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Associations of defect mismatch repair genes with prognosis and heredity in sporadic colorectal cancer

L. Ghanipour [a,*], K. Jirström [b], M. Sundström [c], B. Glimelius [d], H. Birgisson [a]

[a] *Department of Surgical Science, University of Uppsala, Uppsala, Sweden*
[b] *Division of Oncology-Pathology, Department of Clinical Sciences, Lund University, Lund, Sweden*
[c] *Department of Immunology, Genetics and Pathology, Molecular and Morphological Pathology, University of Uppsala, Uppsala, Sweden*
[d] *Department of Radiology, Oncology and Radiation Science, University of Uppsala, Uppsala, Sweden*

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Abstract

Background: Microsatellite instability arises due to defect mismatch repair (MMR) and occurs in 10–20% of sporadic colorectal cancer. The purpose was to investigate correlations between defect MMR, prognosis and heredity for colorectal cancer in first-degree relatives.
Material and methods: Tumour tissues from 318 patients consecutively operated for colorectal cancer were analysed for immunohistochemical expression of MLH1, MSH2 and MSH6 on tissue microarrays. Information on KRAS and BRAF mutation status was available for selected cases.
Results: Forty-seven (15%) tumours displayed MSI. No correlation was seen between patients exhibiting MSI in the tumour and heredity ($p = 0.789$). Patients with proximal colon cancer and MSI had an improved cancer-specific survival ($p = 0.006$) and prolonged time to recurrence ($p = 0.037$). In a multivariate analysis including MSI status, gender, CEA, vascular and neural invasion, patients with MSS and proximal colon cancer had an impaired cancer-specific survival compared with patients with MSI (HR, 4.32; CI, 1.46–12.78). The same prognostic information was also seen in distal colon cancer; no recurrences seen in the eight patients with stages II and III distal colon cancer and MSI, but the difference was not statistically significant.
Conclusion: No correlation between MSI and heredity for colorectal cancer in first-degree relatives was seen. Patients with MSI tumours had improved survival.

Keywords: MSI; MSS; Colorectal cancer; TMA; Heredity; Prognosis

Introduction

Colorectal cancer (CRC) is the third most common cancer in Sweden[e] and worldwide. Approximately 15% of all patients with the disease have a positive family history of CRC.[1,2]

Genetic alterations through many steps of events leading to progression of tumorigenesis in CRC were comprehensively first reported by Vogelstein and Fearon.[3] At least two major pathways of genetic instability have been described; the chromosomal instability (CIN) pathway and the microsatellite instability (MSI) pathway. The CIN pathway is found in approximately 60–70% of all sporadic CRC cases and is characterized by inactivation of many tumour suppressor genes and oncogenes such as adenomatous polyposis coli gene (APC), p53, KRAS and by mutations and loss of heterozygosity (LOH).[5] The MSI pathway occurs due to inactivation of DNA mismatch repair (MMR) genes and accounts for approximately 15% of all sporadic CRC, usually by hypermethylation of MLH1 and silencing of the promoter gene.[6] Inactivation of the MMR system causes inability to repair errors occurring during DNA replication, especially

* Corresponding author.
E-mail address: arezo_g@hotmail.com (L. Ghanipour).
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in the repeat sequences, i.e microsatellites, causing MSI. In Lynch syndrome (hereditary non-polyposis CRC, HNPCC), MSI arises as a result of mutations in MMR genes, most often MLH1, MSH2, MSH6 and PMS2.[7] MSI tumours are associated with good prognosis, proximal colon location, mucinous or signet-ring cell type, poor differentiation, presence of infiltrating lymphocytes and near-diploid phenotype.[8]

The classic technique for MSI analysis is by polymerase chain reaction (PCR). The National Cancer Institute has recommended a panel of five specific microsatellite markers for detection, the Bethesda panel. If two or more of the microsatellite markers show instability, the tumour is classified as being MSI-high (MSI-H) and if only one marker is unstable, the tumour is referred to as MSI-low (MSI-L). Tumours that show no instability are called microsatellite stable (MSS).[9] Another accepted method for detection of MMR mutations is by immunohistochemistry (IHC).[10]

Family history for CRC is a risk factor for development of CRC. Lifetime risk of developing CRC is double among those with a first-degree relative having CRC, and the risk increases significantly if the diagnosis is set at an early age.[11] In a previous study on the present cohort, patients with colon cancer and positive family history of CRC in first-degree relatives had a better survival than those without heredity.[2] The aim of this study was to evaluate whether the improved survival in patients with CRC and family history of CRC in first-degree relatives could be explained by MSI and to bring further insights into the associations between MSI and prognosis in relation to other clinic-pathological variables.

Patients and methods

Patients

Three hundred and twenty patients operated for CRC, between August 2000 and December 2003, at the central district hospital in Västerås, Sweden participated in this prospective study. Information about tumour size, lymph node status, lymphatic or vascular vessel invasion, mucinous cell type and grade of tumour differentiation were received from pathology reports. Information on stage, cancer recurrence, death, and causes of death was received by matching with the Swedish Colorectal Cancer Registry (SCRCR) and from surgical and oncological hospital records. Preoperatively, all patients were asked about family history, and 33 patients had a first-degree relative with verified CRC.[2] Among them 2 patients had Lynch syndrome according to the Amsterdam II criteria, which were excluded from the analysis.

Tissue microarray (TMA) construction

TMAs were constructed from formalin-fixed paraffin-embedded tissue.[12] Prior to TMA construction, all cases were histopathologically re-evaluated on haematoxylin and eosin-stained sections by one pathologist (KJ). Areas representative of invasive cancer, normal adjacent mucosa, and when present, adenomatous lesions and lymph node metastases, were marked on the slides. For each case, a manual arraying device (MTA-1, Beecher Instruments, Sun Prairie, Wisconsin, USA) was used for extraction of two 1.0 mm cores of invasive tumour tissue, one 1.0 mm core from normal mucosa, adenomatous mucosa, and lymph node metastases, as previously described.[13]

Immunohistochemistry and annotation

IHC was performed on 4 μm TMA sections using monoclonal antibodies against MLH1 (Clone ES05, art.nr M3640, Dako), MSH2 (Clone FE11, art.nr NA27, Calbiochem (VWR)), and MSH6 (Clone EPR 3945, art.nr 2846-B, Epitomics (Biosite)). Stained slides were scanned in a high-resolution scanner (ScanScope T2, Aperio Technologies) and separated into spot images representing the different cores in the TMAs. The histological images were analysed and evaluated by a trained annotator (LG) with a web-based annotation system (Imagescope viewer, a digital pathology system). For 19 cases problems emerged regarding interpretation of the results during annotation. Since we had access to fresh frozen tumour tissue from 15 of these cases, re-evaluation with IHC was done by LG.

Normal colonic and rectal epithelium displayed strong nuclear expression of MMR proteins (Fig. 1). Complete absence of nuclear staining was recorded as MSI and positive nuclei staining was considered as MSS (Fig. 1). Surrounding stromal cells and infiltrating lymphocytes functioned as internal controls. Cases without positive staining of controls were considered as non-representative.

Molecular analyses for MSI

As part of another unpublished study, MSI analysis was performed in 32, randomly selected tumours of patients in stage II–III CRC, by the analysis system version 1.2 (Promega, Madison, WI) with 6 ng genomic DNA of fresh frozen tumour tissue, using the Bethesda panel of microsatellite markers (BAT25, BAT26, NR-21, NR-24 and MONO-27). The analyses were performed on a 3130 × 1 genetic analyser (Applied Biosystems, Foster city, CA). The categorization into MSI-H, MSI-L and MSS was as described above.

KRAS and BRAF mutation analyses

Mutation analysis was performed on tumour tissue samples from 207 patients in stage I–IV CRC for analysis of KRAS mutations and from 32 patients in stage II–III CRC (the same tumours as for MSI-PCR) for BRAF mutation analysis, by Pyrosequencing according to the manufacturer's recommendation for PyroMark Q24 BRAF and KRAS v20 assays (Qiagen GmbH, Hilden,

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