PhD Thesis: Role of genetic and epigenetic factors in the development of colorectal cancer

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Abstract

Colorectal cancer (CRC) is the third most common form of cancer and the leading cause of cancer related deaths in the Western world. The etiology of CRC is complex and may involve an interplay of non-genetic, genetic and epigenetic factors. Genes including MutL homolog 1 (MLH1), MutS homolog 2 (MSH2) and O-6-methylguanine-DNA methyltransferase (MGMT) may be involved in the causation and development of the disease. A total of 240 consecutive surgically resected fresh tissue specimens comprising of 120 tumor tissues and 120 adjacent control regions of primary sporadic CRC undergoing upfront surgery for the disease were collected. Blood samples were also collected from each patient and equal number of age and sex matched control subjects. Genomic DNA isolation was performed by phenol chloroform method. For genetic and epigenetic analysis of MLH1, MSH2 and MGMT genes, polymerase chain reaction (PCR), restriction fragment length polymorphism, sequencing, bisulphite modification and methylation specific PCR was performed followed by immunohistochemistry.

The mean age of the patients included in the study was 53 years at the time of diagnosis and the majority of the patients were males (77%). MLH1 (-93 G>A), MSH2 (-118 T>C) and MGMT (-485 C>A) polymorphic genotypes were observed in 71.7% vs. 69.2%, 40% vs. 34.2% and 59.2% vs. 68.3% cases in the patients and control subjects, respectively. It was observed that amongst the patients with MLH1 (-93 G>A) polymorphic genotypes, alcohol intake, tobacco chewing, smoking, fatty diet, irregular intake of fruits, spicy food, non vegetarian diet and lack of physical

exercise were observed in 43%, 17.4%, 43%, 58.1%, 25.6%, 41.9%, 44.2% and 30.2%, respectively. Amongst patients with MSH2 (-118 T>C) polymorphic genotypes, alcohol intake, tobacco chewing, smoking, fatty diet, irregular intake of fruits, spicy food, non vegetarian diet and lack of physical exercise were observed in 37.5%, 27.1%, 33.3%, 58.3%, 10%, 43.8%, 47.9% and 33.3%, respectively. Also, amongst the patients with MGMT (-485 C>A) polymorphic genotypes, alcohol intake, tobacco chewing, smoking, fatty diet, irregular intake of fruits, spicy food, non vegetarian diet and lack of physical exercise were observed in 43.7%, 19.7%, 43.7%, 61.9%, 30.9%, 47.9%, 50.7% and 38%, respectively. With respect to the tumor profile, amongst the patients with MLH1 (-93 G>A) and MSH2 (-118 T>C) polymorphic genotypes, tumor location as colon, TNM stage 3, moderately differentiated grade with absence of lymphatic invasion and metastasis was most commonly observed whereas in case of MGMT (-485 C>A) polymorphic genotypes, tumor location as colon, TNM stage 2, moderately differentiated grade with absence of lymphatic invasion & metastasis was commonly observed.

On epigenetic analysis, the MLH1, MSH2 and MGMT promoter methylation was observed in 43.3% vs. 19.2%, 25% vs. 8.3% and 32.5% vs. 11.1% cases in the tumor and adjacent control tissues, respectively. Among the patients with MLH1, MSH2 and MGMT promoter methylation, alcohol intake, tobacco chewing, smoking, irregular intake of fruits and lack of physical exercise was more commonly observed. With respect to the tumor profile, amongst the patients with MLH1, MSH2 and MGMT promoter methylation, tumor location as colon, TNM stage 3, moderately differentiated grade with presence of lymphatic invasion and absence of metastasis was most commonly observed. On the comparison of occurrence of polymorphism in one gene in the presence of another gene and similarly for promoter methylation, statistical associations were observed. The presence or absence of protein expression was compared with the tumor profile of the patients and correlations were observed with tumor stage and lymphatic invasion. MLH1, MSH2 and MGMT genes may be greatly involved in the causation and development of this disease.