On the colorectal cancer histomorphological and molecular factors predicting lymph node metastasis

Fabio Grizzi[1], Federica Patrinicola[1], Sanja Stifter[2]

[1]Department of Immunology and Inflammation, Humanitas Clinical and Research Center, Milan, Italy; [2]Department of Pathology, School of Medicine, University of Rijeka, Rijeka, Croatia

Correspondence to: Dr. Fabio Grizzi, PhD. Department of Immunology and Inflammation, Humanitas Clinical and Research Center, Via Manzoni 56, 20089 Rozzano, Milan, Italy. Email: fabio.grizzi@humanitasresearch.it.

Comment on: Pai RK, Chen Y, Jakubowski MA, *et al*. Colorectal carcinomas with submucosal invasion (pT1): analysis of histopathological and molecular factors predicting lymph node metastasis. Mod Pathol 2017;30:113-22.

Although a decline in colorectal cancer (CRC) incidence rate has been registered, attributed to increases in screening adhesion rates and linked detection and removal of precancerous lesions, CRC remains one of the most common cancers (1,2). By its frequency, CRC ranks third in men and second in women worldwide. Originally depicted as a multi-step dynamical disease (3,4), it is now recognized that CRC develops slowly over several years and progresses through benign and malignant states, from single crypt lesions through adenoma, to malignant carcinoma with the potential for local invasion and distant metastasis. CRC is a heterogeneous disease; it encompasses different genetic pathways and cellular entities with a wide range of clinical behaviors (5,6). The response to treatment is variable between patients, even when they are diagnosed at the same clinical stage. Such clinical heterogeneity remains an obstacle to the optimization of treatment for each individual.

One of the primary aims of oncological research is the discovery and translation of molecular biomarkers into clinical practice (7,8). However, there is no consensus agreement with regard to the path necessary for the efficient translation of prognostic and/or predictive biomarkers into clinical use that may result in the development of novel therapeutic strategies (9). The term "biomarker" includes biochemical markers, cellular markers, cytokine markers, genetic markers, physiological results, radiological measurements, physical signs and pathological assessment (7).

These can be mainly grouped in four classes: (I) diagnostic biomarkers for early detection; (II) prognostic biomarkers for estimation of disease outcome; (III) predictive biomarkers for adjuvant treatment stratification; and (IV) surveillance biomarkers for disease monitoring and treatment response (10). During the last two decades, several reports have detailed putative prognostic and predictive biomarkers for CRC (11-13). Tumor stage as stated by the American Joint Committee on Cancer/International Union Against Cancer (AJCC/UICC) tumor-node-metastasis (TNM) system is currently considered as the most robust prognostic criterion for CRC patients (14-17). Pathologic assessment of CRC specimens plays an essential role in patient management, informing prognosis and contributing to therapeutic decision making. It is known that submucosally invasive CRCs (pT1) usually have an indolent clinical course. Pathology has a pivotal role in determining the invasive carcinomas that need additional surgical therapy to prevent either local recurrence or to remove draining lymph nodes to prevent metastatic spread. Recently, Pai and colleagues (18) investigated a consecutive series of 116 pT1 carcinomas with and without lymph node metastasis for tumor grade, depth of submucosal invasion, size of invasive component, tumor budding, lymphatic, mucinous differentiation, venous and perineural invasion, and tumor configuration to better define potential histopathological features associated with lymph node metastasis. For all patients, CRC carcinomas were also analyzed for mismatch-repair (MMR) protein abnormalities

using a combination of microsatellite instability (MSI) PCR and MMR protein immunohistochemistry. Furthermore, a subset of 48 carcinomas, including 22 lymph node-positive and 26 lymph node-negative cases, has been evaluated for mutations in 50 oncogenes and tumor suppressors by next-generation sequencing. Interestingly, Pai and colleagues (18) found that pT1 CRCs with lymph node metastasis had deeper submucosal invasion (≥1,000 micrometers), high tumor grade, high tumor budding, and higher frequency of lymphatic invasion. Significant associations between lymphatic invasion, high tumor budding, high tumor grade, and depth of submucosal invasion and the presence of lymph node metastasis were found in univariate analysis. However, multivariate analysis demonstrated tumor budding as the only independent predictor of lymph node metastasis with an odds ratio of 4.3 (P=0.004). According to the 2014 guidelines from the Japanese Society for Cancer of the Colon and Rectum, the presence of one of the investigated histopathological features warrants surgical therapy after endoscopic resection. Depth of submucosal invasion has been shown in various studies as predictive of lymph node metastasis. In their study, Pai and colleagues (18) confirmed that deeper submucosal invasion is significantly associated with more frequent lymph node metastasis; however, this feature was not an independent predictor of lymph node metastasis in their multivariable model.

Tumor budding at the invasive front has been widely shown to be strongly associated with lymph node metastasis across all stages of CRC. In 2004, Ueno and colleagues (19) reported the utility of tumor budding in predicting lymph node in pT1 carcinomas. Pai and colleagues (18) found a statistically significant association between high tumor budding and lymph node metastasis, and identified high tumor budding as the only independent predictor of lymph node metastasis.

Whether molecularly analyzed different CRC subtypes that are predictive of both response to therapy and clinical outcome can be identified. In order to determine if pT1 colorectal carcinomas can be classified molecularly into high-risk and low-risk groups, Pai and colleagues (18) performed next-generation sequencing of a subset of tumors. Although no significant molecular differences were identified between lymph node-positive and lymph node-negative pT1 carcinomas there were some interesting molecular differences based on histological features. High tumor budding was associated with the presence of mutations in TP53 and absence of mutations in the mTOR pathway.

Among the prognostic biomarkers has been recognized the presence of defective DNA mismatch repair (i.e., loss of expression of *hMSH2*, *hMLH1*, *hPMS1*, *hPMS2*, *hMSH6*, or *hMLH3* gene), as assessed by the presence of tumor MSI. However, the study of Pai and colleagues did not found significant differences in molecular mutations or MMR protein/MSI status between lymph node-negative and lymph node-positive tumors.

The term "tumor budding" denotes the presence of isolated single neoplastic cells or small clusters of cells (conventionally, up to five cells) scattered in the stromal compartment at the tumor invasive margin (20,21). Tumor budding was originally introduced as a reliable histopathological hallmark to estimate the aggressiveness of rectal cancer. Subsequently, it has been demonstrated to have a higher prognostic value when compared to other histopathological features, including tumor differentiation and venous invasion. Furthermore, it has been suggested that the frequency of tumor budding increases with more advanced TNM stage (22).

Today, the tumor invasive margin remains poorly described. The advancing edge is, however, where the tumor expresses its aggressive behavior by the processes of de-differentiation, budding formation and vascular invasion and where it is exposed to the host immune response. Although both tumor border configuration and immune reaction have been shown to have an independent prognostic role their assessment is highly subjective with significant inter-observer variability, in particular when specific definitions or diagnostic criteria are not provided.

The majority of CRCs display some degree of budding; hence, attempts have been made at developing scoring systems to identify a prognostically significant degree of budding, termed "high-grade" budding. Definitions of high-grade budding, however, vary substantially among different observers and even among different studies by the same observers.

With respect to setting consensus criteria, studies focusing on budding should be designed to define objective cut-off for meaningful tumor budding.

Histopathological observation of CRC tissues remains the gold standard for cancer diagnosis. Working with human tissues, however, poses several challenges to investigators, including: (I) tissue sampling; (II) selection of the proper preservation technique; (III) tissue complexity; and (IV) ethical and legal rules. A combined approach that integrates histopathology and molecular biology within a unique translational system is a mandatory strategy to

pursue a better understanding of cancer. Such an effort can be achieved only through a more effective incorporation of pathology into clinical research, and conversely by integrating biological research into the pathological assessment, likely through efficient networks of translational researchers joining their data.

Tumor budding promises to be a histopathological prognostic factor in CRC, and although the level of agreement needs to be improved and further investigations are compulsory to confirm any association between the rates of tumor budding recognition and clinical outcome, its evaluation can be improved first by an appropriate training.

It is indubitable that the substantial impediment to the adoption of tumor budding as a routinely reportable feature is the lack of a well-defined, standardized and quantitative assessment. At any event, due to the forceful evidence that tumor budding is one of the most promising prognostic factors actually available, it is compulsory on the clinical community participating to the identification of CRC prognostic factors to move promptly to addressing it and removing the biases to its routine reporting and comparison with other predictive factors.

Footnote

Provenance and Peer Review: This article was commissioned by the editorial office, *Journal of Public Health and Emergency*. The article did not undergo external peer review.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/jphe.2017.02.05). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

References

1. Keane MG, Johnson GJ. Early diagnosis improves survival in colorectal cancer. Practitioner 2012;256:15-8, 2.
2. Siegel R, DeSantis C, Virgo K, et al. Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin 2012;62:220-41.
3. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319:525-32.
4. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990;61:759-67.
5. Sugihara Y, Taniguchi H, Kushima R, et al. Laser microdissection and two-dimensional difference gel electrophoresis reveal proteomic intra-tumor heterogeneity in colorectal cancer. J Proteomics 2013;78:134-47.
6. Al-Sohaily S, Biankin A, Leong R, et al. Molecular pathways in colorectal cancer. J Gastroenterol Hepatol 2012;27:1423-31.
7. Mandrekar SJ, Sargent DJ. Design of clinical trials for biomarker research in oncology. Clin Investig (Lond) 2011;1:1629-36.
8. Deschoolmeester V, Baay M, Specenier P, et al. A review of the most promising biomarkers in colorectal cancer: one step closer to targeted therapy. Oncologist 2010;15:699-731.
9. Butterfield LH, Palucka AK, Britten CM, et al. Recommendations from the iSBTc-SITC/FDA/NCI Workshop on Immunotherapy Biomarkers. Clin Cancer Res 2011;17:3064-76.
10. de Wit M, Fijneman RJ, Verheul HM, et al. Proteomics in colorectal cancer translational research: biomarker discovery for clinical applications. Clin Biochem 2013;46:466-79.
11. Church D, Midgley R, Kerr D. Biomarkers in early-stage colorectal cancer: ready for prime time? Dig Dis 2012;30 Suppl 2:27-33.
12. Legolvan MP, Taliano RJ, Resnick MB. Application of molecular techniques in the diagnosis, prognosis and management of patients with colorectal cancer: a practical approach. Hum Pathol 2012;43:1157-68.
13. Berg M, Søreide K. Genetic and epigenetic traits as biomarkers in colorectal cancer. Int J Mol Sci 2011;12:9426-39.

14. Malesci A, Laghi L. Novel prognostic biomarkers in colorectal cancer. Dig Dis 2012;30:296-303.
15. Ueno H, Mochizuki H, Akagi Y, et al. Optimal colorectal cancer staging criteria in TNM classification. J Clin Oncol 2012;30:1519-26.
16. Shia J, Klimstra DS, Bagci P, et al. TNM staging of colorectal carcinoma: issues and caveats. Semin Diagn Pathol 2012;29:142-53.
17. Gao P, Zhou X, Wang ZN, et al. Which is a more accurate predictor in colorectal survival analysis? Nine data mining algorithms vs. the TNM staging system. PLoS One 2012;7:e42015.
18. Pai RK, Chen Y, Jakubowski MA, et al. Colorectal carcinomas with submucosal invasion (pT1): analysis of histopathological and molecular factors predicting lymph node metastasis. Mod Pathol 2017;30:113-22.
19. Ueno H, Mochizuki H, Hashiguchi Y, et al. Risk factors for an adverse outcome in early invasive colorectal carcinoma. Gastroenterology 2004;127:385-94.
20. Puppa G, Senore C, Sheahan K, et al. Diagnostic reproducibility of tumour budding in colorectal cancer: a multicentre, multinational study using virtual microscopy. Histopathology 2012;61:562-75.
21. Prall F. Tumour budding in colorectal carcinoma. Histopathology 2007;50:151-62.
22. Lugli A, Karamitopoulou E, Zlobec I. Tumour budding: a promising parameter in colorectal cancer. Br J Cancer 2012;106:1713-7.