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EDITORIAL

Colorectal Cancer: Sailing with a T-Cell EMAST

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During the past two decades, considerable progress has been made in understanding and defining the molecular basis of human colorectal cancer (CRC). The genetic heterogeneity of this disease is now well-established, and it has become clear that a distinct subgroup of CRC possesses a unique form of genetic instability, defined as microsatellite instability (MSI). The MSI phenotype is a hallmark of defective DNA mismatch repair (MMR), and inactivation of MMR genes permits increased instability and accumulation of genomic alterations in the sequences containing mono- [e.g.$(A)_n$] and di-nucleotide [e.g. $(CA)_n$] microsatellite repeats. Approximately 12–15% of CRC exhibit high frequency MSI (MSI-H), which includes the majority of Lynch syndrome cancers with germline mutation of a MMR gene and about 12% of sporadic tumors due to methylation-induced transcriptional silencing of the *MLH1* gene [1]. The remaining 85% of CRCs either possess very low levels of MSI (MSI-L) or are microsatellite stable (MSS); however, the molecular basis of these tumors remains poorly understood.

Almost a decade ago, Sidransky and colleagues described a distinct form of genetic instability present in respiratory tumors, which was termed as *elevated microsatellite alterations at selected tetranucleotide* repeats (EMAST) [2]. In contrast to instability at the mono- and di-nucleotide repeats seen in MSI CRCs, EMAST tumors are identified when the frameshift mutations occur at certain tetranucleotide repeat sequences [e.g. $(AAAG)_n$ or $(ATAG)_n$]. Since its initial discovery in non-small cell lung cancers [2, 3], EMAST has subsequently been observed in several other human cancers, including skin [4], ovarian [5], endometrial [6], bladder [4, 7], prostate [8, 9] and colorectal [10–13]. Most microsatellite sequences (either mono-, di- or tetra-nucleotide repeats) are present in the non-coding regions of DNA; however, small minorities of these tandem repeats exist within the coding sequences of growth regulatory genes that play a critical role in driving CRC pathogenesis. Frameshift mutations within this latter class of genes, such as observed with EMAST, have direct functional consequences for the growth and proliferation of tumor cells.

The underlying molecular mechanism(s) causing EMAST remain unclear. The earliest reports in this regard proposed an association of EMAST with mutations in the p53 gene [3, 4]. It was suggested that exposure to environmental carcinogens may exacerbate this phenotype [14]. With the limited evidence at hand, EMAST appears to be distinct from the classic MSI signature that is frequently observed in Lynch syndrome and sporadic CRC. Whether EMAST contributes to tumor progression by specific gene-inactivating mutations within repetitive coding sequences, as is the case with MSI-positive CRC, is a matter of ongoing investigation. Recently, it was shown that EMAST is present in the majority of sporadic CRCs [10]. Haugen et al. demonstrated that EMAST is associated with MSI-L and a deficiency in MSH3 protein expression in colorectal cell lines and tumor tissues [10]. Building upon this evidence, later on it was demonstrated that EMAST can be acquired during the adenoma to carcinoma transformation, as well as upon transformation of well-differentiated carcinomas to moderately and poorly differentiated CRCs [12]. Interestingly in this study, EMAST best correlated in CRCs with ulcerated features, and the authors suggested that inflammation may help drive EMAST [12].

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Along this same line, in this issue of Digestive Diseases and Sciences, Lee and colleagues provide intriguing new insights into this issue, which may help unravel the mystery surrounding the role of EMAST in colorectal neoplasia [15]. Based on the evidence for the presence of tumor infiltrating lymphocytes (TILs) in CRC, the authors asked the logical question whether there was any correlation between the pattern of cytotoxic lymphocytes infiltration and EMAST in CRC. The authors examined 24 adenomas and 84 CRCs for MSI, EMAST and immunochemical detection of CD4+ and CD8+ T cells. CD8+ T cells were more frequently present in tumor cell nests and tumor stroma in moderately- and poorly-differentiated cancers compared to adenomas and well-differentiated CRCs. Likewise, these cells had a higher density in tumor cell nests and stroma of tumors with ulcerated lesions in comparison to the ones with a sessile or polypoid appearance. Lastly, it was noted that both MSI-H and EMAST-positive tumors showed a higher density of CD8+ cells in tumor cell nests and stroma, compared to MSI-L/MSS and EMAST-negative cancers, respectively. The authors did not observe any meaningful associations between any of these characteristics with CD4+ cells, and did not find any prognostic value based upon the density of CD8+ T cells. The authors concluded that increased infiltration of CD8+ T lymphocytes, but not CD4+ cells, in tumor cell nests and tumor stroma are associated with moderately or poorly-differentiated colorectal cancers, ulcerated lesions, MSI-H cancers and EMAST-positive CRCs [15].

The present study by Lee et al. [15] is not only first of its kind to evaluate the association between T cells and EMAST in CRC, but it is very timely, considering the increased recognition that disease progression in cancer patients is not solely determined by tumor characteristics alone, but is significantly influenced by the host response as well. Indeed, accumulating evidence suggests that both local and systemic inflammatory responses play an important role in the progression of a variety of solid tumors, including CRC [16, 17]. It is believed that CRC results from a step-wise accumulation of genetic and epigenetic alterations that lead to the expression of tumor associated antigens, thereby inducing a cellular anti-tumor immune response. It has long been recognized that cytotoxic T-lymphocytes (e.g. CD8+, CD4+ and CD3+ T cells) constitute one of the most significant effector mechanisms of anti-tumor-immunity in CRC [18]. When a specific T cell encounters a tumor cell's antigen/HLA complex, cytotoxic T lymphocytes clonally expand, differentiate into killer cells, and mediate destruction of tumor cells by releasing their lytic components or by disrupting tumor cell membranes and induction of apoptotic pathways. The T-cell infiltration of CRCs with CD4+ or CD8+ T cells, or both, has been associated with improved prognosis of multiple human cancers, including CRC.

One of the most provocative aspects of the findings of Lee et al. is that they discovered a conspicuous association of CD8+ T cells in tumor cell nests and tumor stroma with EMAST and other clinic-pathological features. This is extremely intriguing, and would not only suggest that these cells have higher proliferative activity, but they are in an immunologically active state. Accordingly, the most natural question comes to mind is to address the functional activity of the CD8+ T cells. This question was not addressed in the current study, but, it is certainly an important biological question for future studies. By staining CRC tissues for the presence of cytotoxic mediators such as perforin, granzyme A and B, and granulysin, the answer can be determined.

Due to the small sample size analyzed, there was no association observed between the presence of CD8+ T cells and patient survival. However, the results of Lee et al. provide a reasonable springboard for further evaluation of the role of CD8+ cells and EMAST as prognostic markers in CRC in future large-scale clinical studies. If such associations are found, not only will we have a prognostic indicator for CRC, it may provide the groundwork for a greater understanding of the process of carcinogenesis in the colon.

In contrast to the results observed with CD8+ T cells, this study did not find any association between CD4+ T cells and EMAST and any other clinico-pathological features. Although this may very well be a unique feature specific to the biology of EMAST-positive tumors, it still does not rule out the possibility that a specific subset of the CD4+ cells might be associated with EMAST positive tumors. Unlike CD8+ T cells, CD4+ T-cell populations consist of several subtypes, such as T helper type 1 (Th1), Th2, Th17 and other T-regulatory cells [19]. In view of this, for future studies it may be worth expanding the scope of IHC staining to include markers that represent these specific T-cell subtypes. Further support for this argument comes from the fact that when T-regulatory cells were assessed by staining for the transcription factor, forkhead box P3 (FOXP3), this subtype of CD4+ T cells was significantly increased in MSI tumors [19]. These findings underscore the importance of identification of individual subtypes of CD4+ cells. Along the same lines, quantitative PCRs for T-BET and GATA3 could help determine the ratios between Th1/Th2 cells, which provide additional insights into the proportion of these cancer fighting T lymphocytes.

The results of Lee et al. provide valuable information about the role of infiltrating T cells and their association with EMAST in CRC. Although these results clearly spark interest, additional studies are needed to corroborate the T-lymphocytic patterns observed in this study. Since EMAST progresses with histological advancement of

adenoma to carcinoma in the colon and associates with a specific T-cell infiltration pattern, it may be prudent to analyze these genetic and immune signatures in advanced cancers to better determine their potential prognostic and predictive value. Since we are entering the era of personalized medicine, knowledge of these results would definitely fuel interest in exploring the possibility of whether these genetic and immune markers can be used as prognostic markers for CRC. Lastly, another reassuring aspect of this seminal study is that it not only substantiates the existence of EMAST in CRC, but it is also the first effort in solidifying its association with other facets of cancer biology. In other words, as the pieces of this puzzle are coming together and the evidence is firming, EMAST is definitely emerging as a "bonafide" phenotype in colorectal neoplasia. Thus determination of the role of the T cells in EMAST has allowed us to sail onto a greater understanding on the involvement of immune system in the pathogenesis of human colorectal cancer.

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Conflicts of interest The author has no conflicts of interest to report.

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