**Assessments on the doctoral dissertation of Kenneth Weke:**

1) General theoretical knowledge :

Chapter 1 provides a comprehensive introduction to glioblastoma (GBM), the most aggressive and common primary brain tumor in adults. The chapter begins by discussing the evolution and classification of glioma tumors, emphasizing the high genetic heterogeneity and molecular complexity of GBM. It further explores the pathogenesis of GBM, highlighting the interplay of genetic and epigenetic alterations in tumor development and progression. Additionally, the chapter shed light on the critical role of the tumor microenvironment in GBM, emphasizing the impact of hypoxia, inflammation, and immune suppression on tumor biology and treatment resistance. Also, various proteomics technologies directly related to the thesis works were mentioned.

Overall, Chapter 1 serves as a foundational framework for the thesis, providing a comprehensive overview of GRM, its complexities, and the urgent need for advanced research and therapeutic interventions in the challenging disease.

2) The ability for independently conducting scientific work :

Proteomics researchers face the challenge of mastering diverse technical components, including biological sample preparation, LC-MS/MS analytical techniques, and proteomic bioinformatics. These multifaceted requirements pose a significant hurdle for individuals entering the field of proteomics.

In Chapters 2 and 3, the candidate showcased significant accomplishments in diverse proteomics techniques. These encompass cell culture, single-cell sample preparation, DDA and DIA LC-MS/MS analysis, as well as data analysis utilizing proteomics informatics tools. Additionally, in Chapter 4, the candidate devised an innovative experimental scheme to investigate questions related to GBM biology, yielding promising results. These aspects unequivocally demonstrate the candidate's prowess as an independent researcher.

3) Original solution to a scientific problem : The thesis consists of two technical chapters and an application chapter studying GBM biology. In technical developments, two protocols are establish: a microscale MS-based proteomics method for limited numbers of cells and a workflow for proteome analysis of formalin-fixed paraffin-embedded glioblastoma tissues. In the last chapter, the impact of hypoxia on antigen presentation in glioblastoma was investigated using an integrated proteomics and immunopeptidomics approach.

**General comments on Kenneth Weke's PhD Dissertation.**

In the following comments, the thesis manuscript is assessed with a specific focus on technical considerations.

Chapter 2 :

The efficient execution of LC-MS analysis using a minimal sample size is indeed commendable. Historically, proteomics analysis has faced challenges, primarily the necessity for larger sample volumes compared to genomics analysis. Although high efficient and reproducible LC-MS/MS achieved using microPOTs sample preparation, there are still improvable room in sensitivity compared to macro-scale proteomics. To this end, it is necessary to add the candidate's outlooks or opinions about the further improvement in sensitivity in the discussion section.

A possible improvement can be obtained from multi-emitter technology, heightening the sensitivity without increasing the sample quantity. Introducing multi-emitter technology could potentially yield a decreased flow effect under the same LC settings. The reduction in droplet size contributes to enhanced ionization efficiency. Hence, a brief review of such technology is recommended for its application in future researches.

Chapter 3 :

The overall experiment procedure appears highly reliable. It seems that ample consideration was given to the process of selecting the most efficient analysis method, achieved through a comparison of three sample preparation methods and the section sizes of various FFPEs. The choice of 15 μm

thick section and the application of method 3 applied to actual patient samples are deemed reasonable. However, a question arises regarding what might have occurred if simultaneous analysis were conducted using a TMT kit, etc., instead of a label-free method in the DDA experiment. I recommend your insights on why the label-free method was chosen in the context.

Chapter 4:

Compared to the previous two chapters published in SCI journals, the Chapter 4 should be improved in the higher degree. In Figure 4-1b, the candidate utilized dual approaches of proteomics and immunopeptidomics. While the proteomics analysis results were well-detailed, it is very difficult to locate the LC-MS/MS results of immunopeptidomics in the chapter. It is advisable to independently list and compare LC-MS/MS results of immunopeptidomics with the proteomics results. This will offer valuable insights to other researchers employing the immunopeptidomics approach.

A technical questions :

In chapter 2, 3, and 4, the MS1 scan range is set differently as

- The precursor ion in Chapter 2 has a range of m/z 375-1,600.
- In Chapter 3, DDA m/z 350-1,200, DIA m/z 400 - 1,100 (isolation window m/z 8, number of scan events set 87).
- In Chapter 4, DDA m/z 350-1,200, DIA m/z 350 - 1,100 (isolation window m/z 13, number of scan events set 62).

In addition, why there is differences in the number of scan event set and the isolation window?

**Concluding Remarks :**

- The doctoral dissertation meets the requirements set for doctoral dissertations by The Higher Education and Science Act dated 20 July 2018.

- Work evaluation : positive

- After considering the research results and written articles in the dissertation, *I am applying to the Council of the Biotechnology Discipline for admission of Kenneth Weke to further stages of the doctoral procedure.*

Hookeun Lee

Hookeun Lee Ph. D

Professor, College of Pharmacy,

Gachon University, 191, Hambangmoe-ro,

Yeonsu-gu, Incheon 21936, Republic of Korea

Tel: + 82-32-820-4927, +82-01-5169-1006

Email: hklee@gachon.ac.kr, hookeun@gmail.com