Tumor-associated neutrophils (TANs) as prognostic markers in colorectal cancer: A brief review

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ABSTRACT

Neutrophils are cell protagonists of innate immunity, frequently found in the tumor microenvironment and associated with higher tumor stage and adverse prognosis of patients, especially in hepatocellular, renal cell, pancreatic ductal, and non-small cell carcinomas. The aim of the present review is a systematic appraisal of current literature to examine the prognostic role of tumor-associated neutrophils (TANs) in patients operated for colorectal cancer. We analyzed 9 articles (number = 4606 patients). Although a noteworthy degree of heterogeneity was found in terms of study methodology, 8 out of 9 articles surprisingly showed an improved survival in colorectal cancers with high TAN counts. These data suggest that TANs may be favorable prognostic markers in colorectal cancer. Therefore, there is need for standardization of TAN assessment that will help in more accurately predicting prognosis in patients with colorectal cancer.

Keywords: Colorectal cancer, Prognosis, Tumor-associated neutrophils, Tumor microenvironment

INTRODUCTION

Pathological tumor-node-metastasis (pTNM) staging remains the gold standard for prognostic models for oncologists, but it is mainly focused on tumor cells, neglecting the role of adaptive and innate immune system in the host stromal reaction. Neutrophils are innate immune cells that constitute 50–70% of all circulating leukocytes. A high number of tumor-associated neutrophils (TANs) correlate with higher tumor stage and adverse prognosis, particularly in hepatocellular, renal, pancreatic ductal, and non-small cell carcinomas [1, 2]. In our previous study on gastric carcinomas, the multivariable analysis revealed that TANs, particularly in female patients, were associated with a reduction in their risk of mortality [3]. Similar findings have been confirmed in subsequent studies [4, 5]. Therefore, we performed a review of the literature with the aim of verifying the prognostic impact of TANs in colorectal cancer. In addition, the function, identification, location, and clinical significance of TANs in colorectal cancer will be discussed.

TAN role in cancer

Neutrophils are characterized by a short life span, which makes them difficult to study in human tumors [6]. Therefore, most functional research on TANs is based on animal models of cancer [6]. Fridlender and Albelda [7] have provided experimental evidence of a bipolar
pattern of TAN activation (N1/N2) similar to what has been described in macrophages (M1/M2) and T cell (Th1/Th2) polarization. Briefly, N1 neutrophils exert antitumor activity, whereas N2 neutrophils promote tumor activity [7]. It is possible that N1 and N2 phenotypes do not represent two distinct subpopulations of TANs but only reflect their functional state, which is regulated by molecules secreted in the tumor microenvironment. In particular, interferon beta (IFN-β) induces an antitumoral N1 phenotype, whereas transforming growth factor beta (TGF-β) promotes the polarization of neutrophils into a protumoral phenotype [8, 9]. However, it is necessary to remember that N1/N2 neutrophils have been described in murine models and must be confirmed in human tumors. Therefore, we await further studies to deepen knowledge on the different functional stages of neutrophils in human tumors.

Identification of TANs

Different methods are used in the identification of TANs. Tumor-associated neutrophils were recognized by their trilobed nuclei in hematoxylin and eosin (H&E) stained sections of tumor stroma [10, 11]. Subsequently, immunohistochemical (IHC) staining, such as myeloperoxidase, CD15, CD177, and/or CD66b, has been employed to identify TANs in a more specific manner. However, IHC results must be interpreted with caution. Galdiero et al. [12] pointed out that antibodies against myeloperoxidase highlight not only neutrophils but also monocytes and immature macrophages. CD177 is a neutrophil surface molecule that is restricted to a subset of neutrophils [13], whereas CD15 is expressed in neutrophils, eosinophils, some monocytes, and occasionally in tumor cells [12]. CD66b immunoreactivity is found both on neutrophils and eosinophils and is recognized as a granulocyte “activation marker” [14]. Therefore, we suggest that it could be useful to include a H&E stained serial section as control to establish the percentage of TANs compared to eosinophils.

TAN density

Neutrophils within areas of necrosis or in areas adjacent to ulcerations are usually not counted. Therefore, there are not information about TAN density and tumor ulceration. Tumor-associated neutrophil density may be established by a manual count, i.e., random non-overlapping high-power fields are observed at 400× magnification per slide. Alternatively, automated image analysis can be applied to evaluating TAN in histological sections. Several studies have shown that computer-based analysis provides a more reproducible assessment of the immune cell percentage than visual semiquantitative estimation [15].

Location of TANs

Tumor-associated neutrophils can be found mainly at the invasive front of a tumor (peritumoral location) or in the center of a tumor (intratumoral location). Recent studies suggest that a distinct evaluation of these two areas improved prognostic accuracy compared with single region analysis [12, 16]. Intratumoral TAN distribution shows heterogeneity, being found as a single massive infiltrate in the tumor stroma or as a series of multifocal aggregates scattered throughout the tumor stroma [3]. These data imply that TAN evaluation is more accurate on whole-tissue sections compared to tissue macroarray (TMA) technology that uses small millimeter punches from tumors with an original size of up to several centimeters in diameter [11].

DISCUSSION

Prognostic role of TANs in colorectal cancer

Uehara et al. [10] studied the clinicopathologic significance of microscopic abscess formation in advanced low rectal cancer. Microscopic abscess formation, identified by using H&E staining, contained cell debris and numerous neutrophils. It was usually found at the invasive front of the tumor and remained an independent factor in multivariate analysis, suggesting a favorable prognosis after curative resection for low rectal cancer [10]. Droeser et al. [17] showed that a subgroup of colorectal carcinomas is characterized by high TAN counts, defined as myeloperoxidase and CD15 positive cells per TMA spot. In multivariate analysis, high score myeloperoxidase was positive, but CD15 positive cell infiltration was not independently associated with favorable prognosis. In a following work, these authors further examined interaction between the innate and adaptive immune system in colorectal cancer, studying CD8 and myeloperoxidase positive cells in a TMA [18]. They identified a subgroup of colorectal cancers characterized by low count of both CD8 and myeloperoxidase tumor infiltrates with a severe prognosis. Recently, the same research group noted that myeloperoxidase positivity may be observed in different cells of myeloid lineage. Therefore, in a new study, this research group prefers CD66b IHC on TMA, a classical neutrophil marker [19]. Moreover, they clarified that >90% of CD66b colorectal cancer-infiltrating cells were neutrophils, as this IHC marker is co-expressed by neutrophils and eosinophils. Their results confirmed that CD66b cell infiltration in colorectal cancer is associated with favorable prognosis [19]. Applying CD66b IHC on TMA, Rao et al. [20] showed that a high TAN count was positively correlated with advanced clinical stage and predicted adverse prognosis. By contrast, other authors, applying CD66b IHC on whole section, described a positive correlation with prognosis. Moreover, they added new data regarding the relationship of TANs with carcinogenesis, tumor stage, and tumoral subsites. Zhou et al. [21] studied IHC expression of CD177, a marker mainly revealed in...
neutrophils, in colorectal tumor tissue, in healthy controls as well as in patients with ulcerative colitis and ulcerative colitis-associated colorectal cancer. They showed that high density of CD177-positive TANs predicts favorable prognosis in colorectal cancer. Moreover, density of CD177-positive neutrophils was significantly increased in patients with ulcerative colitis and ulcerative colitis-associated colorectal cancer. Their experimental data showed that depletion of CD177-positive neutrophils facilitated colorectal carcinogenesis, suggesting an important role for neutrophils in the suppression of epithelial cell carcinogenesis in colitis-associated cancer [21, 22]. Berry et al. [11] showed a variability of median TAN density between different stages with consequent different prognostic impact. In particular, they showed lowest TAN density in stage intravenous (IV) neoplasms, whereas high levels of TANs were significantly related with better prognosis in patients with stage II colorectal cancer. Wikberg et al. [16] have studied the prognostic role of TANs at different tumor subsites including the invasive front, the center of the tumor, and in the tumor epithelium of 448 archival human tumor tissue samples from patients surgically resected for colorectal carcinomas. High neutrophil infiltration at the tumor front was mainly found in early stages of colorectal cancers. Moreover, low number of CD66b-positive cells in the tumor front was associated to a worse patient prognosis in stages I–II colon cancers [16]. Similarly, Galdiero et al. [12], using CD66b IHC, showed a significant decrease of TANs in stage IV patients compared to stages I–III. Moreover, at multivariable analysis, high TAN density was associated with better prognosis in patients with colorectal carcinomas (stages I–IV). Their results also demonstrated interactions between pathological stage, TAN density, and postoperative adjuvant chemotherapy with 5-fluorouracil (5-FU) [12]. Interestingly, higher density of TAN was associated with better response to 5-FU chemotherapy in a subgroup of patients with stage III colorectal cancer. Thus, assessment of TAN density may not only be useful for prognostic information, but may also have important therapeutic implications, in particular for identifying patients likely to benefit from 5-FU-based chemotherapy. Future studies are also needed to examine the relationship between neoadjuvant chemotherapy and TANs in colorectal cancer.

CONCLUSION

Although a high number of TANs has been associated with adverse prognosis in several solid tumors, 8 out of 9 publications showed, surprisingly, improved survival in patients operated for colorectal cancer with high TANs. Table 1 summarizes main characteristics of studies regarding TANs in colorectal cancer including TAN identification, cutoff TAN count, tumor compartment, Table 1: TANs in colorectal cancer

| Authors               | TAN identification | Cutoff TAN count | Tumor compartment | Survival endpoints | Prognostic factor |
|-----------------------|--------------------|------------------|-------------------|-------------------|-------------------|
| Uehara et al. [10]    | H&E on whole section | Microscopic abscess | Invasive margin | Disease free survival (DFS) | Favorable, 226 Asian patients |
| Droeser et al. [17]   | Myeloperoxidase IHC on TMA | Regression tree analysis | Tumor stroma | Overall survival | Favorable, 1420 Caucasian patients |
| Däster et al. [18]    | Myeloperoxidase IHC on TMA | ROC curves | Tumor stroma | Overall survival | Favorable, 790 Caucasian patients |
| Governa et al. [19]   | CD66b IHC on TMA | Regression tree analysis | Tumor stroma | Overall survival | Favorable 650 Caucasian patients |
| Berry et al. [11]     | H&E on whole section | Median number/HPF | Tumor stroma | Overall survival | Favorable, 221 Caucasian patients |
| Wikberg et al. [16]   | CD66b IHC on whole section | Semiquantitative evaluation | Intratumoral/peritumoral | Overall survival | Favorable, 421 Caucasian patients |
| Galdiero et al. [12]  | CD66b IHC on whole section | Median number/HPF | Intratumoral/peritumoral | Disease specific survival, DFS | Favorable, 271 Caucasian patients |
| Zhou et al. [21]      | CD177 IHC on TMA | Mean number/HPF | Tumor stroma | Overall survival, DFS | Favorable, 378 Asian patients |
| Rao et al. [20]       | CD66b IHC on TMA | ROC curves | Tumor stroma | Overall survival | Adverse, 229 Asian patients |

Abbreviations: H&E: hematoxylin and eosin; TMA: tissue macroarray; IHC: immunohistochemistry; DFS: disease free survival; HPF: high power field.
survival endpoints, and prognostic factors. These data suggest that organ-specific microenvironment may influence the antitumoral role of TANs and their favorable prognostic impact. However, these studies show heterogeneity in terms of ethnicity (Asian/Caucasian), anatomical location (colon/rectum), stages (I, I–III, I–IV), study methodology (whole sections or TMA), neutrophil identification (H&E stain/IHC; antibodies used), scoring cutoffs and survival endpoints (overall survival, disease specific survival, disease free survival). Further studies are clearly required to standardize the evaluation of TANs in these neoplasms. Standardization of these procedures will be a fundamental step in the eventual implementation of TANs in pTNM-immune staging, as has been suggested for tumor-infiltrating lymphocytes into colorectal cancer pTNM staging.

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