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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Identification of Positively and Negatively Selected Driver Gene Mutations Associated With Colorectal Cancer With Microsatellite Instability

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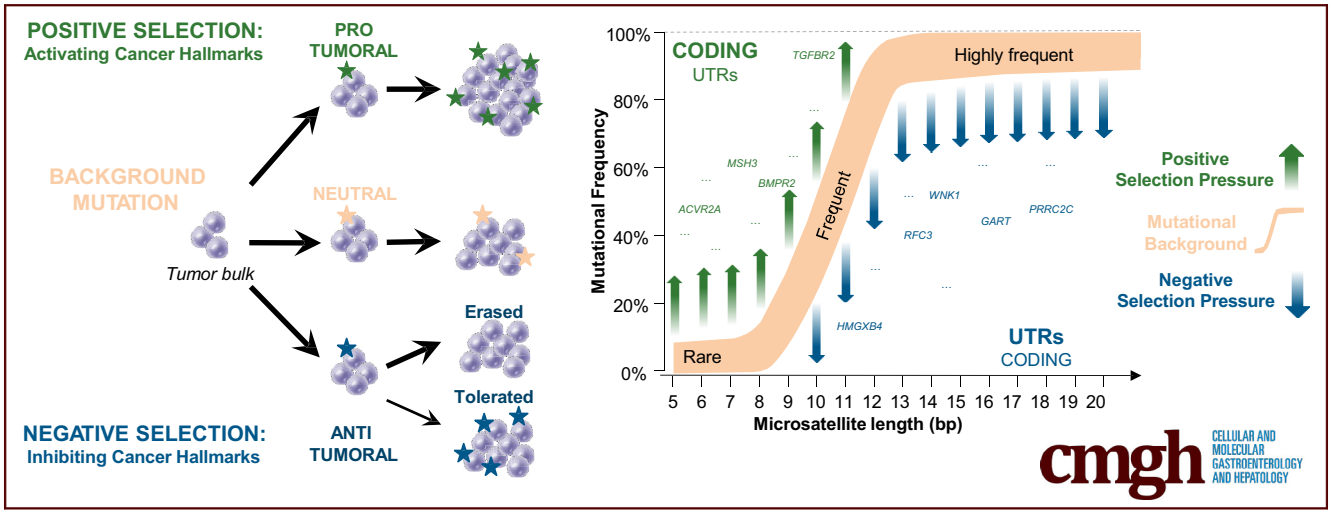

SUMMARY

Recent studies have shown that cancers arise as a result of the positive selection of driver somatic events in tumor DNA, with negative selection playing only a minor role, if any. The present work indicates that in microsatellite instability cancer, the high level of genomic instability generates both positively selected somatic mutations that contribute to the tumorigenic process but also recurrent somatic mutational events that are negatively selected due to their deleterious for the tumor cells.

BACKGROUND & AIMS: Recent studies have shown that cancers arise as a result of the positive selection of driver somatic events in tumor DNA, with negative selection playing only a minor role, if any. However, these investigations were concerned with alterations at nonrepetitive sequences and did not take into account mutations in repetitive sequences that have very high pathophysiological relevance in the tumors showing microsatellite instability (MSI) resulting from mismatch repair deficiency investigated in the present study.

METHODS: We performed whole-exome sequencing of 47 MSI colorectal cancers (CRCs) and confirmed results in an

independent cohort of 53 MSI CRCs. We used a probabilistic model of mutational events within microsatellites, while adapting pre-existing models to analyze nonrepetitive DNA sequences. Negatively selected coding alterations in MSI CRCs were investigated for their functional and clinical impact in CRC cell lines and in a third cohort of 164 MSI CRC patients.

RESULTS: Both positive and negative selection of somatic mutations in DNA repeats was observed, leading us to identify the expected true driver genes associated with the MSI-driven tumorigenic process. Several coding negatively selected MSI-related mutational events (n = 5) were shown to have deleterious effects on tumor cells. In the tumors in which deleterious MSI mutations were observed despite the negative selection, they were associated with worse survival in MSI CRC patients (hazard ratio, 3; 95% CI, 1.1–7.9; $P = .03$), suggesting their anticancer impact should be offset by other as yet unknown oncogenic processes that contribute to a poor prognosis.

CONCLUSIONS: The present results identify the positive and negative driver somatic mutations acting in MSI-driven tumorigenesis, suggesting that genomic instability in MSI CRC plays a dual role in achieving tumor cell transformation. Exome sequencing data have been deposited in the European genome–phenome archive (accession: EGAS00001002477). *(Cell Mol Gastroenterol Hepatol 2018;6:277–300; https://doi.org/10.1016/j.jcmgh.2018.06.002)*

Keywords: Colorectal Cancer; Microsatellite Instability; Tumorigenic Process; Driver Gene Mutations; Positive and Negative Selection.

Acquisition of the multiple hallmarks of cancer mainly is owing to somatic mutations. These hallmarks are a convenient organizing principle to rationalize the growth and complexity of tumors (for review, see Hanahan and Weinberg[1]). Underlying these mutations is the characteristic of genomic instability. This leads to the generation of mutant genotypes that confer advantages or disadvantages to the cells in which they occur, thus allowing the cells to dominate or to involute within the tumor mass.[2] Data obtained from the analysis of thousands of tumors from different primary sites have shown that unlike species evolution, positive selection outweighed the negative selection of somatic mutational events during tumor progression.[3,4]

Different types of genomic instabilities have been described in human malignancies, including a subset of cancers that is characterized by inactivating alterations of mismatch repair (MMR) genes.[5–7] These tumors show a distinctive phenotype referred to as microsatellite instability (MSI). MSI affects thousands of microsatellite DNA sequences, although numerous alterations also occur in nonrepetitive DNA sequences during tumor progression. This phenotype was first observed in tumors from individuals with the familial cancer condition known as Lynch syndrome, and later in sporadic colon, gastric, endometrial, and other cancer types.[8–10] The activating *BRAF* V600E somatic hotspot mutation,[11] affecting a nonrepetitive coding DNA sequence, plays an important role in the progression of sporadic MSI colorectal cancer (CRC). However, most somatic mutations with a postulated role in MSI tumorigenesis are found in microsatellites contained within coding regions, and to a much lesser extent in microsatellites contained within noncoding gene regions (eg, intronic splicing areas, or in the 5' UTR or 3' UTR).[12] Because microsatellites constitute hot spots for mutations in MSI tumors regardless of their location in genes and the function of these genes, such frequent mutations could be neutral or even detrimental to tumorigenesis.[13,14] In accordance with this working hypothesis, we previously reported frequent inactivation of the *HSP110* oncogenic chaperone in MSI CRC.[15–18]

Recent advances in high-throughput sequencing have made it possible to identify all genetic changes in human MSI neoplasms. Kim et al[12] reported a global view in 27 colon and 30 endometrial tumors with the MSI phenotype. With regard to the selection of MSI-driven events, these investigators did not take into account the strong influence of the length and nature of DNA repeats on the frequency of their instability, as shown earlier by several groups.[14,19,20] Furthermore, nucleotide instability outside of DNA microsatellites was not investigated, even though this is an important part of the landscape of somatic changes in MSI CRC.[21] Other studies have attempted to identify driver genes containing selected mutations, or to use various probabilistic models of unselected mutations in MSI CRC while ignoring negative selection, which is more difficult to establish.[8,22] A recent study reported that tumors with a mutator phenotype (including MMR-deficient cancers) acquired more positively selected driver mutations than other tumors, but found no evidence of negative selection.[3] The latter study investigated substitutions at nonrepetitive sequences, without taking into account repetitive sequences that have high physiopathologic relevance in these tumors.

In the present study we performed whole-exome sequencing (WES) of 47 MSI CRCs and validated results in an independent series of 53 MSI CRCs from the The Cancer Genome Atlas (TCGA). Overall, our results shed new light on MMR-deficient tumorigenesis and suggest that genomic instability in MSI CRC plays a dual role in achieving tumor cell transformation. They show hitherto unknown pathophysiological aspects of MSI colon tumors that could lead to

*Authors share co-first authorship; §Authors share co-senior authorship.

Abbreviations used in this paper: bp, base pair; CRC, colorectal cancer; HR, hazard ratio; indel, insertion/deletion; MLH1, MutL Homolog 1; MMR, mismatch repair; mRNA, messenger RNA; MSH, MutS Homolog; MSI, microsatellite instability; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; NR, nonrepetitive; R, repetitive; RFS, relapse-free survival; RTCA, Real-Time Cell Analyzer; shRNA, short hairpin RNA; siRNA, small interfering RNA; UTR, untranslated region; WES, whole-exome sequencing; WGA, whole-genome amplification.

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2352-345X
https://doi.org/10.1016/j.jcmgh.2018.06.002