study is to find alternative way to help chemotherapy agent that already exist which is 5-FU for cure colon cancer. As already know 5-FU still lack of weakness so in this study hope that the sample that test in this study which is lysenin can be co-chemotherapy agent for colon cancer. Lysenin from coelomic fluid earth worm *Lumbricus rubellus* will bind with ligand sphingomyelin with docking method where the result will be new binding complex. After that the new complex will be test with hnRNP M4 membrane complex on colon cancer with docking method too and will see whether there's binding affinity between lysenin sphingomyelin binding complexes with hnRNP M4.

Keywords: Colon Cancer; Lysenin; Sphingomyelin; 5-FU; hnrnp M4

1 Introduction

Colorectal cancer is a malignancy originating from the large intestine, consisting of the colon (the longest part of the intestine) and/or the rectum (the last small part of the large intestine before the anus). According to the American Cancer Society, colorectal cancer (CRC) is the third most common cancer and is the second highest cause of death in men and women in the United States. 2 It has been predicted that in 2016 there will be 95,270 new cases of colon cancer and 39,220 cases of rectal cancer [1]. In 2017 the American Cancer Society predicts the number of colon cancer cases in the United States will be 95,520 new cases and 39,910 new cases of rectal cancer. Whereas in Indonesia itself, in 2012, according to GLOBOCAN (IARC) 12.1% of new cases of colon cancer were the sixth and fourth order as a factor of death from cancer with a rate of 8.9% of deaths. The prevalence between men and women in Indonesia according to GLOBOCAN (IARC) in 2012 was that in men found ±21% of new cases and 10% of deaths of colon cancer patients, while in women found ±7.5% of new cases and ±5.5% of deaths people with colon cancer [2].

Colon cancer treatment can be divided based on the stage into several parts. Stage I recommended therapy: transanal excision (TEM) or trans-abdominal resection + TEM surgical technique if. Stages IIA – IIIC recommended therapy: neo-adjuvant chemoradiotherapy (5-FU/RT short term or capecitabine/short term RT), a second trans-abdominal resection (AR or APR) with TME technique, and adjuvant therapy (5-FU ± leucovorin or FOLFOX or CapeOX). Stage- IIIC: neoadjuvant (5-FU/RT or Cape/RT), trans-abdominal resection and TME techniques or 5-FU and leucovorin

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/FOLFOX/CapeOx. Stage IVA/B treatment: combination chemotherapy or 5-FU/RT pelvis reassess for staging and possible resection. Stage IVA/B: combination therapy with chemotherapy or 5-FU/pelvic RT do a reassessment to determine the stage and possibility of resection and if symptomatic resection continues with palliative chemotherapy for advanced cancer [3].

One of the therapies for colon cancer is adjuvant therapy with 5-FU or 5-fluorouracil. Chemically, fluorouracil, a fluorinated pyrimidine, is 5-fluoro-2,4(1H,3H)-pyrimidinedione. 5-Fluorouracil (5-FU) is a pyrimidine antimetabolite chemotherapeutic drug with a mechanism of action that inhibits the enzyme thymidylate synthase, a deficiency of thymine occurs, thereby inhibiting the synthesis of deoxyribonucleic acid (DNA), and to a lesser extent can inhibit the formation of ribonucleic acid (RNA). DNA and RNA are important in cell division and growth, and the effects of 5-FU can create a thymine deficiency that causes growth imbalance and causes cell death. For the mechanism of inhibition of thymidylate synthase to occur, a reduced folate cofactor is needed so that a strong bond occurs between 5-FdUMP and thymidylate synthase. Reduced folate cofactor is obtained from leucovorin [4].

Treatment of colon, rectum, breast, stomach, and pancreatic carcinomas with 5-FU is successful. Patients with poor nutritional condition, bone marrow depression, severe infections, and fluorouracil hypersensitivity should not take this medication. Patients who take 5-Fu have reported experiencing the side effects including neutropenia, stomatitis, diarrhea, and hand-food syndrome. Each of these outcomes is connected to the patient's administration technique [5]. Cardiotoxicity is the most serious side effect of 5-FU, notwithstanding its rarity [6]. 5-FU has comparatively few side effects and great selectivity for TS action as compared to other chemotherapeutic drugs. The effectiveness of 5-FU as a chemotherapeutic agent has only reached 15%, hence co-chemotherapy medicines must be developed in order to improve the efficacy of 5-FU therapy.

2 Material and methods

2.1 Software used

Software used is PyMOL v.2.2.0 for three-dimensional structure visualization, PatchDock and FireDock for docking Lysenin and sphingomyelin with hnRNP M4 which is a 3-dimensional structure from https://www.rcsb.org/structure/3ZXG, and PyRx for docking to obtain bond affinity and molecular complexes.

2.2 Molecular Docking

The sampling of lysenin and sphingomyelins derived from the PubChem database. Separation of the α and β chains of lysenin using the PyMol program so that only the α chains remain. Then lysenin was docked with α chains and sphingomyelin using the PxRy program. Then a new sphingomyelin-lysenin binding complex will be obtained. Furthermore, the sphingomyelin-lysenin binding complex was docked with hnRNP M4 to see the size of the bond between the two. After the running process is complete, it will come out in the form of the results of the binding and a table containing several values for the binding affinity.

2.3 Data analysis

The end result obtained from docking is the binding affinity of the molecular complex. Low energy (negative) indicates a stable and binding bond, while a high energy value (positive) indicates that the complex formed is less stable and weak. A binding affinity is said to be stable if the binding affinity value is < -6.0 kcal/mol [7].

3 Results and discussion

3.1 The result of lysenin bonding with sphingomyelin

Docking analysis is a computational method to describe the interaction between a molecule acting as a ligand and a molecule acting as a receptor. The interaction between molecules and proteins is important in developing the design of a compound. Several biological processes such as physiological regulation, gene transcription, enzyme reactions, and signal transduction can occur if there are interacting proteins in the ligand bond. Ligands are small molecules that can interact with proteins. Ligand analysis can be done using the docking method.

In this study, the docking began with the separation of lysenin α and β chains, which then the α chain would become the active site that would bind to sphingomyelin. As is well known, lysenin can be an anticancer agent when lysenin binds to sphingomyelin. In the initial docking process, the α and β chains are separated first so that only the α chain remains.

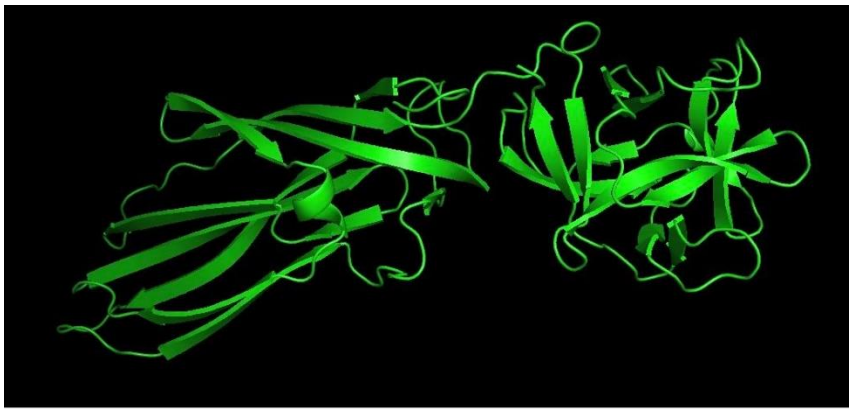

Figure 1 Lysenin α-chain

Furthermore, sphingomyelin will be run together with lysenin α chain, the expected results will be the same or similar to the pre-existing sphingomyelin lysenin bonds.

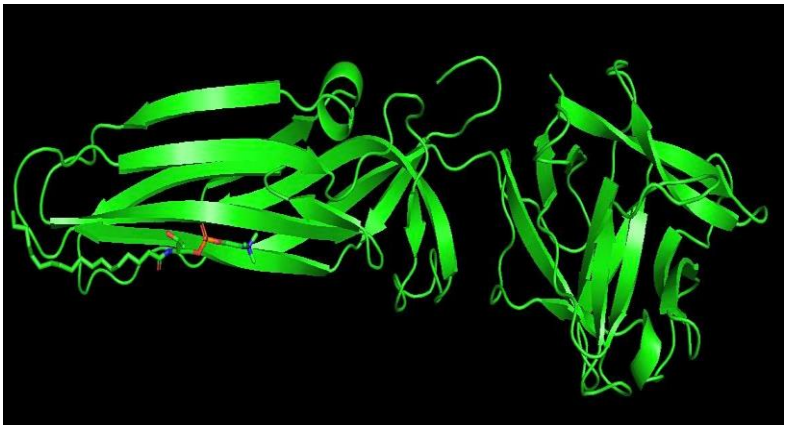

Figure 2 Normal sphingomyelin- lyesnin binding 3D

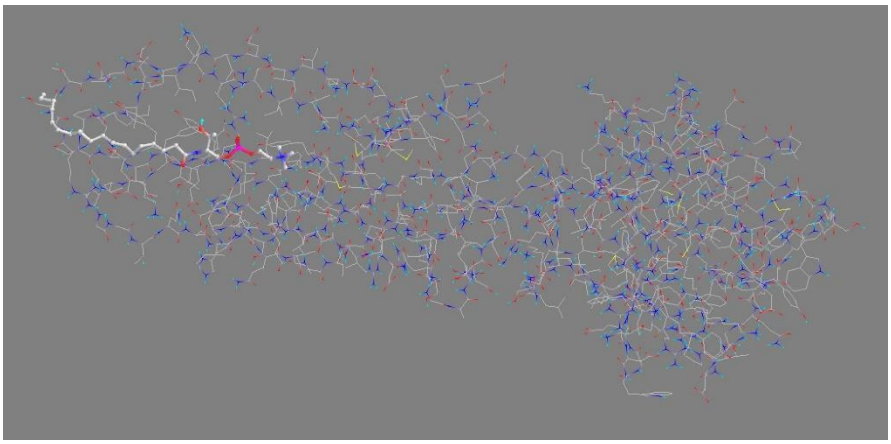

Figure 3 Sphingomyelin vector lyesnin binding was normal

From the results of the docking of lysenin and sphingomyelin, it was found that the bond most closely resembles the normal bond at a binding affinity of -4.0. with a normal distance to the initial docking position, the upper border 0 and

lower border 0 (not a normal picture of lysenin sphingomyelin) is the upper border 2.433 and the lower border 1.373. The meaning of binding affinity -4.0 states that between sphingomyelin ligands and lysenin macromolecules there is an attraction between the two of -4.0 so that the docking results in this study can be used as a reference material for cochemotherapy agents for colon cancer. The similarity between the results of the docking and the control was 61.5%, seen from the equation of the same number of amino acids as many as 8 of the 13 amino acids of the control. These amino acids include threonine, serine, glycine, tryptophan, valine, isoleucine, and arginine with a ratio of the number of bonds with the control 2:4, in the control there are only van der Walls bonds and attractive charge bonds while in the new coupling type, there are van der bonds walls, conventional hydrogen bond, carbon-hydrogen bond, and alkyl.

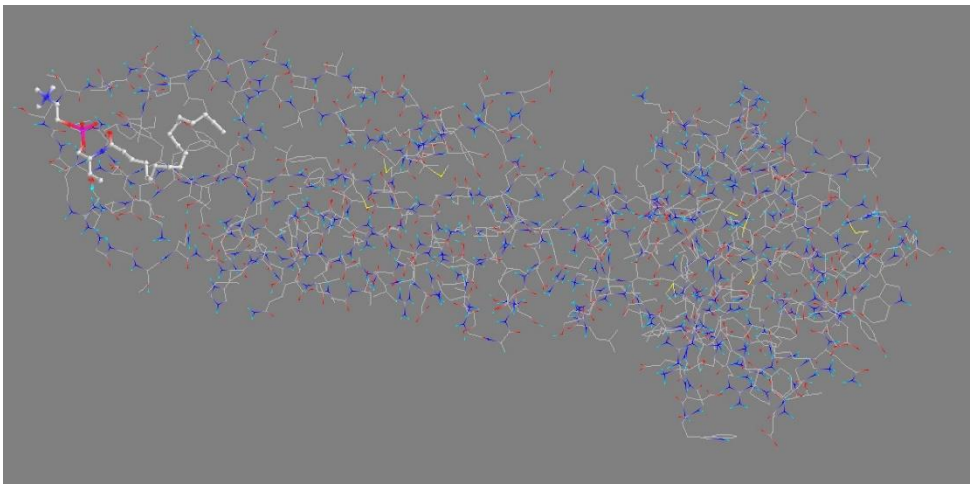

Figure 4 Lysenin- sphingomyelin docking with a binding affinity of -4.0

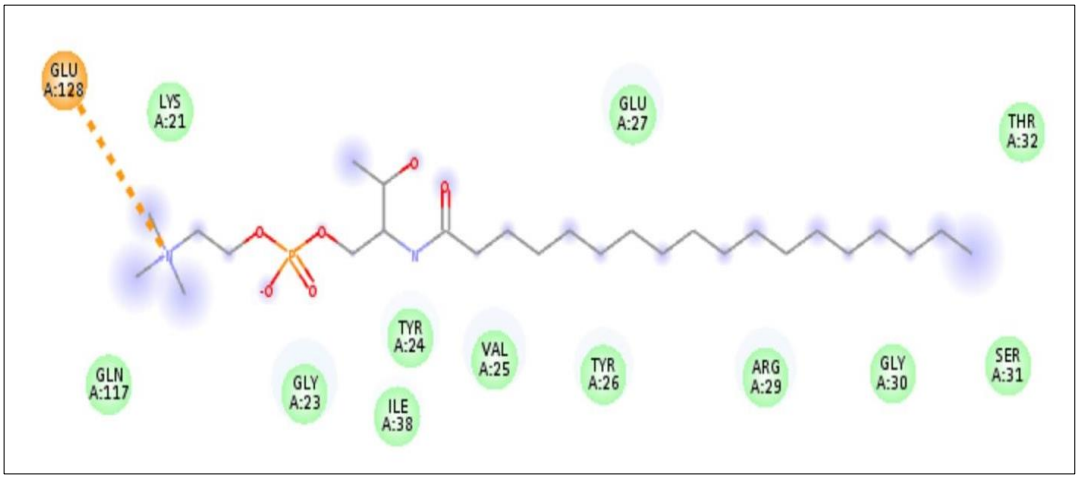

Figure 5 Control of sphingomyelin-lysenin binding

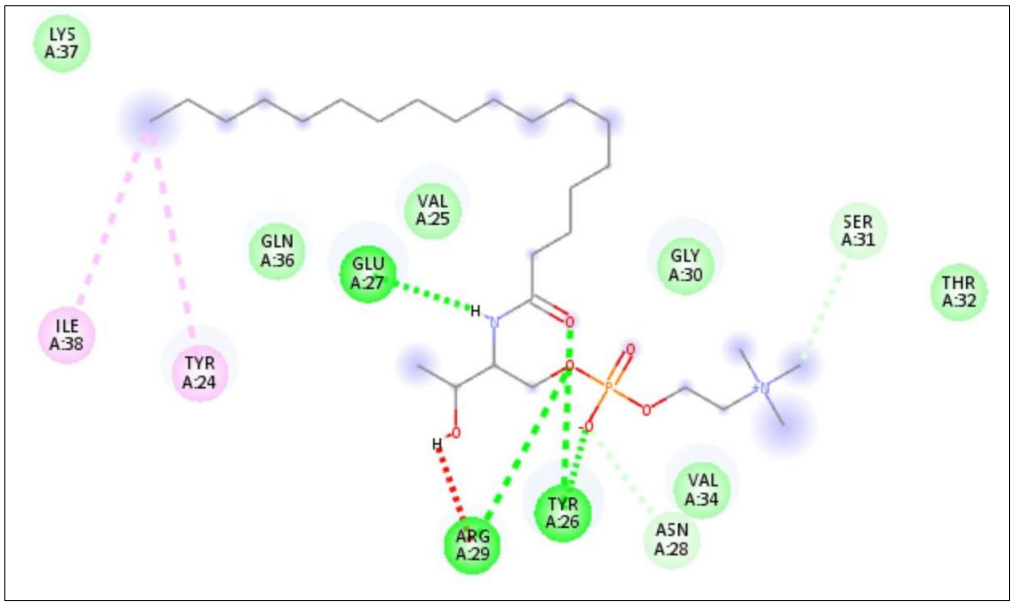

Figure 6 Results of docking of lysenin-sphingomyelin bonds

3.2. Results of Docking Lysenin Sphingomyelin with hnRNP M4

The next stage of the early stage of docking to look for new binding complexes between lysenin and sphingomyelin is docking between the new sphingomyelin lysenin binding complex and hnRNP M4 which is a membrane complex on the surface of the colon cancer membrane where it is hoped that the sphingomyelin lysenin binding complex will be able to bind and penetrate into cells. through this receptor.

From the results of running docking, it was found that the sphingomyelin lysenin binding complex was able to bind to hnRNP M4 which had a binding strength of -4.8 although the result of the binding was considered weak because the normal value of a protein bond with its receptor using this docking technique was -7.3. But the strength of these bonds can prove that the sphingomyelin lysenin bonding complex can work.

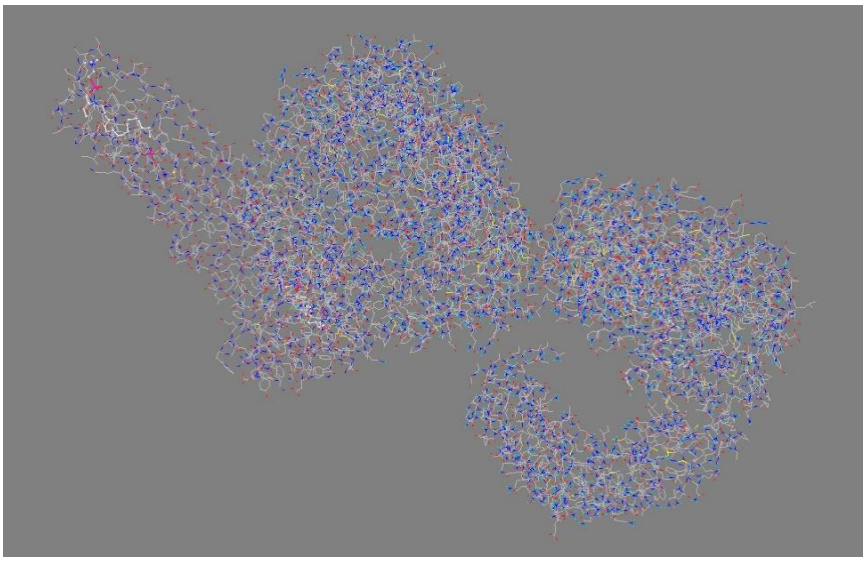

Figure 7 Docking between sphingomyelin- lysenin binding complex and hnRNPn M4

4 Discussion

In this study, two proteins were used, namely lysenin and sphingomyelin, each of which has a function as an agent for apoptosis and lysis of colon cancer cells. The lysenin used in this study came from the earthworm *Lumbricus rubellus* which was extracted from protein isolation from the worm's coelomic fluid. Lysenin is a protein with a total of 297 amino acids and a molecular mass of 33 kDa using sulfate-polyacrylamide or sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [9, 10]. According to the research, it was found that lysenin in the earthworm *Eisenia fetida* is produced from the worm's coelomic fluid which is part of the earthworm's immune system. Lysenin is part of the aerolysin family of small β-pore-forming toxins (β-PFTs) which includes the main virulence factors of several bacterial pathogens, such as aerolysin produced from *Aeromonas* spp, *Clostridium perfringens* epsilon toxin and *Clostridium septicum* α-toxin, while *Bacillus thuringiensis* parasporin-2 has cytokine activity against human cancer cells [11]. As with most PFTs, lysenin is secreted as an inactive protein, a water-soluble monomer. In its bond with sphingomyelin in the eukaryotic cell membrane, the toxin undergoes significant changes to the secondary structure and produces pre-pore oligomer on the surface of the membrane which were identified by electron crystallographic and high-speed atomic force microscopy studies before being inserted into the membrane which triggers pore formation and lysis cell. From the investigation results of the structure of the lysenin monomer that binds to sphingomyelin, it is evident that both phosphocholine heads and one alkyl tail chain of sphingomyelin are required for toxin binding [12].

In this study, the results of the docking between lysenin and sphingomyelin resulted in a bond strength of -4.0, which means that the bond between lysenin and sphingomyelin have meaning and can be used as the basis for this study. The result of the similarity between the control and the new type of bond (lysenin- sphingomyelin) is 61.5%, which means that the bond complex from the docking results is similar to the control, although not significantly so that the new bond complex can be used as the main ingredient in this research [13]. These results were obtained from a comparison of control amino acids with the results of the new docking. In the lysenin and sphingomyelin binding complex, 8 amino acids were obtained which were the same as the control, among others; threonine, serine, glycine, tryptophan, valine, isoleucine, and arginine [14].

This new binding complex is expected to be able to help the work of 5-FU as a cochemotherapy agent for colon cancer even though the pathway of action of this new sphingomyelin lysenin binding complex is different from 5-FU. 5-FU has a mechanism of action by converting 5-FU intracellularly into active metabolites such as fluorodeoxyuridine monophosphate (FdUMP), fluorodeoxyuridine triphosphate (FdUTP), and fluorouridine triphosphate (FUTP), which activation of these three metabolisms interferes with RNA synthesis and the work of thymidylate synthase. The rate-limiting enzyme in 5-FU catabolism is dihydro pyrimidine dehydrogenase (DPD), which converts 5-FU to dihydrofluorouracil (DHFU) [15]. More than 80% of administered 5-FU is normally catabolized early in the liver where DPD is expressed. Meanwhile, the inhibition of thymidylate synthase has a different pathway. It is well known that the thymidylate synthase enzyme can catalyze the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) with 5, 10 –methylene tetrahydrofolate (CH2THF) as a methyl donor [16]. The active metabolite of 5-fluorouracil (5-FU) fluorodeoxyuridine monophosphate (FdUMP) binds to the nucleotide-binding site of thymidylate synthase and forms a stable ternary complex with thymidylate synthase and CH2THF, blocking dUMP access to the nucleotide-binding site and inhibiting dTMP synthesis. The result of an imbalance of deoxynucleotide (dNTP) and increased levels of deoxyuridine triphosphate (dUTP) can cause damage to DNA [17].

The presence of a lysenin sequence capable of forming a transmembrane domain is questionable; therefore the protein can adhere to the membrane surface without penetration of the membrane bilayer. Furthermore, the hydrophobic domain of lysenin can cause local distortion of the lipid layer [18]. In addition, the conversion of lysenin to oligomers cannot be ignored, because fetidine and eiseniapore, another *E. foetida* protein, are shown to undergo oligomerization during interactions with sphingomyelin-containing membranes. It is suspected that the accumulation of sphingomyelin in different membrane microdomains such as sphingolipid-rich/cholesterol-rich rafts can cause the microdomains to be particularly vulnerable to lysenin binding. The lysenin-sphingomyelin complex concentrated in the microdomain can cause local damage to the plasma membrane, followed by cell lysis. From that point of view, studies of lysenin-induced cytolysis could help to reveal if changes in lipid rafts are capable of inducing cell death. There are several ways in which the lysenin/sphingomyelin complex can increase membrane leakage, one of which is related to bilayer destabilization due to local changes in membrane curvature. This mechanism of action has been described for the pro-apoptotic protein tBid in the mitochondrial membrane. Researchers suspect that after permeabilization of the plasma membrane, lysenin can access the sphingomyelin mitochondrial pool, and protein cytotoxicity undergoes augmentation [19]

From the docking results, it was found that the sphingomyelin lysenin binding complex was able to bind to hnRNP M4

which is a membrane complex found in colon cancer (HT-29) which has a bond strength of -4.8 which includes a weak bond (normal -7.3). However, the strength of this bond proves that lysenin sphingomyelin is able to bind to hnRNP M4, after which the lysenin sphingomyelin binding complex will enter and lyse colon cancer cells as described above. But not only that because it is known that hnRNP M4 also binds to CEAR (Carcinoembryonic antigen receptor) which is a receptor of CEA (Carcinoembryonic antigen) which is a protein that is able to protect colon cancer cells from apoptosis and is able to induce induction activation signals. CEA is also used as a marker of colon cancer and also has a role to see the progress of colon cancer itself. CEA can be found in 90% of colon cancer, 60% of breast cancer, lung cancer, and pancreatic cancer [20]. From this study, it is hoped that the presence of a weak bond between the sphingomyelin lysenin binding complex can make a therapeutic agent or co-agent for colon cancer therapy together with 5-FU with different pathways and different actions.

5 Conclusion

Based on the research that has been done, it can be concluded that the sphingomyelin lysenin binding complex has the ability to be an adjuvant agent for colon cancer therapy. Lysenin in the coelomic fluid of the earthworm Lumbricus rubellus binds specifically to sphingomyelin and can work as an anticancer agent. There is lysenin in the earthworm Lumbricus rubellus of 33 kDa. The sphingomyelin lysenin binding complex does not bind to the same receptor as 5-FU in colon cancer (HT-29) because it has its own pathway to damage colon cancer cells. The mechanism of action of the new sphingomyelin lysenin binding complex is by damaging cancer cells after penetrating the surface of the cell membrane, then lysing and appetizing cells from within colon cancer cells.

Compliance with ethical standards

Acknowledgments

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Disclosure of Conflict of interest

The authors declared that there is no conflict of interest.

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