

CHAPTER I

INTRODUCTION

1.1 BACKGROUND

Colorectal cancer (CRC) is the second leading cause of cancer mortality that causes a major public health problem in the United States of America, Europe, Australia and worldwide [1, 2]. An estimated of 500,000 deaths worldwide is due to CRC, with 50,000 occur in the United States alone and 4,000 in Australia [3, 4]. The disease-specific mortality rate is nearly 33% in the developed world.

CRC is a heterogeneous disease in which its pathological precursors exhibit distinctive pathological features and natural histories [5]. CRC progressively occurs as an end result of the accumulation of genetic and epigenetic alterations that force the transformation and progression of normal colonic epithelial cells to cancer [6].

CRC has one of the largest proportions of familial cases out of the many common malignancies known so far. From twin and family members' studies being performed, an estimation of approximately 30% of all CRC cases is an inherited form. Amongst the incidence, 5% of inherited CRC cases are associated with highly penetrant inherited mutations [7]. However, the etiology of the remaining 20-30% is not completely understood. DNA copy number variations (CNVs) are an important component of genetic variation, affecting a greater fraction of the genome than single nucleotide polymorphisms (SNPs) [8]. The

introduction of high-resolution SNP arrays has made it possible to identify CNVs. The role of CNVs as risk factors for cancer is currently unclear. The mitochondrial tumor suppressor gene (*Mtus1*) illustrates a good example of an important CNV with phenotypic effect. Frank et al. [9] found that a small deletion of 1.1 kb in *Mtus1* is associated with a decreased risk of familial and high-risk of breast cancer. Many small CNVs are previously thought to affect many areas that are considered of no significance. However with the emerging of microRNA (miRNA), these small CNVs might actually be involved in familial CRC.

MiRNA is a class of small non-coding RNAs that play important roles in many biological processes such as cell growth, differentiation, apoptosis and gene regulation [10]. They are also involved in cancer and recently abnormal expression patterns of miRNA have been reported in many human cancers. Approximately 50% of human miRNA genes are considered to be located in cancer-associated regions of chromosomes, which are the regular main spots for gene deletion, amplification and mutations [11].

From the studies being performed so far, it was found that miRNA's up regulation or down regulation may possibly play a role in colorectal cancer development, regardless the vague pathway on how it becomes dysregulated in cancer cells itself. MiRNAs may also play a role in the CRC predisposition within the familial cases. Because of the ability to accurately distinguish between tumor and normal tissues, the specific expression patterns of miRNAs can make them promising candidates for a new marker in familial colorectal cancer. Results of previous study in the laboratory show that there are indeed some copy number

variations (CNVs) affecting miRNA genes (unpublished results). To the best of our knowledge so far, no articles have been published about the roles of miRNAs in CRC predisposition, making it a very explorative area.

In this descriptive study we looked for and analyzed CNVs that occur within the miRNA genes in young CRC patients who have been referred to the DNA laboratory of Radboud University of Medical Center (RUNMC), the Netherlands, and suspected to them to be familial CRC that has stable microsatellite and no dysregulation on either *MSH* or *MLH*. Using a custom-made array CGH, screening was performed to identify microRNA CNVs.

1.2 RESEARCH QUESTION

How does the profiling of microRNA copy number variations in patients with familial colorectal cancer appear like?

1.3 RESEARCH PURPOSES

This research aims to characterize the microRNA copy number variations of patient with familial colorectal cancer.

1.4 RESEARCH BENEFITS

This study will provides an alternative way to diagnosed familial colorectal cancer and a possible treatment can be synthesized for the onset of cancer caused by miRNA alteration. Results obtained from this study will be correlated with the clinical and pathological data of the patients and supported through the functional analysis (*in vitro* and *in vivo* models) to obtain the prognostic and predictive markers as well as therapeutic targets in the future. This

study will also enable the early diagnosis of familial colorectal cancer hence can be used as prevention measurement. This will also in turn help in the genetic counseling for the patients and relatives.

1.5 RESEARCH ORIGINALITY

An ongoing studies performed by our colleague Venkatachalam et al. (2010) [12], in the RUNMC laboratory using a 32 patients cohort of colorectal cancer with inclusion criteria of: microsatellite stable colorectal cancer, diagnosed at the age of less than 40 years old or diagnosed at the age of less than 50 years old with at least one 1st degree relative and/or recessive inheritance pattern (affected by CRC) has shown that five new novel candidate genes are affected by CNVs. Amongst the results, two of the cases showed that the CNVs found are not only affecting protein coding genes but also miRNA genes. To this date only a handful of studies have found an association of CNVs with cancer and thus far there is no studies have been done on microRNA copy number variations. This study will be the first study to generate the profile of microRNA copy number variations on patients with early onset colorectal cancer (familial) and do not have any known genetic SNPs or CNVs.