Biological Interaction Mechanism and Relative Medical Application of Synthetic Multivalent Molecules

Transduct in cells and viruses

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Abstract: Nowadays, the increasing use of multivalent molecules for therapy has proved that they play a significant role in clinical application. The multivalent combination provides a wide range of advantages and unique functions that monovalent interactions cannot achieve. Multivalent interaction is collectively much stronger than monovalent interaction. Specifically speaking, multivalent ligands bind tighter to their respective multivalent receptors, including pathogens (viruses, bacteria), toxins, lectins, ion channels, enzymes and cell surface receptors, due to the structure of the molecules, which can ligand with more receptors. Here, this paper introduces the basic mechanism of the multivalent interaction and reviews the applications of the multivalent molecules in cellar targets and bacteria or virus inhibition. If more multivalent molecules would be synthetic to understand cell transduction and treat patients, this technology will have a strong potential in medical application.

1. Introduction

In 1998, Mammen and his colleagues [1] defined the valency of the molecule or entity as the number of independent structural units of similar or the same type that are connected to the molecule or the biological entity, such as bacteria, virus, or cell. The specific simultaneous binding of multiple ligands on the molecules or the multiple receptors on the entity is defined as multivalent interactions (as shown in Figure 1), a common phenomenon in nature [2]. Naturally occurring multivalency is evident in intercellular signaling, viral cell binding, antigen-antibody interactions, etc. Multivalence is an effective strategy to develop highly efficient and selective ligands, especially on the cell, bacteria, and virus surface. Recently, Liu et al. [3] have developed a multivalent, fully synthetic Janus nanotherapeutic platform, in which the cell targeted ligand identified by phage display was on one "face" and FC mimic ligand was on another "face". Moreover, Becker et al. [4] also introduced dextran as a multivalent hydrophilic polysaccharide scaffold for cell-targeted drug polymerization. Besides, they also established a synthetic route to mask the lysine side chain of l17e with a photo unstable protective group, thus opening a way for light-triggered activation of cellular uptake. Here, this paper summarises multivalent molecules that have been applied in cell targets, bacteria and virus targets and discusses the applications of these multivalent molecules.

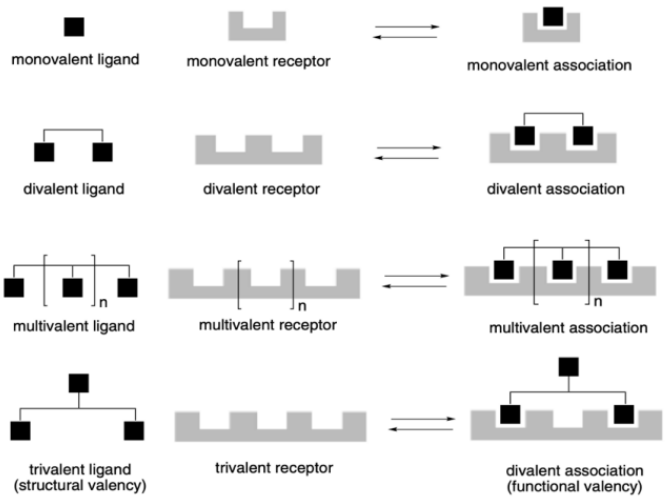

Figure 1. Schematic examples of valency and ligand interactions [2]

Nowadays, multivalent interactions are considered to be a ubiquitous strategy with a wide range of functions that evolved in nature, including selective recognition of multivalent antigens by antibodies. For example, Safenkova et al. [5] investigated the interactions between multivalent antibodies preparations using colloidal gold nanoparticles (GNP) and a multivalent ligand. Besides, Zhang et al. [6] reported a simple, multifunctional, multivalent ligand system that effectively and explicity regulates the aggregation and function of cell surface receptors. Multivalent ligands are composed of polymerized DNA scaffolds modified with biological recognition ligands to interrogate and regulate cellular receptor signals and functions. Moreover, they also have the function of the tight adsorption of virus particles or bacteria on the surface of host cells. These multivalent interactions are more effective and selective than similar monovalent interactions. Therefore, they are only weakly inhibited by most monovalent ligands, especially when the critical gap of monovalent ligands is shallow. Therefore, when designing inhibitors to interfere with multivalent interactions, the use of multivalent molecules seems to be the most effective strategy. This multivalent molecule has been shown to be a very effective inhibitor: especially against surface-surface interactions observed in virus-cell and cell-cell adhesion [2] (as shown in Figure 2). However, the valency and shape of the scaffold have a significant impact on the binding and functional capabilities of the multivalent molecules constituting the scaffold. Over the years, numerous kinds of the scaffold are widely used in the design of multivalent molecules ranging from polymers, dendrimers, liposomes, proteins to gold nanoparticles, as shown in Figure 3.

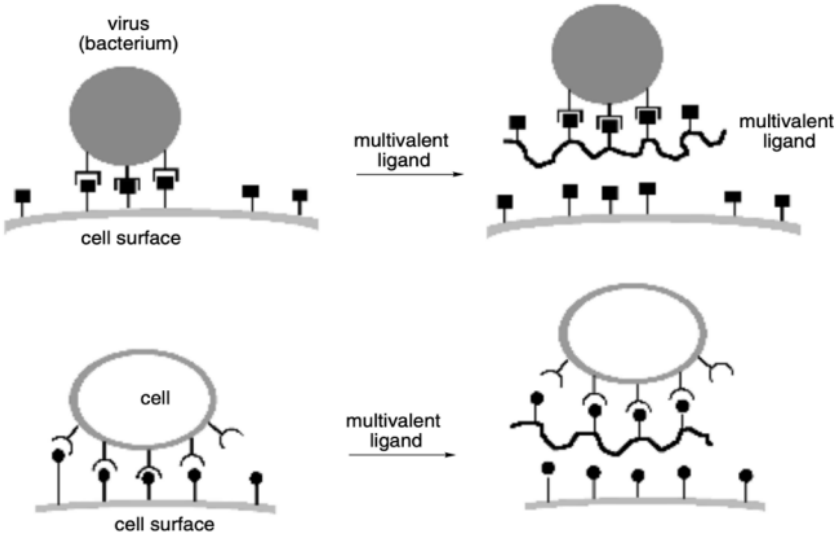

Figure 2. Examples of natural multivalent interactions (virus-cell and cell-cell adhesion) [2]

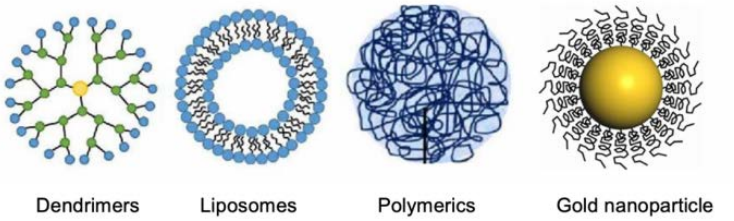

Figure 3. Various scaffolds used in multivalent presentation

2. Mechanism of multivalent molecules interaction

The interaction mechanism of synthetic multivalent molecules includes affinity, kinetics, and thermodynamics. There are fundamental differences in the definition and calculation of bond strength between multivalent interaction and monovalent interaction [2] (Figure 4). Since a monovalent system is constituted by a receptor and a ligand, the affinity constant (K_α) is the binding strength of the complex, which is related to the free energy of association (ΔGmono) according to the Gibbs equation. However, for a multivalent molecule, the interaction between two molecules has N tethered receptors, and N tethered ligands, and the correlation constant is defined as avidity ($K_\alpha$multi). It is a collective binding constant, which considers the multiple interactions between two multivalent entities. It is also related to the binding free energy calculated by the monovalent binding.

$$\Delta G^{mono} = -RTln(K_\alpha^{mono}) \quad (1)$$

$$\Delta G_N^{multi} = -RTln\left(K_\alpha^{multi}\right) \quad (2)$$

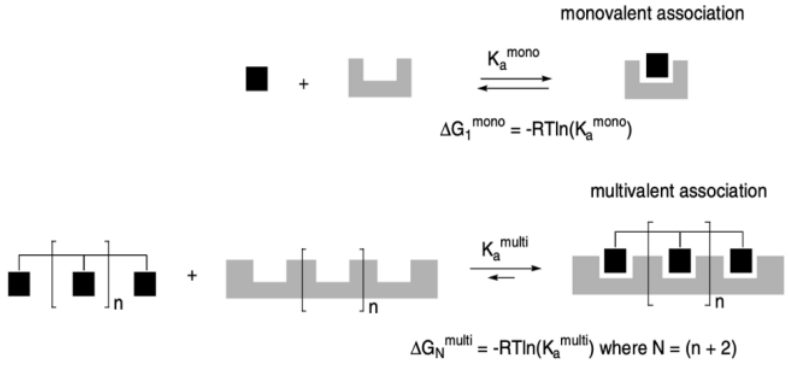

Figure 4. Comparison of free energy of association of monovalent and multivalent interaction [2].

Moreover, as for the thermodynamic basis of multivalent interactions, scientists had done a lot of research. In order to clarify the thermodynamic basis of multivalent interaction, Mammen et al. [1] studied a simple divalent system, and compared the bivalent association complex (Eqn B) with the monovalent association complex (Eqn A1) under some limiting conditions [2] (Figure 5). The binding free energy (ΔG) of the receptor-ligand association complex is composed of enthalpy (ΔH) and entropy (ΔS). The univalent association complex relates to the standard relationship among the three thermodynamic parameters (ΔGmono, ΔHmono, and ΔSmono).

$$\Delta G^{mono} = \Delta H^{mono} - T\Delta S^{mono} \quad (3)$$

$$\Delta S^{mono} \approx \Delta S^{mono}(translational) + \Delta S^{mono}(rotational). \quad (4)$$

In equation (5), the total entropy change involved mainly comes from the change of translation entropy and rotation entropy of the receptor and ligand after binding. This equation represents a qualitative sum. It assumes that translational entropy and rotational entropy make the largest contribution while other possible concentrations are less significant. The binding free energy (ΔGdi) of divalent association complexes is expressed by a standard equation qualitatively related to thermodynamic composition.

$$\Delta G^{di} = \Delta GH^{di} - T\Delta S^{di} \quad (5)$$

$$\Delta H^{di} \approx 2\Delta H^{mono} \quad (6)$$

$$\Delta GS^{di} \approx \Delta S^{mono}(translational) + \Delta S^{mono}(rotational) + \Delta S^{di}(conformational, linker) \quad (7)$$

It is assumed that the association of discrete ligands with each divalent point occurs in a tension-free and independent manner. Under this condition, the change of the enthalpy of bivalent binding is equal to twice the enthalpy of monovalent binding. The entropy change of the bivalent association complex consists of three parts. The first two terms are equal to the translational and rotational components of the monovalent association. However, this estimate is based on some limited considerations. Firstly, the translational and rotational entropies of bivalent and monovalent particles are equal. Besides, in bivalent and monovalent associations, two particles combine to form one particle, which results in the net loss of free translation and rotation of a single particle. What is more, the enhanced binding affinity highlights the thermodynamic aspect of multivalent receptor-ligand interactions for the kinetics aspects of multivalent molecules interaction. However, these aspects are inextricably related to the kinetic characteristics of multivalent binding, which is characterized by the decrease of the dissociation rate of the two interacting entities [2].

3. Medical application of Synthetic Multivalent Molecules

3.1. Sensing cellular targets

3.1.1. Hepatic Mannose/N-acetylgalactosamine-4-SO4 (Man/GalNAc-4-SO4)

Receptors are used to recognize these molecules like lutropin on the surface of the cell. Lutropin is a hormone, which is made by the human's pituitary gland, a small gland located underneath the brain [7]. This hormone plays an important role not only in the healthy growth of children, but also in the sexual development and functioning of adult's.[7] Too much of this type of hormone will be harmful to the healthy development of human sexual organs of adults and the precocious development of children [7]. Therefore, synthetic multivalent molecules on the surface of cellular targets to prevent the overproduction of this hormone are in an urgent matter. The book given by Seok-Ki Choi et al. [2] shows that doing as an agent on the surface of the cell, the Man/GalNAc-4-SO4 receptor, which can exist as a dimer on the surface of hepatic cells or as a monomer on the surface of macrophages, can remove the lutropin from the blood circulation. This process is happened by recognizing and binding the GalNAc-4-SO4 sequences that exist in lutropin, to regulate physiological levels of lutropin, as shown in Figure 5.

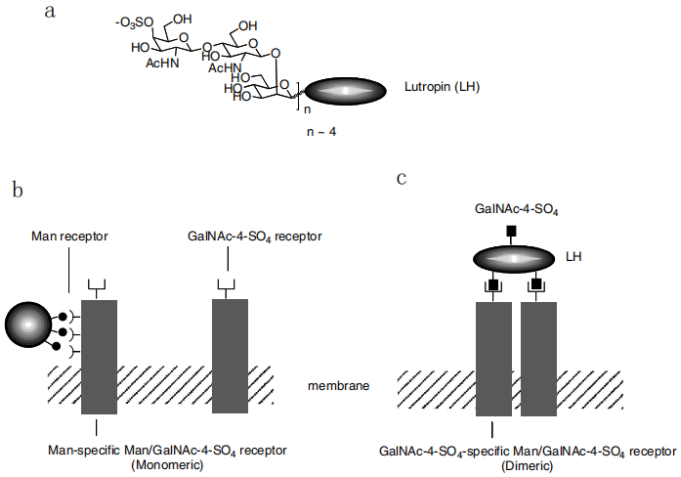

Figure 5. The information about the structures of the Man/GalNAc-4-SO4, and the receptors on the surface of the cell.

(a) The structures of the Man/GalNAc-4-SO4 combined with the lutropin.

(b) The Man/GalNAc-4-SO4 and the GalNAc-4-SO4 combined with the receptors on the membrane of the macrophage as a monomer.

(c) The GalNAc-4-SO4, ligand with the lutropin combined with the receptors on the surface of the hepatic endothelial cell as a dimer.

Figure 5 (a) shows the structures of the Man/GalNAc-4-SO4-lutropin and the information of how the Man/GalNAc-4-SO4, molecules combined with the lutropin. Figures 5 (b) and 5 (c) show that the dimer receptors on the cell membrane interact selectively with lutropin. In contrast, figure 5 (b) shows two monomeric receptors. The left one can combine with both the Man/GalNAc-4-SO4 and the GalNAc-4-SO4, and the right one can only combine with GalNAc-4-SO4. Figure 5 (c) shows that the receptor on the hepatic endothelial cell is present in a dimeric state. While Roseman et al. [8] further showed the information about the function of the Man/GaINAc-4-SO4 receptors in hepatic endothelial cells. To study deeply about these molecules, Roseman and his colleagues have accomplished the experiment to conclude that the sulfate in position 3 is about 12 times faster than that of position 4 in the aspect of the rate of clearance from the blood circulation. Therefore, the GalNAc-3-SO4 will have the potential to replace the GaINAc-4-SO4.

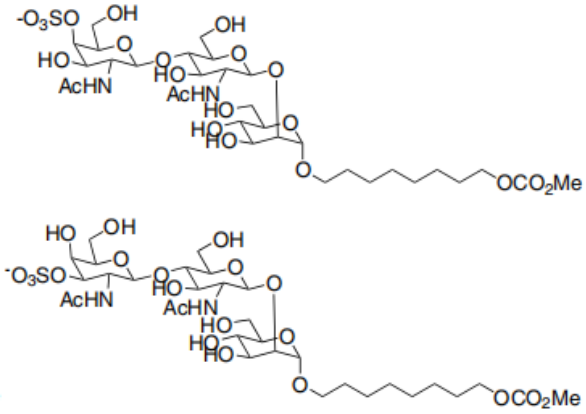

Figure 6. The structure of the GaINAc-4-SO4 (up) and GalNAc-3-SO4 (down).

3.2. Bacteria inhibition

The abuse of conventional antibiotics has led to increasing multidrug-resistant (MDR) bacteria, threatening human health [9]. Traditional small-molecule drugs cannot meet clinical needs. Therefore, the design and development of new antibacterial materials with high antibacterial activity is of great significance in the biomedical field. Recently, gold nanomaterials are more widely used because they are easy to functionalize and have lower toxicity [10, 11]. They also play a significant part in antibacterial applications. Specifically speaking, Zhao et al. [12] found that pyrimidine capped gold nanoparticles destroy bacterial cell membrane by chelating magnesium ion or calcium ion, leading to leakage of cytoplasmic contents (including nucleic acid of the damaged cell membrane), and play its antibacterial role by interacting with DNA and inhibiting protein synthesis by internalized nanoparticles. Yang and his colleagues [13] modified the surface of gold nanomaterials by using several kinds of small molecules to fight against clinical MDR isolates and lab bacterial strains. They had developed multivalent amino saccharide-based AuNPs and investigated their antimicrobial activity. They found that because of their similarity between bacterial peptidoglycans and the amino saccharide, amino saccharide-based AuNPs could specifically antagonize Gram-positive bacteria in vitro. Then they tried to measure the in vivo activity and safety of this kind of multivalent nanoparticles against Gram-positive super bacteria-induced wound infections [9]. They found that the antibacterial activity was much better than that of silver nanoparticles, and they had no obvious toxicity in vivo and didn't cause hemolysis. In addition, they can greatly weaken the viability of bacteria in mature biofilms as well as treat the infection caused by MDR bacteria in vivo. Therefore, the multivalent amino saccharide-based AuNPs have broad clinical application prospects.

Later in 2020, Mei et al. [14] tried to develop multivalent AgNPs-COS to inhibit bacteria. Silver nanoparticles (AgNPs) have a large specific surface area and small particle size and have a strong antibacterial effect against different fungi, viruses, and bacteria [15]. They can contact the bacterial cell membrane and penetrate the cytoplasm, inactivate the necessary respiratory enzymes, and finally

lead to the death of the bacteria [16]. In addition, chitosan oligosaccharide (COS) is a low molecular weight, biocompatible, non-immunogenic, cationic, water-soluble, and FDA-approved adhesive polymer. COS has many biological activities, such as anti-inflammatory, antibacterial, hemostasis. All these effects have been widely studied in the field of biomaterials [17]. The combination of COS and AgNPs surface can increase the surface charge of AgNPs as well as improve its adsorption to the negatively charged bacterial plasma membrane through electrostatic interaction. Therefore, Mei et al. [14] developed a green and simple method to prepare AgNPs-COS as a promising antimicrobial nanomaterial.

AgNPs-COS can be obtained by in-situ surface modification by reduction of AgNO3 in the presence of COS. They optimized the synthesis conditions of AgNPs COS by orthogonal array design (OAD) to obtain high antibacterial activity [14]. The synergistic antibacterial activity was characterized by inhibition fraction (FIC) and inhibition zone test. They found that the nanoparticles could significantly inhibit the growth of Gram-negative and Gram-positive bacteria. Antibacterial mechanism studies had shown that AgNPs-COS could bind to the Mg2+ ions on the surface of the bacteria and interact with the bacterial membrane as well as improve the permeability of the bacterial outer membrane [14]. Then they can damage the membrane of the bacteria and lead to their death. Therefore, it has great potential for bacterial infection and will be widely used in the medical field to prevent a variety of bacterial infectious diseases.

3.3. Blocking virus infection

Multivalent molecules can also be applied to inhibit virus infection, including the influenza virus, Ebola virus, etc. The influenza virus is a highly infectious pathogen, which causes epidemics in humans every year. They can enter host cells through endocytosis after binding to the plasma membrane. The acidification of late endosome induces the conformational change of hemagglutinin (HA), which mediates viral envelope bilayer and endosome membrane fusion. As a result, the viral genome formed by 8 RNA and nucleoprotein complexes was released into the cytoplasm [18]. The infection cycle of the influenza virus begins with the interaction of HA with glycoprotein and sialic acid (SA) residues on the surface of host cells. This process is based on multivalent binding to achieve stability as well as prevent virus separation. Based on this naturally occurring multivalent binding mechanism, scientists introduced a synthetic multivalent entry blocker in the 1990s. High molecular weight scaffolds showed that many SA-derived ligands with low affinity were used to obtain high HA affinity [19]. Over the years, many carrier systems have been used as scaffolds, ranging from liposome proteins, dendrimers, polymers to gold nanoparticles.

Gold nanoparticles (AuNPs) are biocompatible scaffolds that can be used to prepare biological diagnosis, biomolecular detection, and nanostructured materials. They are colloidal stable, but their stability depends largely on pH, total ionic strength, solution, and colloidal concentration. They have great application potential in biology, materials science, physics, chemistry, and related interdisciplinary fields. Besides, AuNPs can combine with drugs, DNA, biological ligands, and small interfering RNA (siRNA) through AuNP surface bonding, and they also have optical properties. Therefore, they are considered excellent drug-delivery systems. In 2010, Papp I. et al. [18] developed a method of using AuNP encapsulated in multiple receptors of SA to compete with viral fusion proteins and studied their potential to inhibit viral infection. They were functionalized by a novel SA terminated glycerol dendrimer to inhibit the binding of influenza virus to the plasma membrane. They chose polyglycerol dendrimer because it has excellent biocompatibility and water solubility, which could provide a suitable scaffold for sialic acid. They obtained dendrites immobilized on gold surfaces of different sizes (2 and 14nm) and measured their ability to inhibit influenza virus infection. They found that sialylated particles of 14 nm showed the great ability to inhibit the infection with influenza virus, while the ones with 2 nm particle size didn’t [18]. Their findings indicated that multivalent interactions are usually strongly dependent on the size of the interacting particle surface. Therefore, these multivalent molecules have the potential to inhibit the influenza virus.

Ebola virus (EBOV) is one of the most lethal pathogens to human beings. It belongs to the Zaire ebolavirus species within the Ebolavirus genus of the family Filoviridae [20]. Ebola virus disease

(EVD) is a severe and fatal disease caused by the Ebola virus (EBOV). The outbreaks of EVD usually begin with possible zoonotic transmission and then spread from person to person through direct contact or contact with infected body fluids or contaminated parasites [21]. Although dendritic cells, monocytes, and macrophages are the primary target cells of infection, EBOV has a broad cell tropism. Entry inhibitors have the advantage of minimizing the drug resistance caused by virus mutation. Therefore, it is an excellent method for antiviral action for EBOV [22]. They can focus on various targets involved in the binding of the envelope viral glycoprotein (GP) with endosome formation, membrane fusion, cell receptors, and viral particles released into the cytoplasm of a target cell. Dendritic Cells Specific ICAM-3 Grabbing Non-integrin (DC-SIGN) is a C-type lectin and present at the surface of dendritic cells. It can recognize highly glycosylated envelope proteins in a calcium-dependent manner. Moreover, it is a common pathogen receptor that has the potential to be a new therapeutic target [23]. The potential role of DC-SIGN expressing cells in animal models of infection has been reported, which indicates that these dendritic cells, in fact, play a significant role in the initial stage of EBOV infection and transmission. Antonio Muñoz et al. [24] synthesized a large spherical polyvalent sugar fullerene. The central C60 core is covalently linked with 12 hex adducts of C60, forming the first tridecane fullerene reported so far. They found that in the subnanomolar concentration range, these tridecane fullerenes can effectively block EBOV infection. Therefore, fullerenes are ideal platforms for studying multivalent interactions, especially because of their biocompatibility and spherical presentation. To sum up, multivalent molecules can specifically bind to multiple ligands or the multiple receptors on the entity and play a significant role in bacteria and virus inhibition.

3.4. Treating cancers

3.4.1. Multivalent tyroserleutide (MYSL) is used to treat breast cancer.

According to the data in 2017, about 9.6 million people were killed by cancer, accounting for one-sixth of the people who died in the same year, which has led to the result that cancer is the second largest killer of human beings [25]. Lynne E. et al. had indicated that the exponential and continued growth rate of the cancer cell and the low viscosity between cancer cells lead to the fact that more cells can evade the immune system to spread to the human's body, which increases the difficulty of cancer treatment, even increases the risk of death. Nowadays, scientists have found some multivalent molecules that can inhibit the transformation and replication of cancer cells [26]. YSL may be used to make the advanced antimetastasis agent during radiotherapy in the future because YSL is a natural peptide that can prevent the growth and metastasis of specific cancers [27]. For example, liver cancer cells' invasiveness and metastatic potential were decreased by avoiding the epithelial-mesenchymal transition (EMT). And Zhuangzhuang Z. et al. assumed that "multivalent interaction may be a strategy to make the interactions between ligands and their receptors increase rapidly" by reading lots of paper [28]. Then Zhuangzhuang Z., with his colleagues, made a comparison between monomeric peptide and multivalent one. They found that multivalent YSL dendrimers have better properties, such as high proteolysis resistance and excellent cytotoxicity inducibility in tumor cells in the human body, leading to the latter having better ability to constrain tumor cell metastasis. Therefore, the MYSL has the potential to treat breast cancer[28].

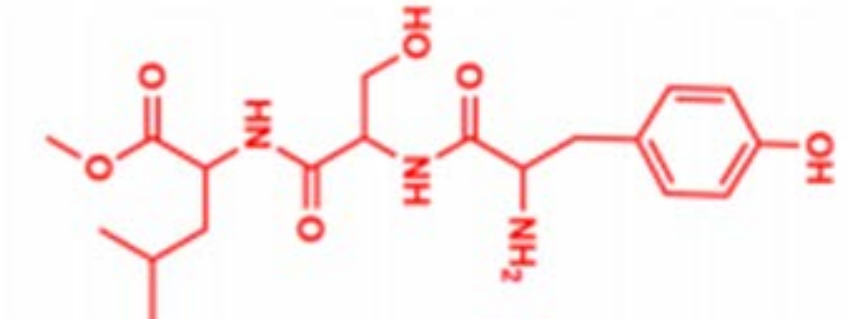

Figure 7. The monomer of the YSL[28]

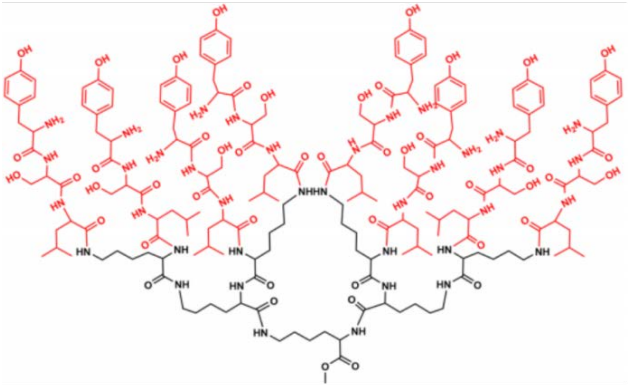

Figure 8. Structure of MYSL [28]

Figure 7 and Figure 8 show that the YSL is a multivalent molecule. From both of these two pictures, information can be gotten that each two YSL can be connected by combined with this molecule, which will be drawn in figure 9. For this reason, the multivalent molecule of the conclusion is proved.

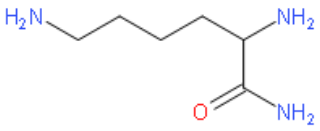

Figure 9. This structure of the molecule is connected with the YSL and they can combine with each other.

This molecule would indirectly estimate the cancer cell. To understand the mechanism of this molecule acted with the cancer cell, Zhuangzhuang Z. et al. have experimented by using the cancer-bearing mice and the different multivalent molecules of YSL with the same YSL monomer molar concentration. They treat these mice with the dosage of 10mg/kg per body weight 6 times [28]. Their experiment results are shown in figure 10.

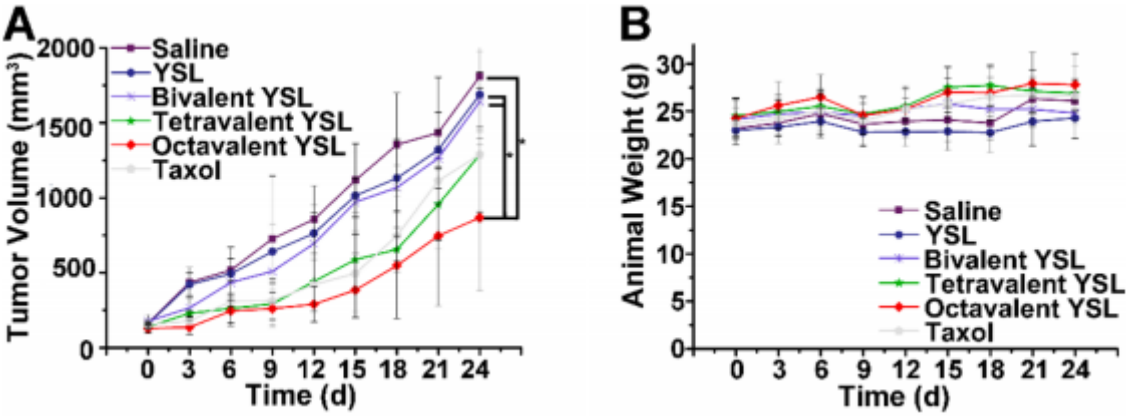

Figure 10. The cancer treatment results of the mice by different multivalent molecules of YSL.

A. The curves about the volume of the tumor growth at the same time.

B. The curves about animal weights in different molecules. [28]

The line chart from figure 10A shows that tumor volume in the mice treated with octavalent YSL is only about 800mm3, only half the same as the mice treated with saline or monomer YSL (about 1700mm3), which indicated that the octavalent YSL[28]. And the more the valent of YSL, the less the tumor volume. Figure B shows that all groups of the mice had almost the same weight, and the average weight had just a slight change, which indicated that these compounds have almost no side effects [28]. After the experiment, Zhuangzhuang Z, et al. had compared with the monomeric YSL and multivalent YSL, and got a conclusion that the multivalent YSL have better abilities to enhance

the serum stability and inhibit the metastasis and growth of breast cancer by cytoskeletal disruption, MMP-9 expression reduction, and cytotoxic tumor cell death promotion[28].

3.4.2. The Glycosphingolipids (GSLs) used for tumor cell marker.

As discussed in section 1.2 on how to treat cancer by using the drugs, the most important method is about how to prevent cancer. Vaccines are medicines which helps fight disease by training the immune system to find and destroy harmful germs and cells by producing antibody [29]. While during the COVID-19, all of the countries were producing abundant vaccines. Net Editorial Board in America discussed the vaccines which can treat cancer [29]. However, before the analysis of how to produce this cancer, it's important to mark the cell tumor. According to the book by Seok-Ki Choi, glycosphingolipids (GSLs) can be grouped into three types according to the structure of attached complex carbohydrates: "globo-, ganglio- and lacto-". And these molecules have lots of application in mediating the adhesion, communication, and signal transduction processes of the cells[2]. While some of the tumor cells have an abnormally high level of specific GSLs on the cell membrane, which will lead to metastasis of cancer cells, such as gangliosphingolipid (GM3) on the melanoma cells [2]. Therefore, specific types of GSL molecules may be used to mark certain types of the tumor cell surface[2].

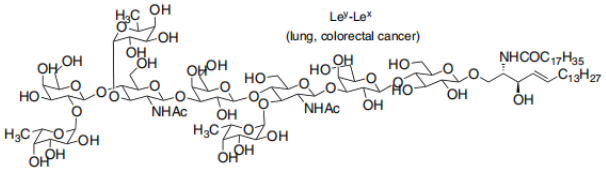

Figure 11. Structures of the GSLs molecules

Figure 11 shows the structures of the GSL. From this figure, we can get the information that this molecule has lots of hydroxyl and ether bonds. GSLs can guide the high production of the antibody via the immune defense mechanism because of the high level of gene expression on the tumor cell membrane. The promotion of the immune system is generally happened by interaction with the tumor-associated antigens GSLs, which had exposed hydrophilic carbohydrate parts. Therefore, the vaccines, including the multivalent molecules, which can carry the tumor-associated antigens, have been innovated for a potential cancer treatment method [30]. Since synthesizing such complex carbohydrate antigens is difficult, this type of vaccine has no progress. Fortunately, with the development of the technology, the large-scale preparation of specific carbohydrate antigens used for vaccines will be achieved [31].

4. Conclusion

We have presented the application of the multivalent molecules in both cellular targets, and viral or bacterial targets since the multivalent molecules have functional valency, which is of great significance due to the structure of the molecules. We have found three multivalent molecules, which can be used for controlling the lutropin hormone in our body, treating cancer, and using as an antigen to innovate the vaccine to study the cell transduction in the aspect of the structure of the molecules. The experiment did on these molecules to understand the action mechanism and how to improve these molecules. We also found some molecules which can be used in viral or bacterial targets to treat this disease. These molecules can treat with more patience because these molecules can treat patients more efficiently with no side effects, and scientists also found some methods to synthetic thus molecules. Although science and technology are very advanced, some high-cost synthetic molecular drugs have not been developed due to the high cost and inaccurate effect. However, in the future, it'll be easier for multivalent molecules synthesis with the development of technology.

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