Application of Fluorescent Probes in Cell Biology

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Abstract: Since the fluorescence phenomenon was first discovered, fluorescent has been wide applied in all aspects of modern society, demonstrating its increasingly important role. As a significant use of fluorescence phenomenon in practice, fluorescent probes also matter a lot in various aspects of the real society. Among all the aspects, cell biology must the one that the fluorescent probes are widely applied in. For example, the fluorescence probe can be used for effectively detecting the sensitivity and selectivity of nitroreductase (NTR) in cancer cells and imaging. Its high-efficiency observation is of great significance for people to better understand the biological function of the nitroreductase (NTR) [1]. Fluorescent probes, when detecting cells, can concurrently provide a great deal of information in vivo about intracellular ion composition, membrane potential, membrane fluidity, polarity, receptor localization, enzymes and enzyme activities. Moreover, they can realize selectable labeling of different components inside and outside cells. The example of in-vivo intracellular pH measurement illustrates the great potential of fluorescent probe technology[2]. Fluorescent probes are also one of the most powerful tools in detecting the dynamic movement of proteins. Protein-based fluorescent probes can effectively monitor the movement and interaction of cell proteins, which can deepen our understanding of protein-related mechanisms in cell biology more intuitively[3].

1. Introduction

As is known to all, when a molecule is excited by excitation light, electrons are possible to jump from the ground state with the lowest energy to the first excited state with high energy. In the process of spontaneous return of the electron in the excited state from the first excited singlet state with high energy to the ground state with the lowest energy, there are two ways: radiative transition and non-radiative transition. Among them, the radiative transition emitted photons is the fluorescence as is often said. The related properties of fluorescence include excitation wavelength, emission wavelength, fluorescence intensity, fluorescence lifetime, etc. Fluorescent probes refer to molecules with these properties very sensitive to the change of the actual environment.

Fluorescent probes are widely used in modern society, including diagnostic medicine, biochemistry, cell biology and many other aspects. Among them, the prospects of fluorescent probes applied in cell biology are quite promising. For example, fluorescent probes can effectively detect the sensitivity and selectivity of nitroreductase (NTR) in cancer cells, image, and observe efficiently, so they have very important significance for people to better understand its biological function. Fluorescent probes, when detecting cells, can concurrently provide a great deal of in-vivo information about intracellular ion composition, membrane potential, membrane fluidity, polarity, receptor localization, enzymes and enzyme activities. Moreover, they can selectively label different components inside and outside cells. The example of in-vivo intracellular pH measurement illustrates the great potential of fluorescent probe technology. Fluorescent probes are also one of the most powerful tools in detecting the dynamic movement of proteins. Protein-based fluorescent probes can effectively monitor the movement and interaction of cell proteins, which can deepen our understanding of protein-related mechanisms in cell biology more intuitively. This review will focus on three important aspects of fluorescent probes in cell biology, including NTR in cancer cells, real-time measurement of intracellular pH in vivo, and dynamic monitoring of protein movement and interaction in cells.

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2. Application of Fluorescent Probes in Cell Biology

2.1 Detection and Imaging of Ntr in Cancer Cells by Fluorescent Probe

As is well-known, cancer is one of the most harmful diseases to human beings. Modern medicine matters a lot in terms of monitoring cancer cells, which can timely and effectively prevent, diagnose and treat cancer. NTR is a class of flavin-containing enzymes that can reduce nitro-containing compounds in the presence of reduced nicotinamide adenine dinucleotide (NADH) as an electron donor[4]. Its abnormally upregulated in cancer cells facilitates us to indirectly observe its presence level in cancer cells. In addition, it has been reported that NTR also helps in activating cancer treatment prodrugs based on nitro compound[5]. Therefore, researchers have studied the fluorescence probe for real-time and effective monitoring of NTR) in cancer cells and imaging of relevant information, which will contribute to people's comprehension about the relevant mechanism and function of NTR in cancer cells. Besides, it can further the exploration of cancer., provide a very pertinent idea to address cancer, arouse the enthusiasm of more researchers for fluorescent probe and cell biology, and then guide more researchers to invest in the research of fluorescent probe and cell biology.

In a word, the research team of Hunan University has developed a new mitochondrial targeted fluorescent probe (benzoindocyanine probe, BICP) for monitoring and imaging the activity of mitochondrial – NTR.

The team modeled the intramolecular charge transfer (ICT) effect of BIC and developed BICP, which can provide a more specific mitochondrial targeting capability and a lower fluorescence background than usual. The results show that the fluorescent probe can quite selectively and sensitively detect nitroreductase in aqueous solution and living tumor cells. BICP not only has been successfully adopted in detecting tumor cells of type I and type II NTR specific activity of the mitochondria, and ensure low cytotoxicity and good biocompatibility. These findings suggest that BICP can better interpret mitochondrial specific nitro reductase activity. Moreover, BICP serves as a new powerful tool for understanding and analyzing mechanisms related to different cell biology, which is of great significance.

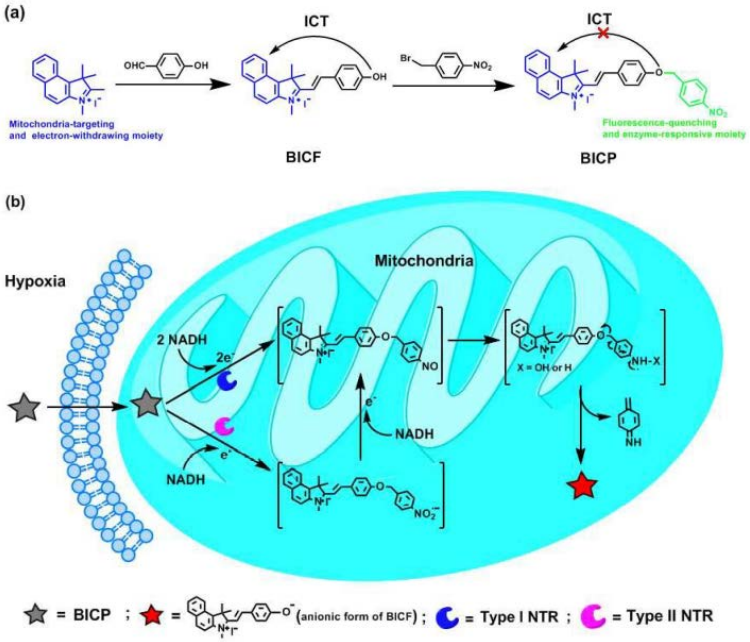

Fig.1 : (a) Design Strategy of Bicp; (B) the Response Mechanism of Bicp to Ntr

2.2 Real-Time Measurement of Intracellular Ph in Vivo by Fluorescent Probe

To measure intracellular pH value by fluorescent probe has always been a hot issue since it will enjoy great prospect in cell biology and biomedical field. Fluorescent probe technology can provide a 2D or 3D pH map of living cells with 0.01 pH resolution, 200 nm spatial resolution, and

millisecond temporal resolution. In 1982, Jan Slavik from Institute of Physiology, Academy of Sciences of the Czech Republic, developed a typical method for measuring intracellular pH changes in vivo using a new fluorescent probe, and this method has been widely used since then.[6][7]

In Jan Slavik's study, BCECF (2',7'-Bis-(2-Carboxyethyl)-5-(and-6)-Carboxyfluorescein) was closely associated with changes in intracellular pH in vivo, as shown in Figure 2. The experimental results show that the response of BCECF (2', 7'-bis (carboxyethyl)-5(6)-carboxyfluorescein)-pH is a very simple and reliable technology in emission, which can be effectively applied to cell biology. Moreover, it has the basic conditions and practical applications for biological application. Jan Slavik has been committed to the practical application of fluorescent probes, and his research team has made great contribution to promoting the practical application of fluorescent probes in various fields.

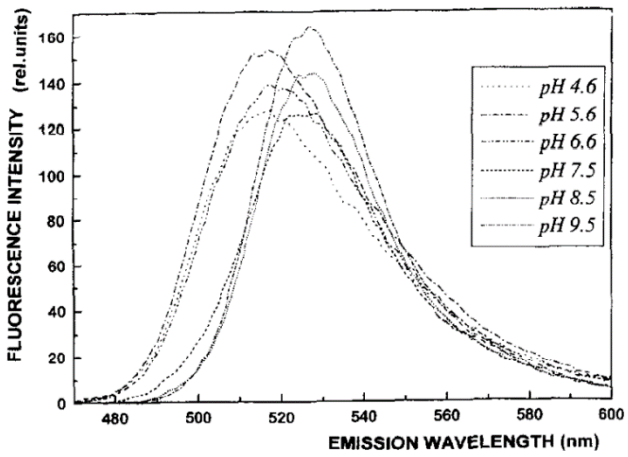

Fig.2 : Relationship between Emission Spectrum of Bcecf (2', 7'-Bis (Carboxyethyl)-5(6)-Carboxyfluorescein) and Ph

2.3 Dynamic Monitoring of Protein Movement and Interaction in Cells by Fluorescent Probes

Fluorescent probe labeling is one of the practical uses. Researchers are fond of applying fluorescent probe labeling to proteins. This is a very prospective research entry point to study the movement and function of various proteins, the interaction between proteins and cell components, and protein information transmission. [8,9]

Labeling techniques based on fluorescent protein (FP) are one of the commonly employed methods to monitor protein localization and function. Among them, the application based on monitoring protein is used more frequently. Therefore, the research team of graduate school from Osaka University and Immunology Frontier Research Center, Osaka University in Japan have reached a consensus that it is quite necessary to develop a new protein fluorescent probe labeling technology based on specificity and pyrogenicity. Apparently, it is a new research perspective, which will promote the multi-field practice and industrialization of protein fluorescent probes. The two Japanese institutions mentioned above have sorted out wash-free labeling strategies for fluorescent probe labeling of proteins, which are all from the previous studies using SNAP-tag-, BL-tag-, and PYP-tag. They have also proposed a number of practical applications, including the development of categorically inducted fluorescent protein systems and the function and role of different proteins in various types of cells to enhance the understanding of cell biology.

The two institutions also summarized how the SNAP-tag, BL-tag and PYP-tag protein labeling systems develop and the photogenic behavior of these protein tags. Among them, the SNAP-tag one is usually adopted in chemical biology studies, which can be introduced in developing wash-free probe method[10]. BL-tag one can be used to label a 29-kDa protein, which is a mutant variant of TEM-1β-peptide lactamase. Reasonable probe design can realize rapid fluorescent protein labeling and real-time pulse tracking analysis in BL label technology[11]. The PYP-tag one, compared with the former two, is smaller. It can be formed by the trans-thioesterification reaction of some specific

thioester derivatives. There are some special mechanisms, such as intramolecular contact quenching, which is a relatively common fluorescence quenching phenomenon [12].

All in all, the research team of the Graduate Schools of Osaka University in Japan, together with researchers at the IFRC, Osaka University, has found that protein fluorescence labeling technology can be adopted to better understand proteins and protein-related mechanisms in cell biology and help people better interpret different mechanisms in cell biology. In the near future, the labeling technology will be further perfected and improved to monitor the movement and interaction of proteins in cells more quickly. The advancement of labeling technology indicates that researchers have a deeper understanding of cell biology, and it also indicates that this technology is an urgent need to solve protein-related phenomena. At present, protein fluorescence labeling technology can not only be used to study various cellular events related to proteins, but also has very important practical significance to understand the related mechanism of proteins.

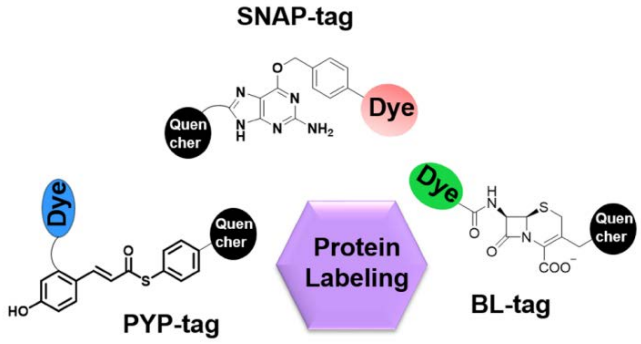

Fig.3 Fluorescent Probes Labeled with Proteins: Snap-Tag Protein, Bl-Tag Protein, and Pyp-Tag Protein.

3. Conclusion

This review has summarized the practical application of fluorescent probes in cell biology, which mainly includes three aspects: detection and imaging of NTR in cancer cells, real-time measurement of intracellular pH, and dynamic monitoring of protein movement and interaction in cells. Through the summary of the practical application of fluorescent probes in these three directions, it is found that the prospect of fluorescent probes in cell biology is very promising.

The sensitivity and selectivity of NTR in cancer cells can be effectively detected and imaging with fluorescent probes. It matters a lot for people to better understand their biological functions by effectively observing the overall level of cancer cells or the dynamic changes of relevant information. Fluorescent probes, when detecting cells, can concurrently provide a great deal of in-vivo information about intracellular ion composition, membrane potential, membrane fluidity, polarity, receptor localization, enzymes and enzyme activities. Moreover, they can selectively label different components inside and outside cells. The example of in-vivo intracellular pH measurement illustrates the great potential of fluorescent probe technology. Fluorescent probes are also one of the most powerful tools in detecting the dynamic movement of proteins. Protein-based fluorescent probes can effectively monitor the movement and interaction of cell proteins, which can deepen our understanding of protein-related mechanisms in cell biology more intuitively.

The development and application of fluorescent probes have greatly promoted the development of cell biology and helped us to understand and investigate the important mechanisms in cell biology. It is widely believed that the development and application of fluorescent probe is far from over, will never stop the pace of progress, in all aspects of cell biology will have a broader practical application.

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