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Micromanaging the classification of colon cancer. The role of the microRNAome

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Abstract

Recent advances in the understanding of the microRNAome provide further insights into the molecular pathogenesis of colorectal cancer and demonstrate the potential role for miRNAs to distinguish molecular subtypes. The mucosa-adenoma-carcinoma model can now integrate miRNAs into the known genetic alterations that drive the progression of colorectal neoplasia.

In a recently published issue of *Clinical Cancer Research*, Balaguer and colleagues showed that hereditary cases of colorectal cancer (CRC) due to Lynch Syndrome can be distinguished from sporadic tumors displaying Microsatellite Instability (MSI) based on miRNA profiles(1). In addition, Bartley and colleagues used a similar high-throughput platform to examine miRNA expression patterns across matched samples of normal mucosa, adenomas with low-grade and high-grade dysplasia, and Microsatellite Stable (MSS) adenocarcinomas(2). Both works represent a step forward in the integration of miRNA biology into the molecular subclassification of CRC and the multistep genetic model of colorectal tumorigenesis(1, 2).

More than two decades ago, Fearon and Vogelstein proposed the model of colorectal carcinogenesis that highlights the sequential somatic acquisition of genetic alterations characterizing the transitions from normal mucosa to an adenomatous colonic lesion, and finally, to carcinoma(3). This seminal description has served as the framework for establishing the molecular classification of CRC.

CRC can be classified in two groups based on the source of genetic instability. Approximately 15% of colorectal tumors present with MSI, arising from either a germline mutation in one of the Mismatch Repair (MMR) genes (Lynch Syndrome) or epigenetic inactivation of *MLH1* (sporadic MSI). MMR deficiency reduces the ability of tumor cells to repair specific DNA errors that tend to occur more frequently within microsatellite tracts. The accumulation of secondary mutations in coding microsatellite sequences will generate further genetic instability, thus promoting rapid cell growth, activation of transcription factors, deficiency of other DNA repair mechanisms, and inhibition of apoptosis(4). Therefore, this pathway of tumor development is called the *Mutator Phenotype*(4). In contrast, ~85% of CRCs are characterized by larger structural abnormalities in the genome,

such as somatically acquired gains and losses (*Chromosomal Instability* pathway). These tumors are almost always MSS and conform to the classic adenoma to carcinoma progression model(4). These cancers arise from an adenomatous lesion that early in its natural history has acquired a somatic mutation in the *APC* gene or another component of the canonical Wnt pathway such as *CTNNB1*, *AXIN1*, *AXIN2* or *TCF4*(5). Genetic alterations classically described in CRCs such as *KRAS* and *PIK3CA* mutations, and loss of 18q and 17p are frequently observed in later stages of tumor development in the *Chromosomal Instability* pathway (Figure 1). This molecular classification has largely driven basic and clinical research in CRC during the last 20 years. For example, multiple studies characterizing the mutational profile of these tumor groups have shown that *KRAS* alterations are more frequently observed among MSS tumors, while *BRAF* and *PIK3CA* are present in MSI CRCs. Moreover, expression profiles have been identified for both tumor subtypes using high-throughput techniques(4). Finally, several research groups have recently uncovered the role of miRNAs in the biology of CRCs(6).

miRNAs are important posttranscriptional regulators that are approximately 18 to 24 nucleotides long. These short non-coding RNAs bind to the 3' untranslated regions (UTRs) of their target messenger RNAs (mRNA) and repress their expression. Bioinformatics and cloning studies have estimated that miRNAs may regulate 30% of all human genes. Furthermore, one miRNA may target hundreds to thousands mRNAs(5). Dysregulation of miRNAs have been detected in CRCs(7) and premalignant colonic lesions using high-throughput platforms(8). In addition, patterns of serum miRNAs in patients with CRCs have been identified compared to healthy controls, thus suggesting their potential role not only in the prognostic setting but also in the diagnostic and screening contexts(8). Differences in miRNA abundance observed in CRCs may be attributable directly to differential expression of the primary transcripts, but other factors may play a role in these profiles(5). First, altered miRNA expression may be a consequence of tumor development that reflects the altered growth and differentiation properties of cancer cells compared to normal cells. Second, somatic mutations in oncogenes and tumor suppressor genes present in CRCs may influence patterns of miRNA expression by dysregulating their processing and cell dynamics. While the impact of these alterations has to be yet defined, it is known that *TP53* mutations may exert specific effects on processing miRNAs. Finally, the impact of recurrent chromosomal lesions in miRNA dysregulation is a subject of ongoing research in the field(5).

Balaguer and colleagues(1) have identified a miRNA profile that can differentiate tumors diagnosed in Lynch Syndrome from sporadic MSI CRCs. Until recently, CRCs with MSI were considered clinically equivalent regardless of whether they arose sporadically or within Lynch Syndrome. However, the clinical and therapeutic differences between these two MSI subgroups are becoming much clearer. As an example, MSI tumors harbor more frequently *BRAF* mutations, which have been associated with worse survival outcomes and lack of benefit from anti-epidermal growth factor receptor therapies(9). Furthermore, it has been recently observed that MSI tumors arising in patients diagnosed with Lynch Syndrome may present different sensitivity to 5-Fluorouracil(10). Therefore, the data presented in the study by Balaguer *et al* may guide further studies of the molecular mechanisms accounting for these differences.

Another interesting observation from the work of Balaguer and colleagues is that patients with a clinical suspicion of Lynch Syndrome but with no identifiable mutation showed similar patterns of miRNA expression to patients with known germline mutations. It is well known that a significant proportion of patients fulfilling clinical Amsterdam criteria for Lynch syndrome do not exhibit identifiable mutations in MMR genes. Several groups have recently described additional molecular alterations leading to MMR deficiency through complex genetic mechanisms. Such mechanisms include the inheritance of *MLH1* germline

epimutations(11) or deletion of the gene *TACSTD1*, upstream of *MSH2*, in combination with methylation of the *MSH2* promoter in EpCAM positive cells(12). *In silico* analysis of the miRNAs dysregulated in both subgroups of hereditary CRCs, but differentially expressed in sporadic MSI tumors, may identify gene targets to be explored as new molecular mechanisms responsible for Lynch Syndrome.

A second important paper by Bartley and colleagues has focused on MSS CRCs(2). Tumors displaying *Chromosomal Instability* are the ideal setting to study the regulatory contribution of miRNAs to the mucosa-adenoma-carcinoma sequence. Here, the dynamics of miRNA expression have been established using matched samples from a total of 20 MSS tumors, their corresponding normal mucosa and adenomas showing different grades of dysplasia. Authors have classified miRNAs in five groups depending on the evolution of their expression across lesions (early *versus* late, and continuous *versus* intermittent patterns). The identification of miRNAs acting on the Wnt and ERK pathways confirms the role of miRNAs in the regulation of these two major pathways of colorectal tumorigenesis(13). These findings may be applicable in prognostic assessment for identification of lesions that harbor greater malignant potential. In fact, clinical application of this technology is feasible, as miRNAs are more stable and less degraded in formalin-fixed paraffin embedded samples compared to DNA or RNA. Furthermore, these findings reinforce the promising future of targeting the Wnt pathway in CRC for therapy and chemoprevention (14). Therefore, micromanaging miRNAs in CRC may open new avenues for translational research in the fields of diagnosis, prognosis, therapeutic and chemopreventive interventions, and highlights the general principle that a fuller understanding of molecular pathogenesis opens new horizons for cancer care.

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Figure 1.

Genetic model of CRC. The figure shows the classical adenoma to carcinoma sequence and the two main genetic instability pathways for colorectal tumorigenesis. Genetic alterations along with the contribution of the miRNAome to the model are displayed. CIMP, CpG Island Methylator Phenotype. Modified from References 4, 15 and 13.