Instability of Non-Standard Microsatellites

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Contributor: Luigi Pasini

Elevated microsatellite alterations at selected tetranucleotide (EMAST) repeats are a genetic signature of colorectal cancers and are caused by somatic dysfunction of the DNA mismatch repair (MMR) protein MutS Homolog 3 (MSH3). There are very few data showing the relation of EMAST presence in the genome with the response to treatment, and there is no information about the metastatic setting. To the best of our knowledge, this is the first study evaluating the correlation between EMAST and response to treatment with chemotherapy or chemotherapy plus bevacizumab in metastatic colorectal cancer (mCRC).

Keywords: colorectal cancer, genomic instability, MSI, EMAST

1. Introduction

Although metastatic colorectal cancer (mCRC) is the third cause of cancer-related deaths worldwide [1][2], the last two decades have seen the introduction of new cytotoxic and biological agents that have improved treatment and overall survival (OS) [3], aided by a better understanding of the molecular mechanisms of the disease and the identification of new prognostic and predictive markers. The introduction of targeted therapies for mCRC, such as the anti-epidermal growth factor receptor (EGFR) monoclonal antibodies cetuximab and panitumumab, or the anti-vascular endothelial growth factor (VEGF-A) bevacizumab (B), has represented an important breakthrough in this setting, but there are still no validated predictive biomarkers for anti-angiogenic treatment [4].

Genomic instability is a landmark of mCRC and is the main effector that leads to the accumulation of mutations in repeated DNA sequences. Microsatellite instability (MSI) is a form of genomic instability caused by impairments in the mismatch repair (MMR) system [5]. MSI is characterized by loss of expression of MMR genes, typically consequent to either genetic mutation or epigenetic inactivation of the MutL Homolog 1 (*MLH1)* and MutS Homolog 2 (*MSH2)* gene promoters [6][7][8]. MSI occurs in around 15% of all sporadic colorectal cancers [9] and is associated with right-sided tumors, lower tumor stage at diagnosis, better prognosis, improved survival, and reduced recurrence of metastasis [10][11]. In the early stages of colorectal cancer, it has been demonstrated that MSI status plays a role in predicting response to adjuvant therapy. In particular, it has been observed that patients with high microsatellite instability (MSI-H) do not benefit from 5-fluorouracil (5-FU) adjuvant therapy [10][12][13], whereas a significant survival benefit has been reported from the administration of B after chemotherapy (CT) [14]. Similarly, in the QUASAR 2 randomized study, the addition of B to capecitabine led to improved survival with respect to capecitabine alone in MSI-H patients and in those with microsatellite stability (MSS) [15]. These data suggest that MSI-H status may be associated with a better response to anti-angiogenic drugs in an adjuvant setting. A study performed in the metastatic setting showed no difference in progression-free survival (PFS) after chemotherapy with bevacizumab therapy in relation to MSI status [16]. Conversely, a recent study performed on a large case series from the Cancer and Leukemia Group B (CALGB)/SWOG 80405 trial reported that patients with MSI-H benefited more from bevacizumab than from cetuximab, highlighting the need to further investigate the role of MSI in relation to the efficacy of B [17].

2. Development

Elevated microsatellite alterations at selected tetranucleotide (EMAST) repeats are considered as a specific, non-standard, subtype of MSI and are caused by the defective translocation of MutS Homolog 3 (MSH3) to the cytosol rather than by genetic or epigenetic alterations in MMR genes. EMASTs are more frequent in colorectal cancers than MSI [18], and their presence is associate with advanced tumor stage, metastasis, poor survival, and intraepithelial inflammation [19][20][21][22]. The predictive role of EMAST has only partially been investigated [23][24], probably because the repeat types and thresholds used in EMAST analysis have still not been standardized. In the present study, we analyzed EMAST status in

relation to prognosis in a series of mCRC patients treated with CT alone or CT + B, and we showed that the presence of EMAST instability was associated with a worse prognosis in the overall case series, with a trend that seemed more significant in the group of patients treated with CT + B.

As far as we know, there are no previous publications reporting similar data on the association between each specific EMAST marker and prognosis and response to mCRC treatments; only a few reports have measured the frequency of individual markers in mCRC patients. Two studies reported that, among EMAST markers, *D20S8* was the locus with the highest frequency of frameshift alterations in mCRC [19][20], and that its instability was a direct consequence of hMSH3 deficiency in tumor cells [25]. This marker is located in the chromosome region of 20p12.3, which is typically associated with cancer susceptibility [26][27][28]. Alterations in the EMAST marker MYCL Proto-Oncogene, BHLH Transcription Factor (*MYCL1*) are strongly related to metastatic recurrence and poor survival of mCRC [29][30]. *MYCL1* is a structurally complex microsatellite consisting of mono-, tetra-, and pentanucleotide repeats [31][32], but it is preferentially mutated in the tetranucleotide locus [33]. Kambara and colleagues hypothesized that this *MYCL1* mutation, which is frequent in mCRC cancer, may indirectly promote tumor growth [34]. In our case series, *MYCL1* was significantly associated with worse prognosis in patients treated with CT, being strongly indicative of poor OS.

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