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Mitochondrial genome alterations in rectal and sigmoid carcinomas

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ABSTRACT

The scarce studies on the molecular pathways involved in the pathogenesis of rectal cancer indicate that these may vary, at least in part, from those relevant for colon cancer. Mitochondrial DNA alterations have been described in several human cancers. We aimed to study D310, *ND1* and *ND5* microsatellite sequence alterations and nuclear microsatellite instability in a series of 38 rectal carcinomas as compared to a series of 25 sigmoid carcinomas. D310 sequence alterations were observed in 34.3% and 37.5% of rectal and sigmoid carcinomas, respectively, whereas *ND1* mutations were present in 2.6% in RC and *ND5* mutations were detected in 5.3% and 8% of rectal and sigmoid carcinomas, respectively. A trend toward an association between nuclear and mitochondrial microsatellite instability was observed in sigmoid but not in rectal cancers. In conclusion, mitochondrial genome alterations are common in both rectal and sigmoid carcinomas and may contribute to their pathogenesis.

1. Introduction

Rectal cancer (RC) represents 25–35% of all large bowel carcinomas. Several studies point to differences between cancer of the right and left colon and rectum concerning etiology, clinical behavior, and pathological features [1,2]. The scarce genetic studies in RC indicate that these tumors, as colon cancer (CC), can develop through chromosomal instability (CIN) and microsatellite instability (MIN) pathways. It is estimated that 80–98% of the RC cases involve the CIN pathway, presenting high mutational frequency in *APC*, *TP53*, and *KRAS* in addition to numerous chromosome changes [1–3].

Much attention has recently been given to the relevance of alterations in the mitochondrial genome for carcinogenesis. Mitochondria have essential functions in cellular metabolism and apoptosis regulation. Human mitochondrial genome (mtDNA) is a 16,569 base-pair (bp), double-stranded, circular DNA that encodes 13 components of the respiratory chain [e.g. *NADH dehydrogenase 1* (*ND1*) and *5* (*ND5*)] and two ribosomal and 22 transfer RNAs. It also presents a noncoding region, named Displacement loop (D-loop), where the heavy chain replication origin is located and binding place of several transcription factors [4]. Mitochondrial genome presents a mutation rate several times higher than the nuclear genome, essentially due to the lack of histones, limited repair mechanisms and high concentration of reactive oxygen species (ROS) [5,6]. Mitochondrial DNA alterations have been described in several human cancers, mostly in the D310 microsatellite sequence located in the D-loop region. In colorectal cancer (CCR) in general, the D310 sequence presents a

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mutational frequency of 28–44% [5,7]. Mutations in genes that encode respiratory chain components, namely *ND1* and *ND5*, have also been described [7]. These alterations may have an impact on mitochondrion function and contribute to carcinogenesis through apoptosis disruption or respiratory chain alterations [7]. The mutational frequency in the D310 sequence in RC is 28–31%, but no mutations have been described in *ND1* and *ND5* microsatellite sequences in tumors at this location [8,9]. An association between mitochondrial microsatellite instability (mtMSI) and nuclear MSI (nMSI) has been also studied in different tumors, but no conclusion has been reached [7,10,11]. Nuclear microsatellite instability is a widespread instability in coding and noncoding microsatellite sequences due to mismatch repair (MMR) deficiency [12]. Mitochondrial microsatellite instability can be defined as change in length in mtDNA microsatellite sequences, but the underlying mechanism is unknown.

The scarce studies on rectal oncogenesis indicate that the mutational spectrum may differ, at least in part, from that observed in CC. We aimed to contribute to the understanding of this issue by characterizing the pattern of certain mitochondrial alterations in RC as compared to sigmoid cancer (SC).

2. Materials and methods

2.1. Patient characteristics and DNA extraction

Tissue samples were obtained from 38 RC and 25 SC after surgical resection at the Portuguese Oncology Institute-Porto. Rectum and sigmoid colon were defined as the large bowel regions up to 15 cm and from 18 to 30 cm from the anal verge, respectively. Tumors from the rectosigmoid junction, defined as the large bowel region between 15 and 18 cm from the anal verge, were not included in this study.

All tumor samples were paraffin embedded and reviewed by a pathologist. Peripheral blood or normal colorectal mucosa as far as possible from the tumor (when peripheral blood was not available) was also collected from the same patients. Clinical data was drawn from hospital records and tumor staging was performed using the American Joint Committee on Cancer (AJCC) criteria (Table 1). This study was approved by the Institutional Review Board.

Table 1
Clinical data on the 38 rectal and 25 sigmoid cancer patients.

Clinical and histopathologic features	Rectal cancer patients (%)	Sigmoid cancer patients (%)
Gender		
Female	20 (53)	10 (40)
Male	18 (47)	15 (60)
Age		
Median	60	61
Range	34–79	47–78
TNM stage[a]		
I	7 (18)	2 (8)
II	14 (37)	15 (60)
III	13 (34)	4 (16)
IV	4 (11)	3 (12)

[a] Data not available for one sigmoid case.

DNA was isolated from paraffin-embedded tumor and normal mucosa as described by Lungu et al. [13] and from peripheral blood using the salt–chloroform extraction method [14].

2.2. D310, ND1 and ND5 mutation screening

The D310 sequence was analyzed only in the cases with matching normal control (35 RC cases and 24 SC cases) by PCR followed by fragment analysis. DNA sequences were amplified under standard PCR conditions and analyzed for length variations on an ABI Prism 310 DNA sequencer (Applied Biosystems, Foster City, CA) [8]. Data analysis was accomplished by GeneMapper software (3.7 version, Applied Biosystems). Differences in fragment lengths between tumor and matching blood or normal mucosa samples were confirmed by direct sequencing on an ABI 310 DNA sequencer using Big Dye Terminator V1.1 Chemistry (Applied Biosystems), according to the manufacturer's recommendations. Data analysis was done by Sequencing Analysis software (5.2 version, Applied Biosystems). The poly-C tract (C_6) located in the *ND1* gene and the poly-C (C_6) and poly-A (A_8) tracts located in the *ND5* gene were also analyzed by direct sequencing as described above.

Our results were compared with published mtDNA reference sequence (GenBank, access number AC_000021) [15] and Mitomap database (MITOMAP: A Human Mitochondrial Genome Database, 2008, http://www.mitomap.org) [16]. Sequence variations found in both healthy and tumor tissue mtDNA were considered as germline polymorphisms. Variations in mtDNA sequences between tumor and matched healthy tissue were considered somatic mutations. The results were independently scored by two observers and a second round of analyses confirmed the results.

2.3. Microsatellite instability analysis

Microsatellite instability was evaluated using the Bethesda panel (BAT25, BAT26, D2S123, D5S346 and D17S250). According to the 1997 National Cancer Institute guidelines, tumors are MSI-high (MSI-H) when at least two out of the five loci are unstable in tumor DNA when compared with the normal counterpart, MSI-low (MSI-L) when one of five loci is unstable, and microsatellite stable (MSS) when none of the loci presents instability.

The Bethesda marker panel was analyzed by PCR and fragment analysis. PCR was carried out as previously described using fluorescence-labeled primers [17]. Fragments were analyzed for length variations on an ABI Prism 310 DNA sequencer and allele sizes were determined using GeneMapper software (3.7 version, Applied Biosystems). The results were independently scored by two observers and a second round of analyses confirmed the results.

2.4. Statistical analysis

Statistical analysis was carried out with SPSS version 15. Results were expressed in absolute frequencies and percentages. Variable comparison was performed by χ^2 and Fisher exact tests. *P* values inferior to 0.05 were considered statistically significant.