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Editorial

Methylation in colorectal cancer

The emerging relevance of colorectal cancer (CRC) in the last few years is well known. In Spain, this is the most prevalent malignancy and the second cause of cancer-related mortality. Focusing on both genders, over 25,000 new cases are diagnosed in Spain, and around 13,000 individuals die from this condition every year (1). Thus, more than ever, this unquestionable significance makes our deeper understanding mandatory, which has evolved on an ongoing basis ever since Fearon and Vogelstein defined their colorectal carcinogenesis model back in 1990 (2). Today, the hypothesis that most CRCs emerge from adenomas via the suppressor –also referred to as chromosome instability (CI)– route, which is initiated by a mutation in gene *APC* –this is the classical description of colorectal carcinogenesis corresponding to the adenoma-carcinoma sequence (2)–, is only met by 60% of cases (3), and two additional alternative, non –excluding routes, are now considered– the microsatellite instability (MSI) pathway, associated with Lynch syndrome and a small proportion of sporadic cases, and the methylator phenotype pathway, most recently identified and referred to as CIMP (*CpG Island Methylator Phenotype*) in the English-language literature. Advances occurred in the understanding of the molecular basis of CRC are doubtless the strongest drive towards a more rational, specific management for this condition via the identification of new, more specific therapy targets, as well as markers to describe the various behaviors of the disease.

Epigenetics is a term used to describe the mechanisms than may modify at various levels the expression of specific genes without altering the corresponding DNA sequence, including DNA methylation, chromatin remodeling, and other processes mediated by non-coding RNA molecules (4). From a general perspective, a tumor would originate from multiple, cumulative changes in the genome of its cells, both epigenetic and DNA sequence changes. These sequence changes include deletions in chromosome regions with gene loss that may be associated with negative cell-cycle regulation (tumor suppressor genes), mutations that may activate or inactivate a number of proteins, gene amplifications entailing an overexpression of specific genes, and even loss or gain of entire chromosomes.

As mentioned above, colorectal carcinogenesis pathways are sometimes mutually excluding, as is the case with MSI and CI, whereas on other occasions there may be some overlap, as occurs with CIMP. CRC is being increasingly classified into various phenotypes according to its molecular profiles (5). Thus, its classification from a molecular viewpoint is based on the predominant cell event (CI, MSI, CIMP) or, equivalently, according to the event-initiating factor (suppressor pathway for CI; mutation pathway for MSI; methylator pathway for CIMP).

CIMP relates to changes at the epigenetic level, more specifically at the DNA methylation level. CpG islands are DNA regions that conform around 40% of gene

Editorial

promoters in mammals. In these regions there is a high concentration of cytosine-guanine pairs linked with phosphate bonds. These CpG sites are unmethylated when genes are expressed; hence their methylation in gene promoters may inhibit genic expression by inactivating the genes involved. Transcriptional inactivation by methylating CpG islands in tumor suppressor gene promoters is a relevant carcinogenesis mechanism, what is usually referred to as methylator phenotype. The mechanism through which methylation occurs in the promoter regions of various genes has been shown to play a role in a proportion of CRCs nearing 35% (6). This is also designated the «serrated pathway to colorectal carcinogenesis», as it seemingly emerges from a serrated precursor lesion (whose histology may include hyperplastic polyps, sessile serrated polyps, and serrated adenomas). In contrast to microsatellite status screening, where Bethesda's panel is used by consensus (7), there is still no agreement on an optimal panel for CpG islands or sites regarding CIMP characterization.

A number of differentiating features may be identified in these tumors. Thus, they seem to prefer the proximal colon, female gender, and older age; poorly differentiated tumors are more common and, from a molecular viewpoint, there is a wider presence of *BRAF* mutations, whereas the mutation rate for *TP53* is lower (8-10).

The mechanisms for CRCs emerging via this pathway seem to stem from a *BRAF* activating mutation, which inhibits physiological apoptosis at the level of epithelial cells in the colon. From this event serrated lesions may give rise to hyperplastic polyps or sessile serrated polyps. These lesions are susceptible to CpG island methylation in the promoter regions of multiple genes, hence they may induce epigenetic silencing –an initially random indirect inactivation of genes. Methylation of the *MLH1* promoter, which is very common in these cases, would originate sporadic CRCs with MSI. In this respect the fact that most sporadic cases with MSI are CIMP positive should be highlighted, whereas the presence of this phenotype in Lynch syndrome patients is uncommon (11,12).

From a clinical standpoint the diagnostic, prognostic, and therapeutic implications of the CIMP pathway are under analysis today. Certain papers focus on the utility of methylated gene identification in plasma or fecal samples for the early diagnosis of CRC (13-18), even in an attempt to predict, from the mucosal wash fluid during colonoscopy, CRC cases with a higher degree of invasiveness at the colonic wall (19). From a prognostic point of view a number of approaches have been essayed. On the one hand, trying to relate the methylation status of certain genes to outcome (for example, *IGF2*, which seems to suggest a poor prognosis) (20). Another instance is the paper reported in this issue of *Revista Española de Enfermedades Digestivas,* by Vengazones et al. (21), where the clinical and prognostic implications of *p16* methylation in CRCs are discussed. Overall studies have also been undertaken to assess the prognosis of tumors with CIMP, which remains blurry given the bias of a common association with mutations in *BRAF*, suggestive of a poor prognosis on their own (22). As can be surmised, also the various epigenetic forms of CRC have been studied regarding the sensitivity-resistance to chemotherapy, but little is known because most studies were performed *in vitro,* among other reasons (23).

As pointed out above, the need to understand the molecular basis of CRC is of paramount importance to correctly design both diagnostic and therapeutic strategies. In this regard, CIMP screening is most relevant, even more so given it is the most recently identified carcinogenesis pathway, as is the screening for specific CpG

Editorial

island methylations in the genes associated with colorectal carcinogenesis, hence the interest arisen by the paper by Vengazones et al. (21), which is an additional contribution in this regard.

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