Development of Novel Approaches to Evaluation of Drug Efficacy Using Cell Function Assay and Real World Data Analysis

金子, 諒右

（様式２）

氏　　名　：金子 諒右

論 文 名　：Development of Novel Approaches to Evaluation of Drug Efficacy Using Cell Function Assay and Real World Data Analysis （細胞機能アッセイおよびリアルワールドデータ解析を用いた新規薬効評価法の開発）

区　　分　：甲

論 文 内 容 の 要 旨

Similar to surgery, a drug is essential for disease treatment and diagnostics, and it also plays an increasingly important role in managing pre-disease conditions. However, high costs, lengthy timelines, and low success rates hinder the drug development process, making it a high-risk investment. In this paper, I address these challenges by proposing a new method for evaluating the effectiveness of drugs. To determine the efficacy of a drug, it is necessary to know its effect on cell functions in detail, and to do so, a method that can evaluate cell functions with high sensitivity and high efficiency is required. In addition, to rapidly explore the unknown effects of drugs, a completely different approach will be necessary. Therefore, in this thesis, I attempted to develop a highly sensitive analytical method for membrane antigens, which are the key to understanding cell functions, for the former category and also attempted to create a method for exploring unknown effects of drugs using real-world data for the latter requirement.

Chapter 2 focuses on improving the sensitivity of flow cytometry for cell membrane antigen detection through the development of fluorescent substrate molecules that are enzymatically rendered hydrophobic, allowing them to permeate cell membranes and stain target cells. To prevent color transfer between cells, a chloroacetyl group was added to the substrate, allowing it to form covalent bonds inside cells. The design resulted in improved staining stability and intensity.

In Chapter 3, I aimed to improve the enzyme sensitization method, which is essential for improving the sensitivity of cell membrane antigen detection. Since conventional diagnostic enzymes have problems with the endogenous activity of human cells, I considered the use of human orthogonal enzymes that do not exist in humans. In 3.1, I established the basis of an enzyme-sensitized membrane antigen detection method using human orthogonal enzymes using α-L-arabinofuranosidase (Araf). It is demonstrated that the detection sensitivity is improved compared to conventional clinical enzymes by using human orthogonal enzymes. In 3.2, I examined the molecular design of substrate of Sulfoquinovosidase (α-SQase) as an example of substrate optimization. Furthermore, in 3.3, a multicolor assay method using human orthogonal enzymes was established using β-D-xylosidase (Xyl) and α-L-rhamnosidase (Rha) and the simultaneous detection of multiple cell membrane antigens was demonstrated.

Chapter 4 utilized a simplified cell counting method to improve lactate dehydrogenase (LDH) activity measurement. A dual-measurement system was designed to simultaneously measure cell count using Nuclear Red dye and total LDH activity using a colorimetric method, allowing easy comparison of normalized LDH

activity per live cell. This method will be useful for accurate evaluation of drug activity to cell.

Chapters 5 explored an evidence-based approach to drug engineering, employing machine learning analysis of real-world data (RWD). This approach is effective for exploring disease risks and generating treatment ideas based on epidemiological evidence, and for discovering unknown drug effects. The analysis identified dietary issues in individuals with fewer teeth as a significant factor associated with frailty in the elderly, indicating the importance of teeth preservation as an interventional target. The other findings suggest that specific medications prescribed for rhinitis could be repositioned as effective drugs for maintaining tooth count.

Overall, this thesis presents multiple new strategies to improve the effectiveness of drug engineering processes.