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Double strand break repair components are frequent targets of microsatellite instability in endometrial cancer

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ABSTRACT

Aim: DNA double strand break (DSB) repair is a central cellular mechanism of the DNA damage response to maintain genomic stability. DSB components are frequently mutated in colorectal cancer with microsatellite instability (MSI). We investigated whether DSB repair is involved in endometrial cancer (EC) with MSI.

Methods: Mononucleotide microsatellite tracts of 14 genes of the DSB repair system were analysed in a series of 41 EC with MSI. Among these genes, the *microcephalin 1* (*MCPH1/BRIT1*) has never been tested as target of MSI in tumour series.

Results: The most frequently mutated gene was *DNAPKcs* ($n = 14$, 34%) followed by *RAD50* ($n = 7$, 17%), *MRE11*, *ATR* and *BRCA1* ($n = 6$, 15%), and by *CtIP* and *MCPH1* ($n = 5$, 12%). While DSB biallelic mutations were infrequent, a high proportion of tumours ($n = 30$, 73%) presented mutations at some component of the DSB repair pathway, and almost half of them showed alterations at two or more components. Tumours with mutations in two or more genes were significantly associated with advanced grade ($p = 0.03$) and vascular invasion ($p = 0.02$) and marginally associated with advanced stage ($p = 0.07$).

Conclusions: Our results suggest that in EC, the DSB repair is a relatively common mutational target of MSI and might contribute to tumour progression, and also that *MCHP1* may be a novel target gene of MSI.

1. Introduction

Microsatellite instability (MSI) is the hallmark of cancer with DNA mismatch repair (MMR) deficiency, and a widespread phenomenon throughout the genome. It consists in the accumulation of hundreds of thousands of somatic insertions/deletion mutations in simple repeated sequences known as microsatellites.[1] Among this bulk of alterations, cancer arises

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when mutations occur at cancer genes, such as those involved in cell growth and survival and DNA repair,. especially in short microsatellite repeats in coding regions present in target genes.[1,2] MSI is the main diagnostic feature of the majority of hereditary non-polyposis colorectal cancer (HNPCC), and also of a subset of sporadic colorectal, gastric and endometrial tumours.[2,3] In endometrial cancer (EC), MSI is present in about 20% of tumours and, as in the majority of sporadic tumours, is mainly caused by epigenetic silencing of the MMR gene *MLH1*.[4]

The DNA damage response (DDR) consists of intricate signalling networks that detect DNA lesions, signal their presence and promote their repair. These sensory networks impact on countless cellular functions (cell cycle, apoptosis, chromatin structure, etc.) and orchestrate cell regulation at multiple levels. DDR preserves genomic stability, and defects in the ability to properly respond to and repair DNA damage increase the risk of tumour development. Several mechanisms are known to participate in the DDR, such as MMR system, double strand break (DSB) repair, nucleotide excision repair (NER), base excision repair (BER), trans-lesion bypass mechanisms, direct lesion reversal and Fanconi anaemia pathway.[5] In the last years a great progress has been made towards understanding the DDR, although studies about how the different participants interact and are controlled are constantly being released.

Cells use two main mechanisms to repair DSBs, non-homologous end-joining (NHEJ) and homologous recombination (HR), depending on the phase of the cycle and the nature of the DSB ends. NHEJ can occur throughout the cell cycle and is the major pathway for the repair of DSBs in multicellular eukaryotes, whereas HR is restricted to late S or G2 since it uses sister-chromatid sequences as a template to repair.[5] DSB repair components are potential targets of MSI. In colorectal cancer (CC), Miquel et al.[6] have recently studied many genes of this DNA repair network. The authors found that a high proportion of tumours carried alterations at one or more components of NEHJ and HR pathways. Such an exhaustive analysis has not been performed so far in EC with MSI, although mutations in several genes have been previously reported.[7–9]

In the present study we investigated 14 DSB repair genes as possible targets of MSI in EC. We also estimated the association between mutations and clinicopathological variables. Among the 14 candidate genes analysed, we included *microcephalin 1* (*MCPH1/BRIT1*), a novel candidate tumour suppressor gene, that has never been tested as target of MSI in tumour series. Although the gene was initially identified as involved in determining the brain size, recent studies suggest that MCPH1 participates in essential biological processes such as the regulation of mitotic entry, the maintenance of genomic stability and the DSB repair.[10–12] Besides, reduced expression of MCPH1 has been reported in ovarian, prostate and breast tumours.[11]

2. Material and methods

2.1. Tumour samples and MSI classification

Our series consisted of 41 EC patients with MSI from a previously described series.[13] The study was approved by the institutional ethical committee and all subjects provided informed consent. MSI determination and classification were performed by using five recommended quasimonomorphic mononucleotide markers (BAT25, BAT26, NR21, NR24 and NR27) according to published conditions and criteria, with some modifications.[14] Briefly, primer sequences of markers were as reported and were amplified in pentaplex in 25 μL reaction volume containing 20 ng of DNA, 240 nM of each primer pair for NR21, NR24, NR27 and BAT25 and 1 μM of each primer pair for BAT26, 200 μM of deoxynucleotide triphosphates, 1× commercial Biotaq Reaction Buffer, 2 mM $MgCl_2$ and 1 U Biotaq DNA Polymerase (Bioline). Thermocycling conditions were: 94 °C, 5 min, followed by 12 cycles of 30 s at 94 °C, 30 s at 72 °C 30 s and 30 s at an annealing temperature that decreased 2 °C every 3 cycles (beginning at 61 °C in the first 3 cycles and decreasing to 55 °C in the last 3 cycles) and 25 cycles of 30 s at 94 °C for 30 s, 30 s at 72 °C and 30 s and 55 °C. A final extension step was carried out at 72 °C for 10 min, followed by storage at 4 °C. The PCR products were diluted 1:50 in distilled water and 1 μL of the dilution was mixed with 9.75 μL of formamide and 0.25 μL of GeneScan 500 LIZ Size Standard (Applied Biosystems), denatured at 98 °C for 5 min and cooled on ice. Fragments were separated by automated capillary electrophoresis in an ABI Prism 3100 16-capillary genetic analyser (Applied Biosystems) and electropherograms were analysed using the Peak Scanner Software v1.0 (Applied Biosystems).

2.2. Frameshift mutation detection in genes of the DSB repair

Frameshift mutations within mononucleotide tracts of 14 genes involved in the DSB repair were analysed. Regions encompassing mononucleotide repeated sequences of genes were amplified in monoplex PCR reactions. For each reaction 20 ng of genomic DNA, 240 nM of each forward and reverse primer, 200 μM of deoxynucleotide triphosphates, 1× commercial Biotaq Reaction Buffer, 2 mM $MgCl_2$ and 1 U Biotaq DNA Polymerase (Bioline) were mixed in a final volume of 25 μL. The forward primer of each pair was marked with the fluorescent dye 6-FAM. Thermocycling conditions were: 94 °C, 5 min, followed by 35 cycles of 30 s at 94 °C, 30 s at the corresponding annealing temperature, and 30 s at 72 °C, with a final cycle of extension for 10 min at 72 °C. Details of PCR conditions for *MCPH1* analysis are shown in Table 1. For the other genes additional information is available upon request. Different groups of amplicons were mixed for simultaneous genotyping. Thus, 1 μL of each amplicon was mixed and diluted up to 50 μL in distilled water, 1 μL of the dilution was mixed with formamide and the size standard, and analysed as explained above. Deletions or insertions in the repeated tracts were identified based on peak pattern alterations when compared to normal DNA sample profiles. Fig. 1 illustrates some electrophoretic profiles of *MCPH1* mononucleotide repeats.

2.3. Statistical analysis

The χ-squared and Fisher's exact non-parametric tests were used to analyse the relationship between the number of mutated DSB components in each tumour and the clinicopatho-