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BREAKING DOWN TUMOR DRUG RESISTANCE: THE LINK BETWEEN CELL MEMBRANE CHANGES AND TREATMENT EFFICACY

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Abstract: There have been significant advances in our understanding of how changes in the fluidity and permeability of the cell membrane can affect drug resistance in cancer. Research has shown that cancer cells often have changes in the fluidity and permeability of their cell membrane that contribute to their resistance to drugs used to treat cancer. These changes may be due to changes in the composition and organization of the lipid bilayer that makes up the membrane, as well as changes in the expression or localization of proteins and other molecules embedded in the membrane. The lipid composition in the tumor cell membrane changes with drug resistance, which can affect the fluidity and permeability of the cell membrane. Reversal of drug resistance can be achieved by altering cell membrane fluidity and permeability. In recent years, there have been numerous studies aimed at understanding the mechanisms underlying these changes and identifying strategies to overcome drug resistance in cancer. This research has led to the development of new drugs and drug delivery systems that are designed to target specific changes in the cell membrane of cancer cells and improve the effectiveness of chemotherapy. Overall, the advances in our understanding of the role of cell membrane fluidity and permeability in drug resistance in cancer have led to the development of new approaches to treat cancer and improve patient outcomes and further research is needed to continue to improve the understanding of these mechanisms and to identify new strategies to overcome drug resistance in cancer. This article highlights the research status and detection methods of cell membrane fluidity and permeability affecting tumor drug resistance.

Keywords: tumor, drug resistance, cell membrane, fluidity, permeability, techniques

Introduction

Today, cancer poses a sombre threat to human health and life. It is projected that there will be more than a million new cancer cases and 609,820 deaths in the US alone by 2023 (Siegel et al., 2023). It is also estimated that the frequency of cancer increases at a rate of 3% to 5% each year. Radiotherapy and chemotherapy are important clinical treatments for cancer. Nevertheless as cancer cells are open to a variety of chemotherapy drugs over time, their sensitivity to these drugs progressively deteriorates, resulting in multidrug resistance. Multidrug resistance (MDR) refers to tumor cells becoming impervious not only to the chemotherapeutic drug, but also to other drugs with different structures and functions as a result of long-term exposure to that drug (Xiang et al., 2020). As a result of

chemotherapy, drug resistance can be distributed into two types: natural drug resistance (drug resistance that exists at the beginning of the treatment) and acquired drug resistance (induced by a chemotherapeutic drug during the treatment process) (Wu et al., 2016).

A major cause of chemotherapy failure is the emergence of multidrug resistance. The main mechanism of tumor drug resistance involves: 1) Abnormal expression of ABC transporter superfamily, such as P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs), lung resistance proteins (LRPs) and mammary gland Drug efflux mechanism mediated by cancer resistance proteins (BCRPs); 2) Abnormal activity of topoisomerase II, reduced expression, reduced or lost targets of chemotherapeutic drugs, resulting in drug resistance (Sauna and Ambudkar, 2007; Huimin, 2005) Increased expression of glutathione-transferase, which catalyzes the combination of glutathione and chemotherapeutic drugs to form a complex, inactivating drug activity to produce drug resistance; 4) drug resistance mediated by enhanced DNA damage repair (Xiang et al.2020). Abnormal receptor expression leads to drug resistance mediated by abnormal apoptotic pathway (Zhou, 2016; Yang et al., 2015). As a result of the complexity and diversity of drug resistance, there are still many areas of unknowns despite the proposed mechanisms of tumor drug resistance mentioned above. One of the most widely studied drug resistance mechanisms is the overexpression of P-gp encoded by MDR1 (Xiang et al., 2020). In order to produce drug resistance, accumulation decreases. Drug-resistant tumor cells overexpressing P-gp have a significantly different membrane lipid composition, which affects its natural properties, such as fluidity, permeability, and phospholipid arrangement order (Baritaki et al., 2007). Researchers have discovered that chemotherapy drugs can be enhanced in sensitivity to tumor drug-resistant cells by altering the fluidity and permeability of tumor drug-resistant cell membranes. This may be due to changing the lipid composition or ratio of tumor drug-resistant cell membranes.

Structure and Composition of the Cell Membrane

The cell membrane, also known as the plasma membrane or cytoplasmic membrane, is a thin, flexible barrier that surrounds and encloses the contents of a cell. Cell membranes provide the structural basis for carrying out life activities. It is made up of a phospholipid bilayer, which is a double layer of phospholipid molecules that is arranged in a mosaic pattern (Singer and Nicolson, 1972). The phospholipid bilayer is composed of two layers of phospholipid molecules, with their hydrophobic tails facing each other and their hydrophilic heads facing outward. The structure of the cell membrane is fluid and dynamic, and the phospholipid molecules are constantly moving and interacting with each other. The fluidity of the cell membrane is determined by the composition and organization of the phospholipid bilayer, as well as by the presence of proteins and other molecules embedded in the membrane. A cell membrane is composed mainly of lipids and proteins, and lipid molecules can be categorized as phospholipids, cholesterol, and glycolipids.

A cell's internal environment cannot only be maintained, but it can also be accountable for the transmission of information and material between it and its external environment as well. The lipid bilayer model was first proposed by Gorter and Grendel, 1925 as a static and symmetrical model of the cell membrane. Over time, the understanding of the cell membrane has advanced to a highly

dynamic and asymmetrical structure. Phospholipids are a crucial component of the membrane and can be categorized into phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidylethanolamine (PE) (Xiang et al., 2020; Zalba and Hagen, 2017). PS, an essential component of the brain cell membrane, regulates various brain functions by influencing membrane fluidity and permeability. PI plays a vital role in metabolic regulation, signal transduction, and cell communication (Xiang et al., 2020; Zalba and Hagen, 2017). The composition and type of phospholipid molecules can differ between different cell membranes, regions on the membrane, and inner and outer phospholipid monolayers in bilayer lipids due to the role it plays in various physiological functions (Xiang et al., 2020). In the outer layer of the phospholipid monolayer, PC is particularly abundant, whereas PE and PS are predominant in the inner layer of the phospholipid monolayer (Alberts et al., 2002). At normal body temperature, cholesterol has a rigid structure because of its high melting point, which helps increase membrane stability, reduce water-soluble substance permeability, and contribute to the fluidity of cell membranes (Baritaki et al., 2007). In addition to completing most of its functions through lipids and proteins, the cell membrane also affects the normal division and proliferation of cells due to differences in composition (Hendrich and Michalak, 2003) . Overall, the structure and composition of the cell membrane are essential for the function of cells, as it controls the movement of molecules in and out of the cell and plays a key role in cell-cell communication and interaction.

Variances between Cancer Cells and Normal Cell Membranes

Cancer cells and normal cells have several differences in their cell membranes that can affect the effectiveness of drugs used to treat cancer and may contribute to tumor drug resistance. During the tumor development, phospholipid content of cell membranes can change. Merchant et al. (1991) used 31 P nuclear magnetic resonance spectroscopy to analyze phospholipids in human benign, malignant, and normal breast tissues. The results showed that compared with the phospholipid content of normal cell membranes, PC, PE, PI in malignant breast cancer cell membranes content increased. Compared with normal cells, the contents of sphingomyelin (SM), β-glycerophosphate and glycerol 3-phosphoserine in the colon cancer cell membrane increased. They measured phosphatidylcholine content in the membrane of malignant colon cancer cells at various differentiation stages by 31 P (P = 1215 MHz) NMR spectroscopy, and found that moderately differentiated colon cancer cells contain phosphatidylcholine in their membrane (Xiang et al., 2020, Merchant et al, 1995). Phosphatidylcholine content was significantly higher than that of poorly differentiated cells. According to Barker and Bowler, 1991, phospholipids do not fully reflect the differences between normal and tumor cell membranes. The membrane phospholipid composition content of liver cancer cells D23 and hepatic sarcoma cells MC7 did not differ significantly from normal rat liver cells (Xiang et al., 2020). However, the fluorescence polarization method proved that the cholesterol content in the cell membrane was significantly reduced. Compared with normal liver cells, the order of the membranes of the above two tumor cells was lower. By fluorescence polarization, Van-Blitterswijk et al. (1982) found that mouse

thymic leukemia cells had significantly higher membrane fluidity than normal thymocytes.

There was a significant increase in phospholipid ethanolamine, as well as an increase in unsaturated fatty acids, in tumor cell membranes compared to normal thymocyte membranes. Increasing fatty acid chains and decreased cholesterol and sphingomyelin are attributed to increased membrane disorder and decreased cholesterol/phospholipid ratio in tumor cell membranes (Xiang et al., 2020). The above studies have shown that the composition of tumor cell membranes is different in different types of tumor cells, and the degree of differentiation of tumors is different, and the proportion of membrane components will also be different.

Changes in the Membrane Composition of Tumor Drug-Resistant Cells

Tumor drug-resistant cells may have changes in the composition of their cell membrane that contribute to their resistance to drugs used to treat cancer. Changes in the composition of the cell membrane can affect the ability of drugs to interact with their target molecules or to enter the cell. Some of the changes in the membrane composition of tumor drug-resistant cells that may contribute to their drug resistance include: 1. Altered expression or localization of proteins: Tumor drug-resistant cells may have different levels or types of proteins embedded in the cell membrane, such as receptors, transporters, or enzymes, which can affect the uptake or efflux of drugs and other molecules. 2. Changes in the lipid composition: Tumor drug-resistant cells may have changes in the composition and organization of the lipid bilayer that makes up the cell membrane, which can affect the fluidity and permeability of the membrane. 3. Increased expression of membrane pumps: Tumor drug-resistant cells may have increased expression of proteins called ATP-binding cassette (ABC) transporters, which can pump drugs out of the cell and contribute to drug resistance (Xiang et al., 2020). During chemotherapy, drugs enter the cell through the membrane and reach their target by crossing the cell membrane. In tumors with drug-resistant cell membranes, drug resistance is facilitated by P-gp overexpression, which reduces drug accumulation intracellularly. The fluidity of the cell membrane is largely determined by the composition of the phospholipid bilayer. Changes in the saturation and length of fatty acid chains can alter the packing of phospholipids in the bilayer, affecting the fluidity of the membrane. For example, it has been shown that increasing the level of unsaturation of the fatty acid chains in the membrane can increase the fluidity of the membrane, resulting in decreased P-gp activity (Hendrich and Michalak, 2003). P-gp is more active in rigid membranes with low fluidity, and its activity decreases as the membrane becomes more fluid. One study showed that increasing the fluidity of the membrane by incorporating unsaturated fatty acids into the membrane decreased P-gp activity, while decreasing the fluidity of the membrane by incorporating saturated fatty acids increased P-gp activity (Drori et al., 1995). Another study demonstrated that cholesterol depletion from the membrane using cholesterol-sequestering agents reduced P-gp activity by disrupting its interaction with the membrane and altering the lateral mobility of P-gp within the membrane (Orlowski et al., 2007). In addition to changes in the lipid composition, alterations in the permeability of the membrane can also affect the activity of P-gp. One mechanism that describes the effect of membrane fluidity on P-gp activity is the role of lipid rafts. Lipid rafts are regions of the membrane that are enriched in cholesterol and sphingolipids and are thought to be important for the localization and

function of P-gp. Several studies have shown that P-gp is localized in lipid rafts, and that the fluidity of these rafts is important for P-gp activity (Siddiqui et al., 2007). Specifically, it has been proposed that the lateral mobility of P-gp within the membrane is influenced by the fluidity of the lipid rafts, and that this mobility is necessary for P-gp to interact with substrates and other components of the membrane. The P-gp expels its substrates into the extracellular medium, which incurs an energy cost for lipophilic species. Alternatively, it may operate as a drug flippase, moving its substrates from the inner to the outer leaflet of the membrane. This mechanism requires that drug molecules have a specific localization within each bilayer leaflet, rather than being randomly distributed. Evidence suggests that P-gp functions as an outwardly directed flippase for various fluorescent phospholipid and glycosphingolipid molecules in both intact cells and reconstituted proteoliposomes. Pgp-mediated drug efflux probably takes place by a flippase-like mechanism, as it requires ATP hydrolysis and is inhibited by known P-gp substrates, and inhibitory potency is highly correlated with their P-gp binding affinity (Sharom, 2014). The flippase model for P-gp function is well-supported by substantial evidence, which indicates that P-gp acts as an outwardly directed flippase for various fluorescent phospholipid and glycosphingolipid molecules in both intact cells and reconstituted proteoliposomes (Sharom, 2014). Cells that overexpress P-gp exhibit altered distribution and decreased accumulation of fluorescent phospholipid derivatives, along with increased outward transport of these analogs, which can be reversed by P-gp modulators. Studies have shown that purified P-gp can act as a broad-specificity, outwardly directed flippase for a variety of both short-chain and long-chain nitrobenzo-2-oxa-1,3-diazole (NBD)-labeled phospholipids and glycosphingolipids in proteoliposomes (Sharom, 2014). Pgp-mediated drug efflux is believed to occur through a flippase-like mechanism, as phospholipid and glycolipid flippase activity required ATP hydrolysis and was inhibited by known P-gp substrates, with inhibitory potency highly correlated with their P-gp binding affinity.

In the process of simulating the insertion of P-gp into the lipid bilayer in phospholipid liposomes, it was found that the fluidity of the membrane was enhanced; and the addition of cholesterol in the phospholipid liposomes could reduce the fluidity of the membrane and increase the ATPase activity of P-gp (Rothnie et al., 2001). By affecting the lipid composition of the cell membrane, the overexpression of P-gp may indirectly affect the biological characteristics of the cell membrane, which may explain the development of tumor drug resistance (Xiang et al., 2020). P-gp expression is commonly found in various types of cancer, including renal and colon carcinomas, melanomas, and central nervous system tumors. P-gp expression has been associated with drug resistance in these tumors, with levels often increasing after chemotherapy. P-gp is the primary cause of multi-drug resistance (MDR) in cancer cells, which is linked to reduced responses to chemotherapy and poor clinical outcomes (Sharom, 2014). Developing compounds that can inhibit Pgp-mediated MDR is a crucial goal in cancer therapy. Modulators, such as verapamil and cyclosporin A, have been identified as chemosensitizers that can reverse drug resistance in MDR cells in vitro. In comparison with mouse leukemia cells (P388), drug-resistant cells (P388/ADR) had reduced PC content and increased SM content, and electron spin resonance spectroscopy showed that the lipid structure of drug-resistant cells was more ordered (Xiang et al., 2020; Ramu et al., 1984; Ramu et al., 1983). Kok et al., 2000 found that in HT29

cells overexpressing MRP1, glucosylceramide and galactosylceramide were increased in the composition ratio of sphingolipids. Human leukemia lymphocytes and vinblastine-resistant cells were analyzed by May et al., 1988, and it was found that the membrane lipid components of drug-resistant cells had a 50% increase in cholesterol and 30% increase in phospholipids, respectively, and a 60% increase in lipoproteins (Xiang et al., 2020). Researchers have shown that phospholipid and cholesterol are embedded between phospholipid molecules in tumor drug-resistant cells, and the polar head of cholesterol is close to that of phospholipid molecules. As a result of the adjacent phospholipid hydrocarbon chains (Peetla et al., 2013), the order and rigidity of the cell membrane are significantly improved (Hendrich and Michalak, 2003, Eytan et al., 1996), resulting in a significant change in the cell membrane's fluidity and permeability, making chemotherapy drugs more difficult to enter the cell, leading to resistance to chemotherapy drugs.

Effect of Changes in Cell Membrane Fluidity on Tumor Drug Resistance

Changes in the fluidity of the cell membrane can affect the effectiveness of drugs used to treat cancer and may contribute to tumor drug resistance. Membrane fluidity refers to the extent to which the lipid bilayer of a cell membrane can move and change its shape. This property is important for various cellular processes such as signaling, transport, and fusion. The fluidity of the membrane is dependent on the composition of its lipids, including the length and saturation of the fatty acid tails, the presence of cholesterol, and the presence of various membrane proteins. Various researches had shed light on the dynamic nature of membrane fluidity and its importance in various cellular processes. For example, studies have shown that changes in the fluidity of the membrane can affect the activity of membrane proteins, such as ion channels and receptors, by altering their conformation and localization.In addition, new technologies such as super-resolution microscopy and single-molecule imaging have allowed researchers to directly observe and manipulate membrane fluidity at the nanoscale level. This has led to a better understanding of the mechanisms underlying the regulation of membrane fluidity, such as the roles of lipid rafts and membrane-cytoskeleton interactions. Cell membrane fluidity is determined by the composition and organization of the lipid bilayer that makes up the membrane (Baritaki et al., 2007), as well as by the presence of proteins and other molecules embedded in the membrane. There are several factors that influence membrane fluidity for cell membrane components, including cholesterol content, saturation levels, fatty acid chain length, lecithin/sphingomyelin ratios and interactions between lipids and proteins (Szlasa et al., 2020, Pallarestrujillo et al., 2000). Moreover, external physical factors, such as temperature, will affect it as well (Zalba and Hagen, 2017). It is the fluidity of the normal cell membrane that allows substances to exchange inside and outside the cell. If the properties of the plasma membrane change in tumor cells, the fluidity of the cell membrane will also change. One of the main mechanisms by which changes in cell membrane fluidity affect tumor drug resistance is through alterations in the expression of drug transporters, such as P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP). These transporters are membrane proteins that play a key role in the efflux of drugs from the cell. Studies have shown that increased expression of P-gp and MRP in tumor cells can lead to decreased intracellular drug accumulation and increased drug efflux, resulting in drug resistance (Sauna and

Ambudkar, 2007). The reduction of drug resistance of tumor drug-resistant cells is associated with changes in P-gp function and membrane fluidity (Callaghan et al., 1993). In addition to drug transporters, changes in cell membrane fluidity can also affect the uptake and efflux of drugs by altering the expression of ion channels and transporters. For example, changes in the expression of aquaporins, which are membrane proteins that regulate the movement of water and other small molecules across the cell membrane, can affect the uptake and efflux of drugs. A study by Frede et al., 2013 showed that the expression of aquaporin-1 (AQP1) was significantly higher in drug-resistant leukemia cells than in drug-sensitive cells, and that inhibition of AQP1 increased the intracellular accumulation of chemotherapy drugs and enhanced drug sensitivity. Cancer cells may alter the fluidity of their cell membrane in a way that affects the effectiveness of drugs used to treat the cancer. For example, cancer cells may have increased fluidity of the cell membrane, which can make it easier for drugs to interact with their target molecules or to enter the cell. On the other hand, cancer cells may also have decreased fluidity of the cell membrane, which can make it more difficult for drugs to enter the cell and may contribute to drug resistance.

Improved cell membrane fluidity is important for improving tumor drug resistance (Baritaki et al., 2007). In tumor-resistant cells, the increase or decrease in membrane fluidity can lead to increased drug uptake (Hendrich and Michalak, 2003) . Ginsenoside Rg3 has been shown to reduce membrane fluidity in human fibroblast carcinoma cells and inhibit the activity of P-gp, thereby reducing drug efflux and reversing drug resistance (Kwon et al., 2008). Tetrandrine reduced membrane fluidity in human breast cancer cells (MCF-7/ADR) doxorubicin-resistant to doxorubicin in a concentration-dependent manner (Fu et al., 2002). In contrast, the membrane fluidity of human leukemia drug-resistant cells (K563/MDR) was increased after microwave treatment, but also effectively reversed the drug resistance of K562/MDR cells (Xiang et al., 2020, Ziru et al., 2002). Although the mechanism by which changing cell membrane fluidity reverses tumor drug resistance is still ambiguous, it is clear that affecting cell membrane fluidity may be a new strategy for improving resistance to tumor drugs. Further, the phospholipid bilayer's structural disruption and changes in cell membrane fluidity may facilitate the entry of cells into apoptosis (Baritaki et al., 2007). The impact of early cell membrane fluidity on tumor cell growth, proliferation, and drug resistance has once again been demonstrated by studies showing that lipid compounds and drugs can enhance early cell membrane fluidity and promote cell apoptosis (Fujimoto et al., 1999). By investigating this relationship between cell membrane fluidity and tumor drug resistance, it is possible to get valuable insights that can potentially lead to innovative approaches for enhancing the efficacy of anticancer therapies.

Detection Method of Cell Membrane Fluidity

1. Fluorescence Polarization Method

Fluorescence polarization is a technique that measures the degree to which the fluorescence emitted by a sample is polarized, or oriented, in a particular direction. Fluorescence polarization is based on the fact that the fluorescence emitted by a sample will be more strongly polarized when the sample is excited by linearly polarized light, as compared to unpolarized light. The fluorescence polarization method involves measuring the intensity of the fluorescence emitted by a sample when it is excited by linearly polarized light, and comparing it to the intensity of the

fluorescence emitted when the sample is excited by unpolarized light. The difference in the intensity of the fluorescence signals is used to calculate the degree of polarization of the sample. One of the most commonly used fluorescent probes for measuring membrane fluidity is 1,6 diphenyl-1,3,5,-ethanetriene (DPH), embedded in the hydrocarbon chain region of the fatty acids in the cell membrane.

By using a fluorescence spectrophotometer to measure the fluorescence intensity of the fluorescent probe and the membrane lipid, fluorescence polarization is used to determine membrane fluidity (Xiang et al., 2020). There is an association between the degree of fluorescence polarization (P) and the degree of fluidity of the cell membrane (Yonggui et al., 2000), where the larger the P value, the weaker the fluidity, and vice versa (Guiqin et al., 2004). As per the findings of Kechun et al., 1981, the fluidity of lipids in the ascites cancer cell membrane is influenced by the microviscosity of the membrane. Conversely, when the microviscosity increases, the fluidity of the cell membrane weakens, as stated by Xiang et al. (2020). In another study conducted by Yuan et al. (2015), the fluidity of erythrocyte membranes was assessed using DPH fluorescence spectrophotometry. Fluorescence polarization is a sensitive method for detecting small changes in the orientation or conformation of molecules, and it is often used to study the interactions between molecules, such as the binding of ligands to receptors or the folding of proteins. It can also be used to study the mobility of molecules in cells or tissues, as the degree of polarization of the fluorescence signal can be affected by the motion of the molecules. This method is widely used because of its rapid detection, sensitivity, and repeatability.

2. Fluorescence Bleaching Recovery Technology

Fluorescencerecovery after photobleaching (FRAP) is a technique that is used to measure the brightness or intensity of a fluorescence signal over time. Fluorescence bleaching occurs when the intensity of the fluorescence signal decreases over time due to the loss of fluorescence from the dye or probe used to label the molecule of interest. Fluorescence bleaching recovery technology involves measuring the intensity of the fluorescence signal before and after bleaching, and using this information to calculate the rate of recovery of the fluorescence signal. In fluorescent recovery after photobleaching, fluorescent substances are labeled on cell membrane proteins or membrane lipids, and then laser light is used to bleach out some of the fluorescence. The fluorescence of the bleached part can be restored after laser irradiation is stopped if the membrane protein or cell membrane has fluidity. Based on the recovery speed, the diffusion rate of membrane proteins or membrane lipids can be calculated and the membrane fluidity can then be calculated (Axelrod et al., 1976; Xiang et al., 2020). In recent years, advances in microscopy and labeling techniques have made FRAP an increasingly powerful tool for studying membrane fluidity. For example, it is now possible to selectively label different types of lipids or proteins within the membrane, allowing researchers to study their mobility and interactions in more detail. Using specific probes to label the lipids of myocardial cell membranes, Zhe et al., 2017 determined the effect of bupivacaine and long-chain lipid emulsions on the fluidity of myocardial cell membranes by using laser confocal microscopy and fluorescence bleaching recovery. Fluorescence bleaching recovery technology can be used to study a variety of phenomena,

including the stability of fluorescent dyes, the dynamics of protein-ligand interactions, and the mobility of molecules in cells or tissues. In addition to studying membrane fluidity in intact cells or tissues, FRAP can also be used to study the properties of artificial lipid bilayers in vitro. This can be useful for understanding how different lipid compositions or modifications affect membrane properties, and for testing the efficacy of potential drugs or therapeutic agents. It can also be used to study the properties of fluorescent proteins, such as their fluorescence intensity and lifetime.

Fluorescence bleaching recovery technology can be used in conjunction with various fluorescence imaging techniques, such as fluorescence microscopy or fluorescence spectroscopy. It is a powerful tool for studying the properties and behaviors of fluorescently labeled molecules, and has a wide range of applications in the life sciences and other fields.

3. Electron Spin Resonance Spectroscopy

Electron spin resonance (ESR) spectroscopy is a technique that can be used to detect the fluidity of cell membranes. ESR spectroscopy detects the motion of unpaired electrons in a sample, which can be used to measure the fluidity of the membrane. The technique involves adding a spin probe, which is a molecule that contains an unpaired electron, to the membrane of interest. The spin probe molecules become embedded in the membrane, and their motion can be detected using ESR spectroscopy. The ESR spectrum of a spin probe in a fluid membrane is broad, while in a more rigid membrane, the spectrum is narrower. The amplitude of the ESR signal reflects the degree of molecular motion of the spin probe, which is in turn influenced by the fluidity of the membrane. By analyzing the ESR spectra of the spin probe in different membranes, it is possible to compare the fluidity of the membranes. One commonly used spin probe for ESR spectroscopy is 5-doxyl stearic acid (5-DSA). The spin probe is added to the membrane of interest, and the sample is then analyzed using an ESR spectrometer. The ESR spectrum is analyzed to determine the fluidity of the membrane. Several studies have used ESR spectroscopy to detect changes in cell membrane fluidity. For example, a study by Pawlikowska-Pawlęga et al., (2014) used ESR spectroscopy to investigate the effects of cholesterol on the fluidity of the plasma membrane in HeLa cells. The authors found that the presence of cholesterol decreased the fluidity of the membrane, as indicated by a narrower ESR spectrum. Another study by Spasojević, 2011 used ESR spectroscopy to investigate the effects of oxidative stress on the fluidity of the mitochondrial membrane. The authors found that exposure to hydrogen peroxide decreased the fluidity of the mitochondrial membrane, as indicated by a narrower ESR spectrum. In addition to its ability to provide information about the fluidity of the membrane, ESR spectroscopy can also provide information about the physical state of the membrane, such as its order or disorder. One of the key advantages of ESR spectroscopy is its ability to directly measure the motion of the lipids in the membrane, rather than relying on indirect measures such as fluorescence recovery after photobleaching (FRAP). This makes it a particularly valuable tool for studying the fluidity of membranes under a wide range of conditions, including those that may alter the behavior of fluorescent probes used in FRAP. ESR spectroscopy works by measuring the behavior of unpaired electrons in the membrane. These unpaired electrons are generated by exposing the membrane to a free radical probe that becomes trapped in the membrane. The unpaired electrons in the probe then interact with the

surrounding lipids, allowing their motion to be directly measured. One common approach in ESR spectroscopy is to use spin-labeled fatty acids, which are fatty acids that have been modified to include a stable free radical group. These spin-labeled fatty acids can be incorporated into the membrane and their motion can be monitored by ESR spectroscopy. The ESR spectra obtained from spin-labeled fatty acids can be analyzed to obtain information about the rotational motion and lateral diffusion of the lipids in the membrane, which can in turn be used to calculate the fluidity of the membrane. ESR spectroscopy has been used to study the fluidity of a wide range of biological membranes, including those from red blood cells, nerve cells, and cancer cells. For example, ESR spectroscopy has been used to investigate the effect of cholesterol on the fluidity of the plasma membrane in breast cancer cells (Zeisig et al., 2007). Another study used ESR spectroscopy to examine the effect of cell aging on the fluidity of the plasma membrane in human fibroblasts (Alonso et al., 2019). In all, ESR spectroscopy is a powerful tool for studying the fluidity of biological membranes, and has been used extensively to investigate the physical properties of cell membranes under a wide range of conditions.

4. Laurdan Two-Photon Microscopy

Laurdan is a membrane probe that exhibits a blue shift in its excitation and emission spectra when it is in a less polar environment and a red shift in a more polar environment. This property makes it a useful tool for detecting changes in membrane fluidity. Two-photon microscopy is a technique that allows for high-resolution imaging of samples using a low-energy, long-wavelength laser, which reduces photodamage to the sample. Laurdan two-photon microscopy uses this probe to measure the polarity of the local environment of the cell membrane. The probe is excited by a two-photon laser, and the emitted fluorescence is collected and used to calculate a generalized polarization (GP) value. GP values range from -1 to 1, with values closer to 1 indicating a more ordered, less fluid membrane and values closer to -1 indicating a more disordered, fluid membrane. Laurdan two-photon microscopy has been used to study membrane fluidity in a variety of biological systems, including cells, tissues, and even live animals. For example, a study by Balogh et al. (2011) used Laurdan two-photon microscopy to measure membrane fluidity in human fibroblast cells. They found that cells treated with a membrane fluidizer had a decreased GP value, indicating an increase in membrane fluidity. Another study by Yamamoto and Ando (2013) used Laurdan two-photon microscopy to investigate the effect of cholesterol depletion on the fluidity of giant unilamellar vesicles (GUVs). They found that the GP value decreased when cholesterol was removed from the membrane, indicating an increase in membrane fluidity. Another study by Jay and Hamilton (2017) used Laurdan two-photon microscopy to measure changes in membrane fluidity during the development of zebrafish embryos. The researchers found that the membrane fluidity of the yolk sac and somatic cells increased during early embryonic development, and that this increase was correlated with changes in the lipid composition of the membranes. Laurdan two-photon microscopy has also been used to investigate the effects of drugs and other agents on membrane fluidity. A study by Erazo-Oliveras et al. (2022) used Laurdan two-photon microscopy to investigate the effects of curcumin, a natural compound with anti-inflammatory and anti-cancer properties, on membrane fluidity in cancer cells. The researchers found that curcumin increased membrane fluidity in cancer cells, which was

correlated with increased cellular uptake of curcumin and increased cytotoxicity. Laurdan two-photon microscopy has several advantages over other techniques for measuring membrane fluidity. It is non-invasive and can be used in live cells and tissues. It is also relatively easy to perform and does not require any special sample preparation. However, it is important to note that the GP value is affected by other factors besides membrane fluidity, such as temperature and probe concentration, so careful controls and calibrations are necessary to ensure accurate results. Overall, Laurdan two-photon microscopy is a powerful tool for investigating membrane fluidity in a wide range of biological systems, from cells to whole organisms. Its sensitivity, specificity, and versatility make it a valuable tool for basic research, drug development, and clinical applications.

The Effect of Cell Membrane Permeability Changes on Tumor Drug Resistance

As a functional characteristic of the cell membrane, membrane permeability refers to how easily substances enter and leave the cell. Changes in the permeability of the cell membrane can affect the effectiveness of drugs used to treat cancer and may contribute to tumor drug resistance. For example, certain cancer cells may have increased permeability of the cell membrane, which can allow drugs to more easily enter the cell and interact with their target molecules. On the other hand, cancer cells may also develop decreased permeability of the cell membrane, which can make it more difficult for drugs to enter the cell and may contribute to drug resistance. It is not just the fact that the P-gp pumps out the drug from the cell that causes tumor drug resistance, but also the physical characteristics of the cell membrane, which reduce the diffusion rate of the drug and make it easier for it to enter the cell, may affect the drug when it enters the cell (Xiang et al., 2020). In cancer cells, the overexpression of P-gp can lead to resistance to chemotherapeutic drugs. This occurs because P-gp pumps these drugs out of the cancer cells, thereby reducing their effectiveness. P-gp achieves drug efflux by using energy from ATP hydrolysis to transport drugs from the cytoplasm of the cell to the extracellular space. The P-gp protein has a large transmembrane domain that forms a channel through which drugs can pass. The channel is lined with hydrophobic amino acid residues that interact with the hydrophobic portions of the drug molecules. Changes in the lipid composition of the membrane can alter the conformation and activity of P-gp, thereby affecting its ability to pump out drugs, while increasing the degree and speed of drug entry into the cell is of great importance for overcoming tumor drug resistance because the drug molecules within are reduced (Hendrich and Michalak, 2003).

Developing drugs that can alter the lipid composition or permeability of the membrane may increase the efficacy of existing drugs by reducing drug resistance. Alternatively, targeting P-gp itself may be more effective in cells with specific membrane properties. With a certain intensity of ultrasound, Jia et al., 2015 improved the permeability of the cell membrane, increased drug accumulation in MCF-7/ADR cells and reversed drug resistance in MCF-7/ADR cells. Both SM and glycolipids reduce membrane permeability in the outer leaflet of a cell membrane, but cholesterol is the decisive factor. By reducing cholesterol content, increasing membrane permeability, and reducing membrane rigidity, these compounds reduce tumor drug resistance. Other changes in cell membrane permeability may also affect the effectiveness of drugs used to treat cancer. For example, cancer cells may alter the expression or localization of proteins on the cell surface, such as transporters or receptors that play a role in the uptake or efflux

of drugs (Xiang et al., 2020). This can also contribute to drug resistance by affecting the ability of drugs to interact with their target molecules or to enter the cell. As phospholipid unsaturation increases, membrane permeability increases, and drug accumulates in the cell, the effect of reversing drug resistance increases (Hill and Zeidel, 2000). Apigenin enhanced cell membrane permeability in a dose-dependent manner and became an important factor in inducing apoptosis (Zhu et al., 2016). The PS component serves not only as an important component of phospholipids in this process, but also as a signal of early apoptosis when the cell membrane is damaged and the membrane permeability increases, PS is flipped from the inner leaflet of the cell membrane to the outer leaflet by flippase (Demchenko, 2012). A pro-apoptotic cationic host defense peptide containing lysine or arginine selectively binds to tumor cells through electrostatic interactions, causing apoptosis and reversing tumor drug resistance in human breast cancer paclitaxel-resistant cells (MCF-7/Taxol) (Xiang et al.2020, Dai et al., 2017). Several drugs targeting ion channels have been shown to be effective in overcoming drug resistance in cancer cells.

For example, a study by Spreckelmeyer et al. (2014) showed that the potassium channel blocker, tetraethylammonium (TEA), enhanced the efficacy of the chemotherapy drug, cisplatin, in cisplatin-resistant lung cancer cells by increasing cell membrane permeability and reducing the expression of drug transporters. By targeting membrane components and transporters, it is possible to enhance drug delivery and overcome resistance, while innovative drug delivery systems can exploit altered membrane permeability for improved therapeutic efficacy.

Detection method of cell membrane permeability

1. Fluorescence Labeling Method

Fluorescence labeling is a widely used technique in biology that has revolutionized the field of molecular imaging and cell biology. This technique offers a non-invasive way to visualize and study biological molecules, and is based on the fact that certain molecules can absorb light of a specific wavelength and emit it at a longer wavelength. The fluorescent dye or molecule is attached to the biological molecule of interest like protein, nucleic acid which then emits a fluorescence signal that can be detected, visualized and imaged using specialized microscopes and cameras. Fluorescence labeling can be used for a variety of purposes, including imaging, identifying specific molecules or cells, and studying the interactions between molecules. Flow cytometry is one of the most commonly used techniques for fluorescence labeling in biological research, and it allows for high-throughput screening of large numbers of cells or molecules. By using this technique, Ling et al. (2016) studied the effect of *Physcomitrella goldenscens* extract on K562 cells' membrane permeability using fluorescein diacetate/propidium iodide (FDA/PI) double staining. This method is based on the principle that fluorescein diacetate can enter live cells and be hydrolyzed by intracellular esterases, resulting in the production of fluorescein, which emits green fluorescence. Propidium iodide, on the other hand, can only enter dead cells with compromised membranes, and it intercalates into the DNA of the dead cells and emits red fluorescence. Guangyao et al., 2017 found that algae-dissolving active substances can destroy the integrity of algae cell membrane through acridine orange/ethidium bromide (AO/EB) double staining method. This method involves the use of acridine

orange, which can enter both live and dead cells, and stains the nuclei of cells with bright green fluorescence. Ethidium bromide, on the other hand, can only enter cells with compromised membranes, and it intercalates into the DNA of the dead cells and emits bright red fluorescence. While fluorescence labeling is a sensitive and easy-to-use technique, it can be affected by several factors during imaging. One of the main challenges in fluorescence imaging is the fast quenching speed of fluorescence, which can result in a loss of signal over time. Other factors that can affect fluorescence imaging include the imaging parameters, such as the excitation wavelength, exposure time, and magnification, as well as the properties of the fluorescent dye or molecule used for labeling.

2. SEM

Scanning electron microscopy (SEM) is a imaging technique that uses a focused beam of electrons to produce detailed, high-resolution images of the surface of a sample. SEM is often used to study the morphology, or shape, of cells and tissues, as well as their surface features and textures. According to Goldstein et al. (2018) SEM can provide high-resolution images with high depth of field, allowing for detailed observation of surface morphology, topography, and texture. The images generated by SEM can also provide information on chemical composition and crystallographic structure through the use of various detectors, such as energy-dispersive X-ray spectroscopy (EDS) and electron backscatter diffraction (EBSD). SEM can be used to detect changes in cell membrane permeability, as the permeability of the membrane can affect the appearance of the cell surface. The permeability of the cell membrane is responsible for controlling the movement of molecules in and out of the cell, and changes in this permeability can have a significant impact on the overall function of the cell. One of the key advantages of SEM is that it can be used to visualize changes in the size or shape of cells, as well as changes in the distribution of proteins or other molecules on the surface of the cells. These changes may be indicative of changes in the permeability of the cell membrane, and SEM can provide detailed images that allow researchers to study these changes in great detail. Surface irregularities are studied by bombarding them with atomic rays, which emit secondary electrons and other signals that are detected by detectors after they are received. The changes on the surface of the cell membrane were observed by SEM after ganglioside antibody was injected into mouse leukemia cells (Roquenavarro et al., 2008). SEM can clearly observe morphological changes on cell surfaces including microvilli, pseudopodia, and perforation, as well as changes in microvilli under different conditions (Xiang et al., 2020). It is worth noting that SEM is a surface imaging technique and cannot provide information about the interior of cells or tissues. Other techniques, such as transmission electron microscopy or fluorescence microscopy, may be needed to visualize changes in the interior of cells or tissues or to study other aspects of cell membrane permeability.

3. Electrophysiology

Another method to measure cell membrane permeability is electrophysiology, which involves the measurement of changes in the electrical properties of cells in response to changes in membrane permeability. Electrophysiology is a powerful tool that allows for the measurement of the electrical properties of cells in real-time. The technique is based on the fact that ion channels, which are transmembrane proteins that regulate the flow of ions across the cell membrane, play a critical role in regulating cell membrane permeability.

Changes in ion channel activity can alter the electrical properties of the cell membrane, which can be measured using electrophysiological techniques. This technique can provide real-time information about changes in membrane permeability and ion transport (Deng et al., 2004). Patch-clamp electrophysiology is a widely used technique to investigate ion channels in cell membranes, including the effects of drugs and toxins on membrane permeability (Zheng et al., 2004). One widely used electrophysiological technique for investigating ion channels is the patch-clamp technique, which was first developed by Erwin Neher and Bert Sakmann in the 1970s (Neher and Sakmann, 1976). The patch-clamp technique involves attaching a glass pipette to the surface of a cell and forming a high-resistance seal between the pipette and the cell membrane. This allows for the measurement of the electrical properties of a small patch of cell membrane, typically on the order of a few square micrometers. Patch-clamp electrophysiology can be used to investigate the effects of drugs and toxins on membrane permeability. For example, the technique has been used to study the effects of certain antibiotics on bacterial cell membranes (Kreir et al., 2008). Additionally, the patch-clamp technique has been used to study the effects of toxins on ion channels, such as the effects of tetrodotoxin on sodium channels (Brau et al., 1988). In addition to the patch-clamp technique, other electrophysiological techniques can be used to measure changes in membrane permeability, such as impedance spectroscopy (Matthews and Judy, 2003). Impedance spectroscopy measures changes in the electrical impedance of a cell or tissue sample in response to changes in membrane permeability, and has been used to study the effects of drugs and toxins on cell membranes (Crowell et al., 2020). These techniques have applications in drug discovery, toxicology, and basic research in cell biology.

4. Atomic force microscopy

Atomic force microscopy (AFM) is a high-resolution imaging technique that can be used to study biological samples, including cells and cell membranes. AFM is a powerful tool for studying membrane permeability because it can directly measure the mechanical properties of the cell membrane, including its stiffness and elasticity. In this way, AFM can provide valuable insights into the permeability of the cell membrane, which is critical for many cellular processes, including drug uptake and cell signaling. The basic principle of AFM is to use a small probe with a sharp tip to scan the surface of a sample in a raster pattern, similar to how a record player reads the grooves on a vinyl record. The tip is attached to a cantilever that deflects as it interacts with the sample, and the deflection is measured with a laser beam. By moving the sample and the probe relative to each other, a 3D image of the sample surface can be generated with nanometer-scale resolution. To study membrane permeability with AFM, the first step is to immobilize the cells on a substrate. This can be done by attaching the cells to a surface through a process called cell adhesion, which involves coating the substrate with molecules that the cells can bind to. Once the cells are immobilized, the AFM probe can be used to indent the cell membrane and measure its mechanical properties. There are several ways that AFM can be used to study membrane permeability. One approach is to measure the stiffness of the cell membrane using a technique called force spectroscopy. In force spectroscopy, the AFM probe is used to apply a small force to the cell membrane, and the resulting indentation of the membrane is measured. By analyzing the relationship between the applied force and the resulting

indentation, the stiffness of the membrane can be calculated. Changes in membrane stiffness can be an indicator of altered permeability, as increased permeability can lead to changes in the organization and composition of the membrane. Another approach is to use AFM to measure the force required to rupture the cell membrane, which is called membrane disruption force spectroscopy (MDFS). MDFS involves increasing the force applied to the cell membrane until it ruptures, and measuring the force required to do so. Changes in the rupture force can be an indicator of changes in membrane permeability. AFM can also be used to directly visualize the effects of membrane-permeabilizing agents on the cell membrane. For example, AFM has been used to study the effects of chemotherapeutic drugs on cancer cell membranes. One study used AFM to show that the drug doxorubicin caused changes in the stiffness and elasticity of the cell membrane, which could be indicative of increased membrane permeability (Agarwala et al., 2022). In another study, AFM was used to visualize the formation of nanoscale pores in the cell membrane caused by the drug cytochalasin B (Lamprecht et al., 2014). Overall, AFM is a powerful tool for studying membrane permeability in cancer cells.

5. Lactate Dehydrogenase Release Assay

A lactate dehydrogenase (LDH) release assay is a laboratory test that measures the amount of LDH in a sample. LDH is an enzyme that is involved in the anaerobic metabolism of glucose and other sugars because it does not require oxygen as a co-factor for its enzymatic activity, and is found in many tissues in the body, including the liver, heart, and skeletal muscles. Cell membrane damage is often detected by lactate dehydrogenase release (lactate dehydrogenase, LDH) and by the enhanced permeability of the membrane caused by the damaged membrane. The assay involves adding a sample containing LDH, such as cell culture supernatant or tissue homogenate, to a solution containing specific enzymes and substrates. The LDH in the sample catalyzes the conversion of the substrate to a product, and the amount of product produced is measured and used to calculate the amount of LDH in the sample (Xiang et al., 2020). When the cell membrane is damaged and its permeability is increased, LDH can be released outside the cell. LDH is an extremely stable cytoplasmic enzyme, which normally cannot pass through the membrane. As a result, lactic acid is converted into pyruvate, which reacts with tetrazole to produce red. A microplate reader can detect the formazan compound after dissolution, and the percentage of LDH activity in the total LDH can be calculated, which reflects the extent of damage to the membrane (Xiang et al., 2020). In a time-concentration-dependent manner, polypeptide BF-30 caused pancreatic cancer cells to release LDH under its action at different concentrations and times, suggesting that polypeptide BF-30 damages pancreatic cancer cell membranes in a time-concentration-dependent manner (Wang et al., 2017). Using the LDH release assay, Chang et al. (2011) also examined the effect of hepcidin 1-5 on human fibrosarcoma cells and human cervical cancer cells. This method has the advantages of being simple to use, highly sensitive, low in cost, and highly detectable. The LDH release assay can be used to evaluate the effectiveness of a treatment or to monitor the health of cells or tissues. It is often used in cancer research, as cancer cells are often more sensitive to certain treatments and may release more LDH in response to treatment. The LDH release assay can also be used to evaluate the effectiveness of a drug or to monitor the health of cells or tissues in other contexts, such as in drug development or toxicology studies. The LDH

release assay is relatively simple, quick, and cost-effective compared to other cytotoxicity assays such as the MTT assay and the trypan blue exclusion assay (Fotakis and Timbrel, 2006). However, the LDH release assay has some limitations. For example, the release of LDH is not always indicative of cell death, and some cells may release LDH without losing their viability. In addition, certain experimental conditions, such as pH, temperature, and the type of culture medium, can affect the accuracy and reproducibility of the assay.

6. Magnetic resonance imaging

Magnetic resonance imaging (MRI) is a non-invasive imaging technique that uses magnetic fields and radio waves to create images of the body. It has been widely used in medical diagnosis and research due to its high spatial and temporal resolution, and the ability to provide information on the structural and functional properties of tissues. Recently, MRI has been explored as a tool to evaluate membrane permeability in cancer cells. One of the most commonly used methods to assess membrane permeability using MRI is dynamic contrast-enhanced MRI (DCE-MRI). DCE-MRI involves the injection of a contrast agent, typically a gadolinium-based compound, into the bloodstream, and the subsequent measurement of the signal intensity in the tissue of interest as a function of time. The contrast agent leaks out of the blood vessels and accumulates in the extravascular space, leading to an increase in signal intensity. The rate of contrast agent uptake and washout can provide information on the membrane permeability and perfusion of the tissue.By analyzing the time course of contrast agent uptake and clearance from the tumor, researchers can estimate parameters such as the volume of the extravascular extracellular space (EES), the permeability-surface area product (PS) of the tumor vasculature, and the fractional plasma volume (fPV) of the tumor. DCE-MRI has been used to evaluate the membrane permeability of various types of cancer cells, including breast, prostate, and brain tumors. For example, in a study of breast cancer patients, DCE-MRI was able to distinguish between benign and malignant lesions based on differences in membrane permeability and perfusion (Choyke et al., 2003). Similarly, in a study of prostate cancer patients, DCE-MRI was able to predict the likelihood of tumor recurrence based on differences in membrane permeability and perfusion (Alonzi et al., 2007). Another method that has been used to assess membrane permeability using MRI is diffusion-weighted imaging (DWI). DWI measures the diffusion of water molecules in tissue, and can be used to evaluate changes in the extracellular space associated with membrane permeability. In cancer cells, increased membrane permeability can lead to an increase in the extracellular space, which can be detected using DWI.DWI has been used to evaluate the membrane permeability of a variety of cancers, including breast, prostate, and liver tumors. For example, in a study of breast cancer patients, DWI was able to differentiate between benign and malignant tumors based on differences in membrane permeability (Sinha et al., 2002). In short, MRI has been explored as a tool to evaluate membrane permeability in cancer cells which is quite useful in the diagnosis, prognosis, and monitoring of various types of cancer, and has the potential to improve patient outcomes.

7. TEER

Another commonly used methods to study cell membrane permeability is the measurement of transepithelial electrical resistance (TEER) using a voltmeter. TEER is a measure of the integrity of the cell monolayer like Caco-2, HT29, MDCK, A549 and is a

reflection of the tight junctions between the cells. It is an indirect measure of cell membrane permeability, as increased TEER indicates tighter junctions, which reduces the paracellular transport of drugs across the cell monolayer. The TEER method has been used extensively to study the effects of various drugs and compounds on cell membrane permeability in Caco-2 cells (Anderberg and Artursson, 1993). To measure TEER in Caco-2 cells, the cells are grown to confluency on a permeable membrane, such as a polycarbonate filter or a Transwell insert. The membrane separates two compartments, apical and basolateral, which represent the luminal and blood sides of the intestinal epithelium, respectively. The voltmeter is then used to measure the electrical resistance across the membrane, and the readings are recorded. The values are expressed as ohm*cm^2 to account for the surface area of the membrane. The measurement of TEER is a non-destructive method that can be used to monitor the integrity of the cell monolayer over time. A decrease in TEER value indicates an increase in paracellular transport, which could be due to the disruption of tight junctions between the cells. Conversely, an increase in TEER value indicates tighter junctions, which reduces paracellular transport.TEER measurement has several advantages over other methods of studying cell membrane permeability. It is non-invasive and does not require the use of exogenous markers or probes, which can interfere with the cellular processes being studied. Moreover, TEER measurement can be performed in real-time, allowing for the monitoring of drug effects on cell membrane permeability over time. Several studies have used TEER measurement to study the effects of various compounds on cell membrane permeability in Caco-2 cells. For example, it has been shown that the addition of polyphenols, such as quercetin and resveratrol, to the apical compartment of Caco-2 cells increased TEER values, indicating tighter junctions (JanssenDuijghuijsen et al., 2017). Similarly, it has been shown that the addition of the anti-inflammatory drug, dexamethasone, to the apical compartment of Caco-2 cells increased TEER values and reduced paracellular transport (Beck et al., 2007). One advantage of the TEER method is that it provides an indirect measure of cell membrane permeability, as increased TEER indicates tighter junctions between cells, which reduces the paracellular transport of drugs across the cell monolayer. This makes the TEER method a useful tool for evaluating the effects of various drugs and compounds on cell membrane permeability in Caco-2 cells (Anderberg and Artursson, 1993). Another advantage of the TEER method is that it is a non-destructive and non-invasive technique, which means that the cells can be used for further experiments after the TEER measurement. This makes the TEER method a useful tool for monitoring the integrity of cell monolayers over time, as well as for evaluating the effects of different culture conditions on cell membrane permeability. However, the TEER method also has some disadvantages that need to be considered. One disadvantage is that the TEER measurements are affected by a number of factors, such as temperature, pH, and culture medium composition, which can affect the tight junctions between cells and the overall integrity of the cell monolayer (Srinivasan et al., 2015). This means that it is important to standardize the experimental conditions when using the TEER method to ensure accurate and reproducible results. Another disadvantage of the TEER method is that it does not provide information on the permeability of specific molecules or drug compounds across the cell membrane. Instead, it provides an overall measure of cell membrane permeability, which can be affected by a number of factors. This means that it is

important to complement the TEER measurements with other assays, such as the microfluidic permeability assay (MPA), to obtain a more comprehensive understanding of cell membrane permeability and drug transport across cell membranes.

8. Microfluidic Permeability Assay

The microfluidic permeability assay (MPA) is a new method for studying drug permeability across cell membranes. This method uses microfluidic channels to create a continuous flow of drug molecules across a monolayer of cells like Caco-2, HT29, MDCK, A549 cells. The permeability of the drug molecules is measured by monitoring their concentration in the effluent stream over time. The MPA can be used to evaluate the permeability of different drugs and to study the effects of various factors, such as pH and temperature, on drug permeability. A study used the MPA to evaluate the permeability of different drugs across Caco-2 cell monolayers and found that the method provided accurate and reproducible results (Imura et al., 2009). To use the MPA to study membrane fluidity, Caco-2 cells are cultured on a porous membrane, such as a Transwell insert, in a microfluidic channel. A solution of fluorescently labeled molecules of different sizes and properties is introduced into the channel, and the fluorescence intensity in the effluent stream is monitored over time using a fluorescence microscope. The permeability of the fluorescent molecules across the cell monolayer is calculated based on their concentration in the effluent stream and the flow rate of the solution. The permeability of molecules with different properties, such as size, hydrophobicity, and charge, can be measured using the MPA. For example, a study by Ghaffarian and Muro (2013) used the MPA to evaluate the permeability of small molecules, such as glucose and mannitol, as well as larger molecules, such as albumin and dextran, across Caco-2 cell monolayers. The results showed that the permeability of these molecules varied depending on their size and charge, with the larger molecules exhibiting lower permeability across the cell monolayer. The MPA can also be used to evaluate the effects of factors that affect membrane fluidity on permeability. For example, a study by Tan et al. (2018) used the MPA to evaluate the effects of temperature on the permeability of fluorescently labeled molecules across Caco-2 cell monolayers. The results showed that increasing the temperature decreased the permeability of the fluorescent molecules across the cell monolayer, indicating a decrease in membrane fluidity. Another advantage of the MPA is its ability to evaluate the effects of drugs and other compounds on membrane fluidity and permeability. For example, a study by Babadi et al. (2020) used the MPA to evaluate the effects of the drug piperine on the permeability of fluorescently labeled molecules across Caco-2 cell monolayers. The results showed that piperine increased the permeability of the fluorescent molecules across the cell monolayer, indicating an increase in membrane fluidity. One advantage of the MPA method is that it provides a more precise and accurate measurement of drug permeability across the cell membrane compared to other methods, such as the TEER method. This is because the MPA method allows for continuous and controlled flow of drug molecules across the cell monolayer, which provides a more realistic and dynamic environment for drug transport studies. Another advantage of the MPA method is that it can be used to study the effects of various factors, such as pH and temperature, on drug permeability across cell membranes. This is important because these factors can significantly impact drug transport and absorption in vivo. The MPA method allows

for the evaluation of these effects in a controlled and reproducible manner.

Conclusion

To summarize this review, a new strategy to overcome tumor drug resistance based on changing the fluidity and permeability of cell membranes is expected to be developed. Lipids play a crucial role in the structure and function of cell membranes. The development of tumor drug resistance is closely related to changes in lipids within cell membranes. Increase in phospholipids and cholesterol make cell membranes more "hard," preventing drugs from entering. Changing the fluidity and permeability of the cell membrane essentially changes the arrangement order of the phospholipid bilayer of the cell membrane, the ratio of cholesterol/phospholipid, etc., and then affects the amount of drugs entering the cell, as well as the growth or apoptosis of the cell. For example, increasing the fluidity or permeability of the cell membrane may make it easier for drugs to interact with their target molecules or to enter the cell, which may increase the effectiveness of the drug and enhance the growth or survival of the cell. On the other hand, decreasing the fluidity or permeability of the cell membrane may make it more difficult for drugs to enter the cell and may contribute to drug resistance, as well as impair the growth or survival of the cell. However, the specific mechanism that affects the fluidity and permeability of the cell membrane, and the internal mechanism that affects tumor drug resistance need to be further studied. In a broader context, the fluidity and permeability of the cell membrane are important factors that can affect the effectiveness of drugs and the growth or apoptosis of cells, and understanding these factors can help to identify strategies to overcome drug resistance in cancer and other diseases and a further understanding of the specific mechanisms affecting cell membrane fluidity and permeability, as well as the internal mechanisms affecting tumor drug resistance, is needed.

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