

Review

Loss of heterozygosity in colorectal cancer

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Colorectal cancer (CRC) is one of the most common malignancies in the world. The development and progression of CRC is a multistep process, which involves many dietary and environmental factors. A great number of oncogenes, tumour suppressor genes and DNA repair genes contribute to molecular and biological features of CRC, providing us much essential information about the pathogenesis of this disease. Loss of heterozygosity (LOH) of tumour suppressor genes was observed at various loci on different chromosomes like 1p, 1q, 4q, 5q, 8p, 9q, 11q, 12p, 14q, 15q, 17p, 17q, 18p, 18q and 22q in CRCs. In this review, the loss of heterozygosity in patients with colorectal cancer and the interrelationship between tumour suppressor genes and colorectal cancer initiation and progression are discussed.

Key words: Colorectal cancer, tumour suppressor gene, loss of heterozygosity (LOH).

INTRODUCTION

Colorectal cancer (CRC) is the third most common cause of death in the world (Shibuya et al., 2002). Cases of CRC can be classified as sporadic (75%), familial (20%) and genetic syndromes such as Hereditary Nonpolyposis Colorectal Cancer (HNPCC) and Familial Adenomatous Polyposis (FAP) (5%) (Hendon and Dipalma, 2005). CRC develops as the result of the progressive accumulation of genetic and epigenetic alterations that lead to the transformation of normal colonic epithelium to colorectal adenocarcinoma (Grady, 2005). CRC occurs through a multi-step process, which is characterized by the inactivation of tumour suppressor genes and activation of proto-oncogenes by mutations and/or allelic loss or mismatch repair deficiency (Fransen et al., 2004). The risk of getting colorectal cancer increases with age. Nearly 90% of colorectal cancer patients are over the age of 50 (Robins and Kumar, 1987).

As allelic loss at a certain region of chromosome is thought to indicate the presence of a tumour suppressor gene, loss of heterozygosity (LOH) analysis is presently the most common method used to identify potential locations for these genes. Although LOH is a common phenomenon in a variety of human cancers, a high frequency allelic loss at a specific chromosomal region indicates the

location of a candidate tumour suppressor gene. According to this hypothesis, there are still several undiscovered tumour suppressor genes that play potentially important roles in cancer development (Pylkkänen, 2002). Genome-wide searches for LOH have been performed successfully to localize putative tumour suppressor gene loci. The observation that LOH may have prognostic implications has created a situation in which the study of this abnormality provides information significant enough to suggest that, for clinical purposes, tumours of certain types should be typed routinely for LOH at specific loci (Massa et al., 1999).

LOH analysis is a sensitive genetic method that is used to detect micro deletions on chromosomes. A frequent deletion in a chromosomal region may suggest the existence of a candidate tumour suppressor gene (Cetin et al., 2008). Here, we reviewed the positive correlation between tumour suppressor genes and CRC development. In consequence, the importance of LOH needs to be re-evaluated.

COLORECTAL CANCER

Colorectal cancer is one of the four most prevalent cancer types in the world, which affects the majority of elderly people (Boyle and Langman, 2000). A cascade of cellular, biochemical and genetic events is known to occur in the development and progression of tumours,

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leading to malignancy and ultimately metastatic dissemination. In all human cancers, the molecular genetic alterations in colorectal cancer are among the best understood (Kapitanovic et al., 2004). Colorectal carcinogenesis is a multistep process in which a series of genetic and epigenetic events leads to the transition from normal mucosa polyps to carcinoma. Together, these events allow cells to bypass several of the normal regulatory processes and develop traits specific to malignant cells, such as limitless replication, evasion of apoptosis and tissue invasion. Because the spontaneous somatic mutation rate is not sufficient to account for such a number of mutations, acquisition of a mutant phenotype is generally presumed to be an early event in tumour progression, with additional mutant phenotypes likely to enter later in its progression. This development is most likely the result of mutations or losses in genes that maintain genetic stability by mediating precise replication, damage repair, or checkpoint activation (Brenner et al., 2007). Colorectal tumorigenesis is a multistep process in which many oncogenes and tumour suppressor genes are involved (Cacev et al., 2005; Zheng et al., 2005).

Colorectal cancer represents a group of heterogeneous epithelial malignancies, of which familial adenomatous polyposis coli (FAP) and hereditary nonpolyposis colon cancer (HNPCC) are two major colorectal cancer predisposition syndromes. FAP is usually present in the second decade of life and is characterized by the presence of 100s to 1000s of adenomatous polyps carpeting the large bowel. Malignant change usually takes place in one or more polyps after age 50. Almost all FAP cases result from truncating mutations in the APC gene. In contrast, HNPCC patients have a normal or only slightly elevated tendency to develop adenomas, but the probability and rate of progression to carcinoma is increased and an increased risk of other carcinomas is also a recognizable feature of this syndrome. Germ line defects, in one or more of a group of DNA mismatching repair genes such as MLH1, MSH2, MLH3, PMS1, PMS2, MSH6 and GTBP, are associated with HNPCC. Approximately 15% of colorectal cancer is caused by dominantly inherited predisposition to the disease. Only 2 – 6% of cases have been attributed to FAP or HNPCC, suggesting the presence of additional predisposition genes (Mao et al., 2006).

TUMOUR SUPPRESSOR GENES AND LOSS OF HETEROZYGOSITY

Cancer results from successive accumulation of alterations in essential genes that play important roles in cell signaling, proliferation, apoptosis, invasion, or angiogenesis. Some of these genes get over expressed, producing abundant supply of their gene products, whereas others are suppressed or even deleted. Genes of the former group, in which alterations lead to gain of function,

are the so-called oncogenes. Oncogenes are variations of normal genes (proto-oncogenes) that play important roles in normal cells by activating cell signaling or proliferation. These genes become active because of specific abnormalities such as gene amplification, over expression, point mutation, insertion mutation, or translocation (Velasco et al., 2008).

A second group of genes involved in cancer development and progression are tumour suppressor genes (TSGs) (Velasco et al., 2008). TSGs have a normal physiological role of retarding cell division. TSGs work with the DNA repair system, which makes them necessary in the maintenance of host genetic stability. TSGs in mutated form can be passed on as germline heritable DNA defects. They are the cause of syndromes of genetic predisposition to cancer (Ross, 1998).

Inactivation of TSGs causes cells to display one or more phenotypes of neoplastic growth. Knudson's definition of a classical tumour suppressor gene requires the inactivation of both alleles of a candidate gene in tumours. Inactivation of these classical TSGs usually occurs either in the deletion of the one own allele or mutation in the rest alleles. However, a new class of TSGs with haploid insufficiency, in which one allele is lost and the remaining allele is haploid sufficient, has been described recently and these hemizygous tumour suppressor genes show a tumour-prone phenotype when challenged with carcinogens (Knudson, 1971; Gunduz et al., 2005).

Several tumour suppressor genes associated with colorectal carcinoma have been identified: these include TSGs mutated in colon cancer (MCC) and adenomatous polyposis coli (APC) on chromosome 5q, p53 on chromosome 17p, nm23-H1 on chromosome 17q, and TSGs deleted in colon cancer (DCC) and deleted in pancreatic carcinoma (DPC4) on chromosome 18q. The adenomatous polyposis coli (APC) tumour suppressor gene was identified 10 years ago through its association with an inherited syndrome of colorectal cancer known as familial adenomatous polyposis coli (FAP). APC is mutated in the germ-line DNA of patients with familial adenomatous polyposis. Somatic mutations or allelic deletions of APC, or both, have also been described in sporadic colorectal cancer (Kapitanovic et al., 2004).

One of the critical steps for the identification of TSGs is loss of heterozygosity analysis (LOH) (Gunduz et al., 2005). LOH, identified by comparing patterns of polymorphisms in normal and tumour cells from one individual (Cacev et al., 2006), is observed at many loci in tumours. LOH of tumour suppressor genes is believed to be one of the key steps to carcinogenesis of colorectal cancer. LOH, the loss of one allele at a specific locus, is caused by a deletion mutation or loss of a chromosome from a chromosome pair. When this occurs at a tumour suppressor gene locus where one of the alleles is already abnormal, it can result in neoplastic transformation (Zheng et al., 2005; Zhou et al., 2008; Wan et al., 2006;

Zhou et al., 2004). At loci showing LOH, two alleles are observed in normal cells, while only one allele is detected in tumour cells because the other has been lost (Cacev et al., 2006).

Loss of heterozygosity can be identified in cancers by noting the presence of heterozygosity at a genetic locus in an organism's germline DNA and the absence of heterozygosity at that locus in the cancer cells. This is often done using polymorphic markers, such as microsatellites or single nucleotide polymorphisms, for which the two parents contributed different alleles (Cacev et al., 2006).

COLORECTAL CANCER AND LOSS OF HETEROZYGOSITY

Loss of heterozygosity in colorectal cancer was first reported by Vogelstein et al. (1989). It was then explored by many investigators. Attempts to establish the relationship between colorectal cancers and allele losses at different chromosome loci have been made. In one study aimed to screen LOH in the colorectal cancer, the frequency of allelic losses were determined at 17p (28%), 18p (26%), 18q and 5q (25%), 9q and 15q (24%), 8p (23%) and 14q (20%) throughout the genome (Mao et al., 2006). Nevertheless, Mao et al. (2006) reported six different LOH areas as 4q (42%), 1p (36%), 11q (31%), 17p (28%), 18p (26%) and 1q (25%) in sporadic colorectal carcinomas.

PTPRJ (protein tyrosine phosphatase receptor type J) on chromosome band 11p11 is a gene exposed to deletion among several cancer types including colorectal cancer. Ruivenkamp et al. (2003) reported a strong correlation between the LOH of PTPRJ and the loss of chromosomal region 18q12-21 in a more progressed form of colorectal adenomas (Ruivenkamp et al., 2003).

In a study, allele loss at 15q14-q22 was studied in 277 cases of early-onset colorectal cancers using four microsatellite markers (D15S970, D15S117, D15S971 and D15S1028) (Popat et al., 2003).

The observation of high frequency LOH on chromosome 1 in patients with sporadic colorectal cancer by Zhou et al. (2004) may suggest the presence of putative tumour suppressor genes associated with sporadic colorectal cancer on 1p36.31-36.33. TP73, a member of p53 gene family located on chromosome 1p36, is a tumour suppressor gene (Kapiteijn et al., 2001). However, due to the fact that many other genes are also located in 1p36, the effect of TP73 on colorectal carcinogenesis needs to be further determined (Zhou et al., 2004). In another study on long arm of chromosome-1, (1q31.1-32.1), high-frequency LOH was determined in sporadic colorectal carcinoma. No significant association between clinic pathological findings (patient gender, age, tumour size and the stages of tumour) and LOH in each selected marker was observed. One or more tumour suppressor genes may be located within the targeted

region. After searching databases, CSR1 was presumed to be involved in the carcinogenesis of colorectal carcinoma. However, no functional evidence exists (Zhou et al., 2008).

The neurofibromatosis type 1 (NF1) gene is a large gene located on chromosome 17q11.2. The NF1 protein turns active form of Ras protein into an inactive form. A well-defined transition is needed from NF1 to Ras. Ras protein plays a central role in cellular growth and differentiation and its aberrant activation is involved in the development of a variety of human cancers. Negative regulation of Ras activity classifies NF1 gene as a tumor suppressor gene (Cacev et al., 2005). Loss of heterozygosity at NF1 locus was determined in colon tumor tissues. NF1 isoform type I was found to significantly increase in tumor tissue compared with normal tissue. Thus, NF1 isoform type I may play a role in the development and progression of colon cancer (Cacev et al., 2005).

Loss of heterozygosity on long arm of chromosome 22 was also investigated. In these studies, LOH on chromosome 22 was observed in the oral (40%), brain (40%), ovary (55%), breast (40%), pancreatic endocrine (30%) tumours and gastrointestinal stromal tumours (77%). An allele loss at average rate of 28.38% on 22q13 region was also reported in colorectal cancer (Zheng et al., 2005).

Chang et al. (2005) investigated prognostic value and frequency of LOH at 14 genetic loci near regions containing important genes in colorectal cancer formation. In one of such genetic locus, LOH occurred in 78.8% of the tumours. Regions observed at highest frequency of LOH were determined as tumour suppressor gene locus (TP53 (65%), DCC (64.3%), D8S254 (51.7%) and APC (47.8%)) (Chang et al., 2005). High-frequency LOH was also found to correlate with high metastatic potential of colorectal cancers. In another study, the relationship between LOH in regions where APC and DCC genes are located and micro metastasis in per colonic lymph nodes was also studied (Zauber et al., 2004) and no correlation was found. However, Tanaka et al. showed interaction between LOH of region 18q21 in colorectal cancers and lymph node metastasis (Tanaka et al., 2008). Parallel results from different studies may suggest a positive correlation between loss of heterozygosity and the metastatic potential of colorectal cancers.

While the positive correlation between clinic pathological findings and LOH in colorectal cancers was shown in some studies, the prognostic value of LOH is still not clear. To determine the correlation between allele loss at the BRCA1 locus and patient prognosis in sporadic colorectal cancers, Garcia et al. (2003), reported that the 1st and 2nd stages of LOH in gene locus BRCA1 could be used as a free prognostic factor for colorectal cancer. In one study 40 microsatellite markers in eight cancer-related chromosomes 3p, 4p, 5q, 8p, 9p, 13q, 17p and 18q were analyzed. Unilateral chromosomal loss was

found to correlate with clinical and pathological findings (Choi et al., 2002). In another study, Kapitanovic et al. (2004) detected LOH at the APC gene loci in 30.1% of tumours examined. The APC gene LOH was significantly higher in B stage of the Dukes (55.6%) and in the moderately differentiated tumours (Kapitanovic et al., 2004).

Activated Ras proto-oncogenes, especially Kras2, play an important role in the carcinogenesis. Mutation of Kras2 gene was detected in breast, rectum, stomach, pancreas, ovarian, lung and liver tumours (Wan et al., 2006). Wan et al. reported allele losses in chromosome region 12p12-13 where Kras2 gene is located in Chinese colon carcinoma tissues. Just like other studies, in this study no correlation was observed between the occurrence of LOH and age, patient sex, size of tumour and metastases of cancer cells (Wan et al., 2006). In another study, no correlation was found between clinic pathological findings and LOH observed in loci D4S3013 (4p15.2) and D4S405 (4p14) (Zheng et al., 2005).

CRC develops after a long carcinogenesis cycle and multiple stages. A number of tumour suppressor genes are involved in this process. Some are shown to be correlated with the progression of colorectal cancer. However, it is possible that many unidentified tumour suppressor genes may exist. In these studies, the correlation between LOH analyses and candidate tumour suppressor genes, as well as their roles in the tumorigenesis of colorectal cancer were investigated. However, clinical and pathological findings are also important for the progression of colorectal cancers like that in other cancers. In this review, the correlation between clinical and pathological findings with allele losses on various chromosome regions in colorectal cancer was discussed. As a result, the candidate tumour suppressor genes can be determined by LOH analyses. In addition, allele losses chromosome regions, where candidate tumour suppressor genes are located, may be important in terms of the progress of the disease.

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