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Research Paper

A Specific Mutational Signature Associated with DNA 8-Oxoguanine Persistence in MUTYH-defective Colorectal Cancer

Alessandra Viel [a,1], Alessandro Bruselles [b,1], Ettore Meccia [c,1], Mara Fornasarig [d], Michele Quaia [a], Vincenzo Canzonieri [e], Eleonora Policicchio [f], Emanuele Damiano Urso [g], Marco Agostini [g,h], Maurizio Genuardi [i,j], Emanuela Lucci-Cordisco [i], Tiziana Venesio [k], Aline Martayan [l], Maria Grazia Diodoro [l], Lupe Sanchez-Mete [l], Vittoria Stigliano [l], Filomena Mazzei [c], Francesca Grasso [c], Alessandro Giuliani [c], Marta Baiocchi [b], Roberta Maestro [a], Giuseppe Giannini [m], Marco Tartaglia [f,2], Ludmil B. Alexandrov [n,o,p], Margherita Bignami [c,2,*]

[a] *Functional Onco-Genomics and Genetics, CRO Aviano National Cancer Institute, Aviano, Italy*
[b] *Department of Haematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy*
[c] *Department of Environment and Primary Prevention, Istituto Superiore di Sanità, Rome, Italy*
[d] *Unit of Gastroenterology, CRO Aviano National Cancer Institute, Aviano, Italy*
[e] *Unit of Pathology, CRO Aviano National Cancer Institute, Aviano, Italy*
[f] *Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, Rome, Italy*
[g] *First Surgical Clinic, Department of Surgical, Oncological and Gastroenterological Sciences, University of Padua, Padua, Italy*
[h] *Nano Insperid Biomedicine Lab, Istituto di Ricerca Pediatrica (IRP) Padua, Italy*
[i] *Institute of Genomic Medicine, Catholic University, Rome, Italy*
[j] *Fondazione Policlinico Universitario "A. Gemelli, Rome, Italy*
[k] *Unit of Pathology, Candiolo Cancer Institute-FPO, IRCCS, Candiolo, Turin, Italy*
[l] *Division of Gastroenterology and Digestive Endoscopy, Regina Elena National Cancer Institute, Rome, Italy*
[m] *Department of Molecular Medicine, University La Sapienza, Rome, Italy*
[n] *Theoretical Biology and Biophysics, Los Alamos National Laboratory, Los Alamos, NM, USA*
[o] *Center for Nonlinear Studies, Los Alamos National Laboratory, Los Alamos, NM, USA*
[p] *University of New Mexico Comprehensive Cancer Center, Albuquerque, NM 87102, USA*

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ABSTRACT

8-Oxoguanine, a common mutagenic DNA lesion, generates G:C>T:A transversions via mispairing with adenine during DNA replication. When operating normally, the MUTYH DNA glycosylase prevents 8-oxoguanine-related mutagenesis by excising the incorporated adenine. Biallelic *MUTYH* mutations impair this enzymatic function and are associated with colorectal cancer (CRC) in MUTYH-Associated Polyposis (MAP) syndrome. Here, we perform whole-exome sequencing that reveals a modest mutator phenotype in MAP CRCs compared to sporadic CRC stem cell lines or bulk tumours. The excess G:C>T:A transversion mutations in MAP CRCs exhibits a novel mutational signature, termed Signature 36, with a strong sequence dependence. The MUTYH mutational signature reflecting persistent 8-oxoG:A mismatches occurs frequently in the *APC*, *KRAS*, *PIK3CA*, *FAT4*, *TP53*, *FAT1*, *AMER1*, *KDM6A*, *SMAD4* and *SMAD2* genes that are associated with CRC. The occurrence of Signature 36 in other types of human cancer indicates that DNA 8-oxoguanine-related mutations might contribute to the development of cancer in other organs.
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1. Introduction

DNA repair provides a major protection against cancer, and germline DNA repair defects in inherited syndromes confer a significant cancer predisposition. DNA replication errors are a major source of mutations, and early-onset colorectal and other cancers are associated with germline mutations in DNA mismatch repair (MMR) genes (Lynch et al., 2015) or in the proofreading exonuclease domains of DNA

* Corresponding author.
E-mail address: margherita.bignami@gmail.com (M. Bignami).
[1] These authors equally contributed to this work.
[2] These authors jointly supervised this work.

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polymerases POLE and POLD1 (Palles et al., 2013). In a distinct mutational mechanism, endogenously or exogenously generated reactive oxygen species (ROS) induce pre-mutagenic DNA lesions. Most ROS-induced base damage is repaired by base excision repair (BER) initiated by DNA glycosylases. 8-Oxoguanine (8-oxoG), one of the most common oxidative DNA lesions can mispair with adenine during DNA replication to generate G:C>T:A transversion mutations. 8-oxoG-induced mutagenesis is prevented by the cooperative action of the DNA glycosylases encoded by the *OGG1* and *MUTYH* genes. OGG1 removes 8-oxoG from 8-oxoG:C pairs and MUTYH scans the newly-synthesized daughter strand to locate and remove incorporated adenine mispaired with 8-oxoG (Mazzei et al., 2013; Markkanen et al., 2013; Tsuzuki et al., 2007). A hydrolase encoded by the *MTH1* gene provides a third level of protection against oxidative mutagenesis by degrading 8-oxodGTP to prevent the incorporation of 8-oxodGMP into DNA (Mo et al., 1992). Inactivation of any of these genes increases steady-state DNA 8-oxoG levels and confers a mutator phenotype (Klungland et al., 1999; Tsuzuki et al., 2001; Hirano et al., 2003).

To date, no human disease has been associated with defective OGG1 or MTH1 activities. In contrast, germline biallelic *MUTYH* mutations underlie *MUTYH*-associated polyposis (MAP), a recessively heritable colorectal polyposis with a predisposition to colorectal cancer (CRC) (Al-Tassan et al., 2002; Sieber et al., 2003). CRCs in MAP patients bear distinctive somatic G:C>T:A transversions in the *APC* gene (Al-Tassan et al., 2002; Sieber et al., 2003; Jones et al., 2002). The relationship between defective repair of oxidized DNA and CRC susceptibility was strengthened by the recent report that germline mutations in *NTHL1*, encoding a DNA glycosylase involved in the BER of oxidized pyrimidines are associated with a polyposis clinically similar to MAP (Weren et al., 2015). Thus, two repair pathways counteract CRC susceptibility by acting at replication to process mismatches containing oxidized DNA bases.

Mutational signatures in cancer genomes provide indications of the mechanisms underlying neoplastic transformation (Alexandrov, 2015; Alexandrov et al., 2013a,b, 2015; Helleday et al., 2014). Thus, CRC from MAP patients offer the unique opportunity to identify a mutational fingerprint of persistent 8-oxoG:A mismatches. Here, we report that whole-exome DNA sequencing identifies a distinct mutational signature of G:C>T:A transversions in MAP CRC. The mutational signature is reflected in the specific pattern of oncogenes/tumour suppressors involved in colorectal carcinogenesis and associated with inactive MUTYH. Our findings also indicate the possible involvement of DNA oxidation-related mutations in other types of human cancer as the *MUTYH* mutational fingerprint is present in cancers of other organs.

2. Material and Methods

2.1. Subjects and Tissue Samples

All subjects included in this study gave informed consent for gene testing and related research (Table S1). The local scientific IRB approved the collection and usage of tumour samples for this research (CRO Aviano IRB-03-2016). Blood, normal intestinal mucosa, adenomas and CRCs were retrospectively obtained from MAP patients with confirmed constitutional biallelic mutations in the *MUTYH* gene. CRCs were collected at surgery, while adenomas and normal mucosa were harvested at colectomy or endoscopic polypectomy. In all cases histopathological evaluation was performed by a pathologist.

DNA from frozen tissues was extracted using the Qiagen DNeasy Tissue kit (Qiagen, Hilden, Germany) with a protocol including RNase treatment. FFPE tissues were deparaffinized by two washes in Noxyl (15 min at 56 °C) and DNA was then obtained using the FFPE Qiagen Tissue kit (Qiagen). DNA quality and concentrations were assessed using Nanodrop spectrophotometer (ThermoFischer Scientific, MA, USA) and Qubit Fluorometer (Invitrogen, CA, USA).

2.2. Whole Exome Sequencing, Data Analysis and Validation

Whole Exome Sequencing was performed by the Beijing Genomics Institute (BGI, Hong Kong). Target enrichment was performed using in-solution technology (NimbleGen SeqCap EZ Library v.3.0, Roche) and the resulting target libraries were sequenced by Illumina sequencing technology (HiSeq2000) (Illumina, San Diego, CA, USA). Raw image files were processed by Illumina base calling software (CASAVA 1.7) using default parameters. Paired-end reads were aligned to the human genome (UCSC GRCh37/hg19) with the Burrows-Wheeler aligner (BWA v. 0.7.10). Presumed PCR duplicates were removed using Picard's MarkDuplicates. The Genome Analysis Toolkit (GATK 3.2) was used for realignment of sequences encompassing indels and for base quality recalibration. Somatic single-nucleotide variants were detected using muTect software v.1.1.6, and small indels were identified through a comparison between indels called in individual CRCs and their matched non-tumoural samples by means of the GATK Haplotype Caller algorithm, applying the following quality filters: quality score > 100 and quality-by-depth score > 1.5; indels below these thresholds or resulting from four or more reads having ambiguous mapping (this number being >10% of all aligned reads) were discarded. The resulting SNVs and small indels were annotated by SnpEff v3.6 and dbNSFP2.8 in terms of functional impact of variants (missense or nonsense, coding or non-coding, location with respect to exon-intron junction, depth, reference/variant reads ratio, dbSNP ID, amino acid change and position, cancer association data and structural/functional impact). Variant validation and genotyping were performed by Sanger sequencing. Amplicons were directly sequenced using the ABI BigDye Terminator Sequencing kit (Applied Biosystems, Foster City, CA, USA) and an automated capillary sequencer (ABI 3500, Applied Biosystems). Sequence electropherograms were analyzed using Sequencing Analysis Software v.5.4 (Applied Biosystems).

2.3. Microsatellite Instability Analysis

Five mononucleotide and two pentanucleotide markers were co-amplified using the MSI analysis system (Promega Madison, WI, USA) according to the manufacturer's protocol. The fluorescent-labeled PCR products obtained from constitutional DNA and tumour DNA were separated by capillary electrophoresis using an AB3130 xl sequencer and evaluated with the GeneMapper software (Applied Biosystems/Life technologies). The 5 quasi-monomorphic mononucleotide markers included in the kit have high sensitivity and specificity in detecting alterations in tumour samples with mismatch repair defects. Criteria for definition of MSS and MSI are according to the Bethesda guidelines (Umar et al., 2004).

2.4. Mutational Signature Analysis

Prior to performing mutational signatures analysis, the mutational catalogues of all examined samples were generated. These catalogues include the six types of substitutions (C>A, C>G, C>T, T>A, T>C, T>G; mutations are referred to by the pyrimidine of the mutated Watson–Crick base pair) as well as the base immediately 5′ and the base immediately 3′ of each somatic mutation. The immediate 5′ and 3′ sequence context for each somatic mutation was extracted using the ENSEMBL Core APIs for human genome build GRCh37.

Mutational signatures analysis was performed based on somatic substitutions and their immediate sequence context. Briefly, mutational signatures were deciphered for all cancer types together using our previously developed computational MATLAB framework (Alexandrov et al., 2013a).

The computational framework for deciphering mutational signatures is freely available and it can be downloaded from: http://www.mathworks.com/matlabcentral/fileexchange/38724. The algorithm deciphers the minimal set of mutational signatures that optimally explains

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