

# Expression and Anti-breast Cancer Effect of Immunotoxin scFv-Mmut

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**Abstract:** In this experiment, the *Pichia Pastoris* expression plasmid pPIC9K/scFv-Mmut was constructed, and the immunotoxin scFv-Mmut was successfully expressed in the eukaryotic expression system of *Pichia Pastoris*. The effect and mechanism of immunotoxin scFv-Mmut on inhibiting activity and inducing apoptosis of breast cancer cells were analyzed by in vitro assay. Through this experiment, it can be found that after the treatment of BT474 cells with a certain concentration of scFv-Mmut, the cell cycle will be affected and be blocked in the S phase, which proves that scFv-Mmut can induce the apoptosis of BT474 cells and has a significant anti-breast cancer effect.

**Keywords:** Breast Cancer; Immunotoxin; Single Chain Antibody; Melittin Analogues; Express; Apoptosis.

## 1. Introduction

Breast cancer contains a variety of different subtypes, among which triple-negative breast cancer accounts for about 15% of the total breast cancer population [1]. Advanced triple-negative breast cancer is not sensitive to targeted therapy and endocrine therapy, and chemotherapy is the main clinical treatment, but most of the curative effects are not satisfactory [2]. In recent years, targeted therapy, immunotherapy and other methods have begun to be applied in the treatment of triple-negative breast cancer patients [3]. Human epidermal growth factor receptor-2 (HER-2) is closely related to the occurrence, metastasis and prognosis of breast cancer. In the targeted therapy of breast cancer, HER-2 is a good choice of target [4]. In the experimental study, combined with the characteristics of breast cancer, in the process of constructing immunotoxin, it is necessary to scientifically select corresponding effector molecules and select appropriate guiding vectors. Among them, scFv is an ideal vector type in the selection of guiding vector. This type of carrier has the characteristics of anti-HER-2, small molecular weight, weak immunogenicity, can maintain the antigen affinity activity, and has strong tissue penetration [5]. Melittin is the main component of bee venom and the main bioactive substance. With relatively low immunogenicity and strong membrane-breaking activity, melittin can affect the cell membrane of tumor cells, causing their lysis and cell death [6]. Therefore, melittin has become an ideal toxin molecule. In this study, the characteristics of melittin and the restriction of hemolytic activity were comprehensively analyzed, and the overall structure of melittin was actively optimized to construct a melittin analogue (Mmut) as effector molecule. The melittin looklike has good apoptosis-inducing ability and no hemolytic activity, which can meet the needs of experimental analysis.

## 2. Methodology

### 2.1. Reagents and Instruments

The cell line used in the experiment was BT474 cell line, provided by Wuhan Shanen Biotechnology Co., LTD. *Pichia* *Pichia* GS115 strain, provided by Ningbo Mingzhou

Biotechnology Co., LTD.

### 2.2. Inhibitory Effect of scFv-Mmut on Breast Cancer Cells

MTT assay was used to detect the inhibition rate of BT474 cells under different concentrations of scFv-Mmut, and the specific results were shown in Table 1.

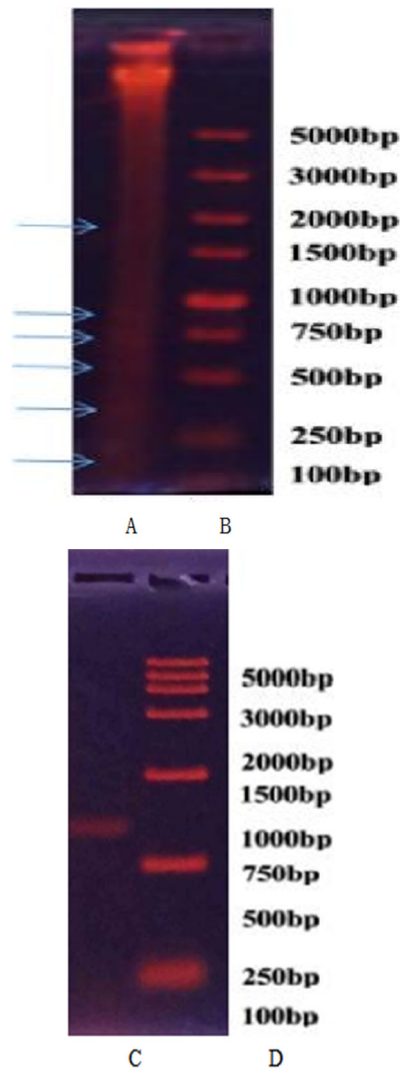
**Table 1.** Statistics of BT474 cell inhibition rate under different concentrations of scFv-Mmut ( $\bar{X} \pm S$ , %)

| scFv-Mmut concentration ( $\mu\text{g} \cdot \text{mL}^{-1}$ ) | BT474 cell inhibition rate |
|----------------------------------------------------------------|----------------------------|
| 0.05                                                           | 16.35 $\pm$ 3.14           |
| 0.1                                                            | 21.02 $\pm$ 2.45           |
| 0.2                                                            | 35.16 $\pm$ 2.13           |
| 0.3                                                            | 42.36 $\pm$ 1.15           |
| 0.4                                                            | 51.96 $\pm$ 1.03           |
| 0.5                                                            | 66.95 $\pm$ 3.14           |
| 0.6                                                            | 81.65 $\pm$ 2.49           |
| 0.7                                                            | 84.65 $\pm$ 2.47           |

Through the analysis of the data results in Table 1, it can be found that with the continuous increase of scFv-Mmut concentration, the corresponding inhibition rate of BT474 cells shows a trend of increasing. When the inhibition rate of scFv-Mmut on BT474 cells reached 50% (IC<sub>50</sub>), the corresponding scFv-Mmut concentration was about 0.4 $\mu\text{g} \cdot \text{mL}^{-1}$ .

### 2.3. DNAladder Experimental Result

In this experiment, identified by agarose gel electrophoresis, the experimental results of DNAladder are shown in Figure1:



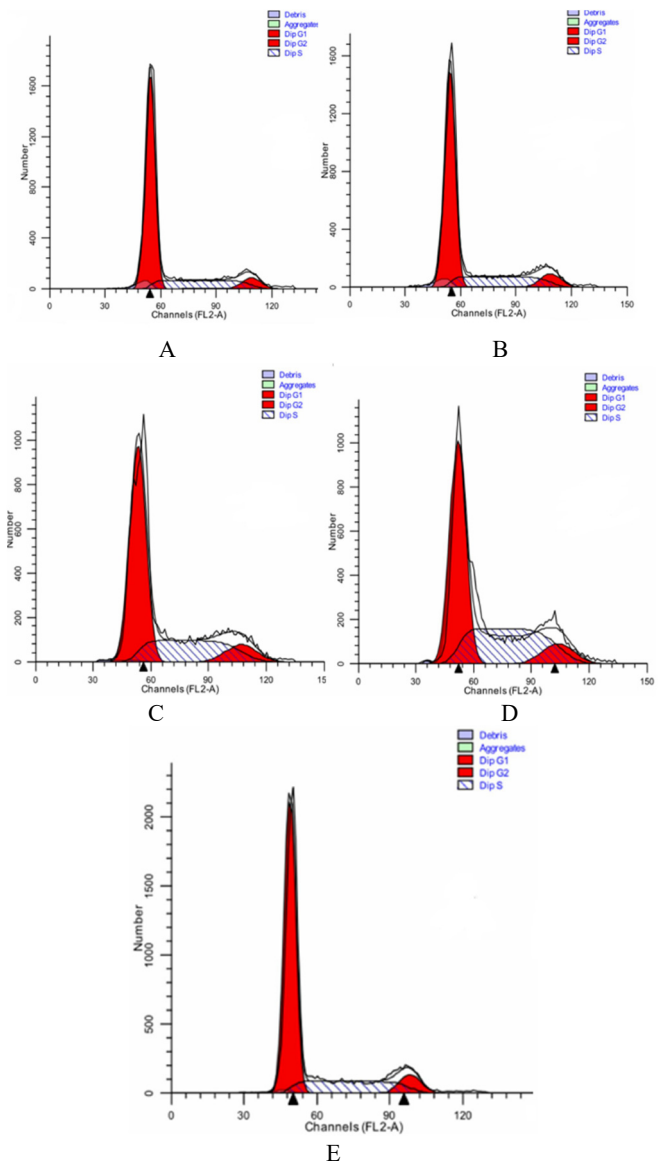
**Figure 1.** Results of DNALadder

Lane A: DNA fragments of BT474 cells treated with scFv-Mmut for 48 h; C: BT474 cells treated with scFv-Mmut for 1 h; DNA segment lane B, D: DL5000 Marker

In the process of apoptosis, the expression of new genes and synthesis of some biomacromolecules are often used as regulatory factors [7-9]. Through the analysis of the experimental results in Figure 1, it can be found that with the standard molecular weight as a reference, chromatin DNA fragments present multiple parallel bands on the gel, namely DNALadder. The results showed that immunotoxin scFv-Mmut could induce apoptosis of BT474 cells.

#### 2.4. Cell Cycle was Detected by Flow Cytometry

BT474 cells were treated with different concentrations of scFv-Mmut for 48 h and stained with PI. The changes of cell cycle were detected by flow cytometry, and the results were shown in Figure 2.



**Figure 2.** BT474 cell cycle detected by flow cytometry

A-D: BT474 cells treated with 0.1, 0.2, 0.4, 0.6 $\mu\text{g}\cdot\text{mL}^{-1}$  scFv-Mmut for 48 h; E: Blank control group BT474 cells

By observing the detection results in Figure 2, it can be found that the S phase of treated BT474 cells showed a gradually increasing trend with the increasing concentration of added scFv-Mmut. The results showed that after scFv-Mmut treatment, BT474 cells were blocked in the S phase, indicating that scFv-Mmut can induce the apoptosis of BT474 cells.

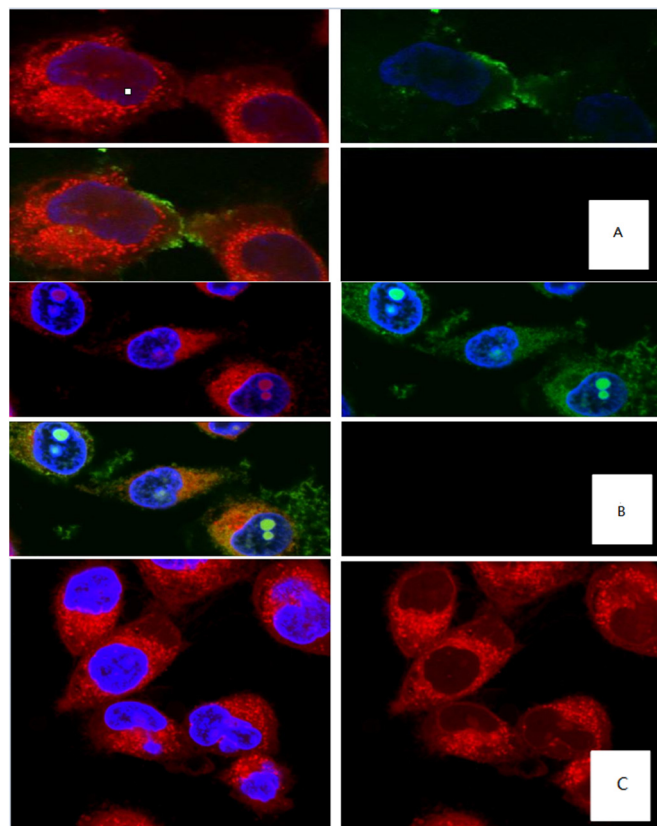
#### 2.5. The Distribution of scFv-Mmut in Cells was Observed by Laser Confocal Method

The distribution of scFv-Mmut in cells was observed by laser confocal microscopy, and the results were shown in Figure 3.

Through the analysis of the detection results in Figure 3, it can be found that:

(1) Figure. 3A shows the staining results of BT474 cells treated with FITC-scFv-Mmut for 1 h. By observing the detection results in the figure, it can be found that in the upper left area of the picture, there are red and blue channels, cell nuclei emit blue light, and mitochondria emit red light. In the lower left area of the picture, there are red and blue channels, scFv-Mmut emits green fluorescence, some of which enter

the cytoplasm, and some of which are scattered in the surrounding area of the cell. In the upper right area of the image, there are green and blue channels, scFv-Mmut emits green fluorescence, cell nuclei emit blue fluorescence, and scFv-Mmut is scattered in the surrounding area of the nucleus.



**Figure 3.** Laser confocal detection results

A: BT474 cells were treated with FITC-scFv-Mmut for 1 h; B: BT474 cells were treated with FITC-scFv-Mmut for 48 h; C: Blank control group

(2) Figure 3B shows the staining results of BT474 cells treated with FITC-scFv-Mmut for 48 h. Through observation of the detection results in the figure, it can be found that in the upper left area of the picture, there are red and blue channels, cell nuclei emit blue light, mitochondria emit red fluorescence, and mitochondria surround the region around the nucleus. In the upper right area of the image, there are green and blue channels, and scFv-Mmut gives off green fluorescence, which can be clearly observed in the area around the nucleus compared with the culture for 1 h [10]. In the lower left area of the image, there are red, blue and green channels, scFv-Mmut emits green fluorescence, and mitochondria emit red fluorescence. Most scFv-Mmut enters the cytoplasm and overlapped with mitochondria. Through it, the nucleus showed significant changes, significantly wrinkled, granular. It can be seen that scFv-Mmut can target HER-2 positive breast cancer BT474 cells, enter the cytoplasm and play a role in inducing cell apoptosis.

(3) The observation results of BT474 cells in the blank control group by laser confocal method are shown in Figure 3C. Through observation of the picture, it can be found that the nucleus of BT474 cells is colored blue by Hoechst 33342. The mitochondria of BT474 cells were stained Red by MitoTracker® Red CMXRos and showed a uniform distribution around the nucleus.

### 3. Conclusion

In this experiment, the *Pichia Pastoris* expression plasmid pPIC9K/scFv-Mmut was constructed, and the immunotoxin scFv-Mmut was successfully expressed in the eukaryotic expression system of *Pichia Pastoris*. After that, the inhibitory activity of immunotoxin scFv-Mmut on breast cancer cells was tested in vitro. The effect and mechanism of inducing cell apoptosis were analyzed.

Through this experiment, it was found that after the treatment of BT474 cells with a certain concentration of scFv-Mmut, the cell cycle would be affected and be blocked in the S phase, which proved that scFv-Mmut could induce the apoptosis of BT474 cells and had a significant anti-breast cancer effect.

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