CHAPTER 1

INTRODUCTION

1.1.   Problem Background

Primary brain tumor has become the 10th leading cause of death in 2017 in the United States (American Society of Clinical Oncology (ASCO), 2017). Furthermore, in early 2018, American Cancer Society made some prediction and stated that approximately 23,880 persons in the US, including adults and children, are estimated to be diagnosed with brain or central nervous system tumors in 2018. Mortality caused by overall cases of brain and central nervous system is predicted to reach almost 75%. Moreover, among all of the incidences of brain and nervous system tumor, about 85% to 90% of cases are account for brain tumor incidences.

The most common type of brain tumor that occurs globally is glioma, which accounts for almost 30% of all primary tumor of the brain. Gliomas derived from glial cells of the brain, thus, it can be characterized into four types based on the origin of the tumor cells; including astrocytomas, ependymomas, oligodendrogliomas, and mixed gliomas (Weller et al., 2015).

Even though glioma incidence is low in Indonesia, the most common type of primary malignant brain tumor, i.e. glioblastoma, has a poor prognosis in the country. Around 70% of patient diagnosed with glioblastoma will encounter rapid progression of the disease within one year upon the diagnosis. Further, those patients have a pretty slim survival rate. Only less than 5% of the patient could survive for five years after diagnosis (Davis, 2016).

The golden standard of brain tumor diagnosis is histological diagnosis from sample biopsy. Besides histological diagnosis, additional diagnostic procedures such as molecular diagnostic on genetic and/or epigenetic alteration of the sample are also needed. The detection of epigenetic changes on specific genes, such as MGMT gene, have been widely used to assists the determination of therapeutic options for the patients (Weller et al., 2015). Nevertheless, methylation changes have become one focus of study that holds great potential as predictive and prognostic markers in diagnosis

and treatment for cancer. Furthermore, both clinical diagnosis and research required fast, reliable, and cost-effective procedures for methylation detection.

MLH1 (MutL Homolog 1) is one gene which expression is known to be affected by methylation. As a tumor suppressor gene, *MLH1* is involved in mismatch repair (MMR) mechanism of DNA repair, which is also used as biomarker in colorectal cancer detection (Genetic Home Reference, 2018). Since methylation of *MLH1* gene promoter may lead to the development of colorectal carcinoma, the same mechanism may also operate in the sequelae of gliomagenesis. However, epigenetic mutation of *MLH1* in glioma is mostly unknown. Since there is inadequate data of brain tumor genetic profiling, particularly on glioma, this project will focus on the investigation of the methylation changes of *MLH1* gene from glioma, a common form of brain tumor, samples in Indonesia.

DNA methylation status on the specific gene can be analyzed using several techniques, including digestion-based assay followed by PCR, methylation-specific PCR (MSP), methylation-sensitive HRM PCR, bisulfite sequencing, pyrosequencing, and bead array (Kurdyokov & Bullock, 2016). In all of the techniques except digestion-based assay, the DNA template needs to be pre-treated by bisulfite conversion just like in MS-HRM PCR technique. Meanwhile, the digestion-based assay does not require bisulfite conversion treatment on the DNA template. Instead, the methods utilize specific endonuclease(s) to perform selective digestion of the DNA based on the methylation condition on the gene. This method usually will be followed by PCR or qPCR technology for further assessment of the methylation condition (Kurdyokov & Bullock, 2016).

One of the most common procedures used to analyze DNA methylation condition is by using MSP. This procedure requires two pairs of primers specific for the methylated and unmethylated template, which is used on bisulfite-modified DNA template (Cui et al., 2011). Another method that commonly used in identifying methylation status is bisulfite sequencing technology, which also considers as the golden standard procedure. The procedure involves sequencing the PCR product that has been pre-treated by bisulfite conversion. This procedure can be used to assess the methylation status of individual CpG sites within specific CpG islands, which provide detail information on the

location of methylation (Kurdyokov & Bullock, 2016). However, this procedure has limitations in term of cost and complexity since next-generation sequencing (NGS) technology is included in the procedure. In comparison, MS-HRM PCR procedure is less complex and faster in detecting methylation condition by utilizing real-time PCR technology.

One of the reliable methods that can be used to analyze promoter methylation in clinical samples is based on PCR high resolution melting (HRM). This technique is usually used for genotyping of single nucleotide polymorphism (SNP). Later, advancement in two areas has made the high resolution melting method has been adjusted to detect promoter gene methylation (Wojdacz & Dobrovic, 2007). The first one is the discovery and utilization of intercalating dyes which do not affect the polymerase chain reaction (PCR) process. The second one is the advancement of instrumentation that has high accuracy in detecting the fluorescence changes (Wojdacz & Dobrovic, 2007). This approach has been applied in several studies for methylation detection by comparing the sample's melting profile with the reference profile of unmethylated gene (BT549 cell line) and fully methylated standard (universal methylated human DNA standard) (Wojdacz & Dobrovic, 2007).

Since this procedure has not been implemented in Indonesia optimally, this project will focus on optimizing the protocol in detecting methylation in *MLH1* gene in glioma with the expectation to develop a reliable research protocol. The final result of this study will be compared to Immunohistochemistry data of *MLH1* protein expression in glioma, which had been determined from the previous study by the host institution. The comparison will be used to evaluate whether *MLH1* methylation correlates to lose expression of *MLH1* protein showed by immunohistochemistry in glioma.

1.2.   Problem Formulation

Indonesia is lagging behind regarding biomarker development for cancer diagnostic, prognostic, or epipharmacologic biomarker detection in glioma. Therefore, implementation of fast, reliable, and cost-effective procedure, such as for methylated *MLH1* detection in glioma, may help investigates the

genetic profiling of glioma in Indonesia. Thence, Methylation-Sensitive High Resolution Melting (MS-HRM) PCR analysis is a reasonable procedure to be used in this study.

1.3.   Research Objectives

- Optimization protocol for methylation detection using MS-HRM qPCR
- Application of methylation detection (MS-HRM) on *MLH1* gene from glioma in Indonesia

1.4.   Scope of Research

- Literature review
- Bisulfite Conversion PCR
- Methylation Sensitive High Resolution Melting Protocol Optimization for *MLH1* gene
  - PCR mix optimization
  - qPCR HRM optimization
  - Electrophoresis
- Detection of methylated *MLH1* gene from glioma sample
  - qPCR HRM using optimized protocol
  - Electrophoresis