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Editorial

On the prognostic & predictive impact of immune cells system in colorectal cancer

Colorectal cancer (CRC) remains the third most commonly diagnosed cancer in males and the second in females worldwide[1], despite improvement of our understanding of the natural history of disease progression, the advancements in prevention, early diagnosis, surgical and post-surgical treatment. Recent progresses in tumour biology highlighted that non-neoplastic cells, including endothelial cells, cancer-associated fibroblasts, mesenchymal stem cells and cells of the innate and adaptive immune system actively participate in the pathogenesis, surveillance and progression of CRC[2,3]. Nevertheless, a relevant issue remains to unravel the discrepancies between the inhibitory effects on cancer growth exerted by the local immune response and the promoting effects on cancer proliferation, invasion, and dissemination induced by some inflammatory cell types[4,5]. The need to investigate the complex interactions between the tumour cells and their surrounding microenvironment, and the potential prognostic and predictive impact of immune cell system has lead to an increasing number of studies aimed at exploring the density, type and location of various inflammatory cell subtypes during the progression of CRC[2,6-13]. However, it is still not possible to recommend any specific "immune score" for CRC because of the existing controversies among different studies[14,15]. At the molecular level, CRC encloses a complex array of gene alterations, affecting supra-molecular processes. Like individual fingerprints, each tumour arises and behaves in a unique fashion that is unlikely to be exactly recapitulated by any other tumour. It has been ascertained that genetic and epigenetic features, such as microsatellite instability (MSI), chromosomal instability, CpG island methylator phenotype (CIMP) or global DNA hypomethylation lead to alterations of gene function on a genome-wide scale. The suppressor pathway is disrupted in CRC with chromosomal instability occurring in the majority of CRCs (~85%). Differently, CRCs of the mutator pathway (~15%) have a defective DNA mismatch repair system, which leads to accumulation of thousands of unrepaired mutations[16,17]. It has been shown that CRC with MSI has distinctive features, including a tendency to produce abnormal peptides that, by acting as tumour neo-antigens, could induce an adaptive immune response effective in limiting tumour growth and metastasis[18-23]. Additionally, a pronounced lymphocytic infiltration has been more markedly evidenced in MSI than in microsatellite stables (MSS) CRC[10,24,25]. This makes essential to comprehensively control for tumour molecular variables to avoid biased survival-effect estimates. Even though the majority of CRCs are MSS, there may have been over-representation of MSI-negative tumours in the population, potentially skewing the data toward favourable prognoses[21]. It is also known that the lymphocytic reaction to tumours is linked with many of these molecular variables, suggesting the relevance of the host immune response in specific pathways of carcinogenesis. Also, the inter-relationships between tumour molecular variables and host immune response complicate the survival analysis. Actually, an apparent prognostic effect of the immune response could simply reflect the molecular variables, or the presence of host immune response might merely indicate an indolent tumour subtype[14]. To define the independent prognostic effect of an immune reaction, a large database of CRC with extensive molecular characterization is needed. In addition to the tumour-genetic background, the discrepancies can be mainly attributed to (*i*) the heterogeneous pattern distribution of the immune cell types, (*ii*) the anatomical site, and (*iii*) the method applied to assess the density of immune cells, *i.e.* qualitative, semi-quantitative or quantitative scoring systems. With the employment of accurate methods for analysing the immune infiltrate (*i.e.* computer-aided image analysis systems), it is becoming

evident that distinct infiltrating cell types have distinct prognostic and predictive significance[15]. Attention has been focused on the predictive values of T-lymphocytes located in the centre of the tumour, along the tumour invasive margin and in tertiary lymphoid aggregate mainly detectable in proximity of the tumour[25,26]. It has also been shown that tumour-associated macrophages localized to different regions of the carcinoma have variable effects on tumour cells.

Caution is further needed before incorporating tumour-infiltrating T-lymphocytes into tumour staging system, (*i.e.* Tumor Node Metastasis, TNM). To minimize the risk of inappropriate tumour downstaging at diagnosis, survival data need to be confirmed in independent series of patients studied in the past decade. Laghi *et al*[8] investigated the relationship between the density of infiltrating $CD3^+$ T-lymphocytes along the tumour invasive margin, and the occurrence of metachronous distant-organ metastases after potentially curative resection in a consecutive series of patients (n=286) with deeply invading (pT3 or pT4) MSI-typed CRC, and no evidence of distant organ metastasis at diagnosis. They found that large areas covered by $CD3^+$ T-lymphocytes were associated with a low-risk of metachronous metastasis and consequently a survival advantage, only in patients with node-negative CRC, but not in patients whose cancers involved lymphnodes (TNM Stage III). Additionally, the prognostic advantage conferred by a high density of $CD3^+$ T-lymphocytes was independent of tumour MS-status in patients with TNM stage II CRC. Therefore, CD3-immunostaining of CRC tissues could be a potential biomarker for selecting stage II patients who, because they are at very low risk for cancer progression, could be spared adjuvant treatments.

As tumours are complex heterogeneous cell populations[27] that show distinctive genetic and epigenetic profiles, there may not be a single biomarker that will provide sufficient information for predicting treatment response and patient outcome. It remains to solve several critical issues related to the discrepancy among the current studies, in terms of sample size, study setting, disease stage, the presence *versus* absence of treatment data, and treatment modality (no therapy to chemotherapy, radiation therapy, or both)[14]. Additionally, laboratory methods to assess immune response represent primary topics to be considered when comparing results from different studies. In particular, these include (*i*) tissue microarray *versus* whole surgical tissue sections; (*ii*) objective image analysis *versus* subjective pathologist qualitative or semi-quantitative interpretations; (*iii*) immunophenotyping markers (*i.e.* cluster of differentiation, CD); (*iv*) covariates and potential confounders assessed (in particular the presence *versus* absence of tumour molecular characteristics); and (*v*) statistical method and multivariate analysis models. It is clear that to standardize research methods and appropriately evaluate evidence, we need to develop general and specific consensus on immune-cell evaluation in oncology research[14]. It is also plausible that the quantification of type, density and location of the infiltrating immune cell sub-population using standardized computer-aided image analyses systems, coupled with automation of immunohistochemical procedures, becomes a primary step in understanding CRC natural history, and, in a clinical perspective, its prognostic or predictive determinants[28]. The use of computer-assisted quantification software to analyse histological sections may also be valuable since the computer evaluates the whole slide, thus reducing the risk of observer bias in choosing sections to evaluate. Furthermore, a comprehensive analysis of all the components of the immune infiltrate in the context of their localization, structural organization remains largely, if not entirely, to be reported to prospective studies. In parallel, understanding the mechanisms underlying immune reaction to CRC, its mediators (cytokines and chemokines), and its impact at different disease stages should provide compulsory information in making decisions of patient prognosis and management, and new tools to develop more appropriate and effective mired therapeutic strategies.

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