CHAPTER 1

INTRODUCTION

1.1. Background

Colorectal cancer (CRC) is one of the most common cancers worldwide affecting both genders and both developed and developing regions (International Agency for Research on Cancer, 2012). CRC is an emerging field of research to understand the underlying mechanisms of carcinogenesis that can be used to establish more effective methods for diagnosis and treatment. As of other types of cancer, the molecular mechanisms of colorectal carcinogenesis that are more common and widely discussed are genetic aberrations, especially DNA mutation. Other than oncogenes and tumor suppressor genes, DNA mismatch repair (MMR) genes are also prone to be altered and inactivated in CRC, causing MMR deficiency (dMMR) that leads to microsatellite instability (MSI). MSI is the main hallmark of Lynch syndrome, a condition caused by a germline mutation of an MMR gene (Poynter et al., 2008). The inactivation of MMR genes is not only caused by mutations but can also result from the epigenetic mechanisms, such as DNA methylation (Tariq & Ghias, 2016).

Apparently, MSI-associated CRC is most commonly caused by DNA hypermethylation of mutL homolog 1 (*MLH1*) promoter (Vaiopoulos, Athanasoula, & Papavassiliou, 2014). Because of that, *MLH1* has become one of the main interests in the research of CRC and this study. The promoter region is the main focus in this study due to its crucial role in affecting gene expression by acting as the start site of the transcription process. The mechanism of *MLH1* promoter methylation in CRC has indeed been widely observed and discussed. Nevertheless, there is not any data regarding its occurrence and frequency in Indonesia yet, as well as its association with different ethnic groups. A data profiling of CRC patients is essential to elucidate the pattern and frequency in the molecular mechanism of colorectal carcinogenesis in Indonesia.

Even though most cases of CRC with MSI phenotype are sporadic and associated with *MLH1* promoter methylation, there is still the other cause that comes from a hereditary origin. If CRC patients are tested for *MLH1* methylation and comes back with a negative result, they might need to be submitted to genetic testing to look for a germline mutation (Pérez-Carbonell et al., 2010). The results of this study could become an important indicator to determine the necessity to undergo genetic testing.

The study of *MLH1* promoter methylation has been performed using various methods of detection, such as bisulfite sequencing and pyrosequencing. However, most of those methods are costly, time-consuming, and have a low sensitivity (Pérez-Carbonell et al., 2010). For this reason, a methylation detection method that provides rapid, cost-effective, sensitive, and reliable detection is needed for identifying the methylation status of a certain region of interest. Methylation-sensitive high-resolution melting (MS-HRM) has been developed and emerges as a methylation detection method with high potential as the most suitable method that meets these requirements. The analysis of MS-HRM enables us to distinguish between fully and partially methylate samples with cost-effective and fast detection (Wojdacz, Dobrovic, & Hansen, 2008). Therefore, MS-HRM becomes a good method of choice for methylation analysis and interest to be investigated further in this study.

1.2. Objectives

The objective of this research thesis project is to optimize the detection of methylation of *MLH1* promoter in CRC samples using MS-HRM analysis.