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論 文 要 旨

THESIS SUMMARY

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要旨（英文800語程度）
Thesis Summary (approx.800 English Words)

The present study has developed a high-performance two-dimensional gel electrophoresis (2-DE) technique and described its practical implementations. This technology allows comprehensive analysis of proteins in the samples of interest, illustrated by the present study employing human hair, tear, serum and zebrafish embryos, which are attractive specimens that are readily accessible and could be collected almost non-invasively. This classical approach is still a powerful tool in the context of proteomics until today because it allows visualized mapping analysis to be performed, where hundreds to thousands of proteins could be resolved in a single gel. More notably, it enables separation and identification of post-translational modifications (PTMs) and protein isoforms, where the patterns could also be easily recognized on the acquired gel images. These modifications are the critical modulator of biological activities in a cell, which could closely reflect the state of biological system at a given time.

This study also described the limitations found in the conventional proteomic protocol, and how they were ameliorated in the modified protocol. Conventional 2-DE is often laborious, time-consuming and extremely prone to technical reproducibility error. However, the current technology was modified and optimized to raise its reproducibility, gel-resolution as well as reduce the amount of time required to generate 2-DE gels. The down-sizing of 2-DE device has largely contributed to the overall improvement of the gel resolution. This has enabled a more consistent electric field passing across the gel as well as consistent temperature control throughout the electrophoresis. Besides, its ability to be miniaturized is also an essential feature for the future practical use of 2-DE in clinical settings.

Employing this technology, high-quality gel images of human hair, tear, serum and zebrafish embryos were attainable. Human hair has unique ability to act as a health record where it captures information about health and diseases of an individual and thus has great potential to be used for long-term monitoring of health conditions. Therefore, other than the non-invasive collection of hair samples, the present study also demonstrated that only small amount of hair shafts is required to be subjected for protein extraction using a strong alkaline-based lysis buffer. After which, over hundreds of proteins spots were detected on the high-resolution 2-DE gel image using a modified proteomics protocol. Besides, human tear, which is a body fluid consisting of vast amount of molecular information, is also extremely useful for the study of ocular surface diseases. Similarly, using only a small amount of tear samples, over hundreds of proteins could be resolved by 2-DE. Overall, the high-sensitivity performance of this 2-DE technology requires the use of only small sample amount, which leads to low invasiveness for practical use.

The present study has also successfully established protocol for the depletion/removal of high-abundant proteins (HAPs) in both human serum and zebrafish embryos. This has enabled the execution of longitudinal proteomic analysis using blood serum, where time-series samples were obtained to reveal multiple candidate biomarkers for post-hematopoietic stem cell transplantation (HSCT) and sepsis progression. In both studies, multiple protein biomarkers were reported, some of which were commonly observed. This includes markers related to coagulation, haemolytic anemia and inflammation. Interestingly, in the study of post-transplant complications, majority of the proteins are shown to be associated with metabolic complications, insulin resistance, iron overload as well as thrombosis, which are possibly linked to the development of acute graft-versus-host disease (aGvHD). On the other hand, more proteins related to the innate immune system such as inflammation were reported in sepsis progression. While this could indicate there was a severe body-wide inflammatory response to infection in sepsis patients, such finding also corroborates with the fact that individual marker has limited specificity and sensitivity, as they are

altogether released during the pathogenesis of many diseases, which, in this case, was sepsis and post-transplant complications. The identification of multiple proteins associated with disease pathology may not only improve the sensitivity and specificity for diagnosis of these complications, it may also help to reveal proteins of potential therapeutic use.

The viability of these studies also insinuates that the development of reliable proteomic protocol for various sample types is a pivotal first-step for these samples to be potentially used as biomarker sources in various settings. Therefore, ongoing efforts were also made to deplete/remove HAPs present naturally in human hair and tear. Lastly, similar time-course analysis will also be carried out in the future to evaluate teratogenicity using zebrafish embryo, where it would serve as an alternative sustainable toxicology model in human pharmaceuticals. All in all, the high-performance two-dimensional gel electrophoresis technology employed in this study is a powerful and versatile tool that holds great promises in diagnostics, prognostics and therapeutics. Future strategy involving the integration of AI with proteomic image data is also anticipated to be a major advancement in proteomics research.