Cancer Letters 332 (2013) 3–10

Contents lists available at SciVerse ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

Mini-review

Tumor-associated macrophages as potential diagnostic and prognostic biomarkers in breast cancer

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ARTICLE INFO

Article history:
Received 6 November 2012
Received in revised form 11 January 2013
Accepted 14 January 2013

Keywords:
Tumor-associated macrophage
Diagnosis
Prognosis
Breast cancer

ABSTRACT

Breast cancer development largely depends upon the essential contributions from the tumor microenvironment, where several inflammatory cell populations (e.g. macrophages) orchestrate breast cancer development. The majority of tumor-associated macrophages (TAMs) exhibit alternatively activated M2 properties, produce abundant anti-inflammatory factors and facilitate tumor development. Clinical evidences compellingly indicate the association between high TAMs influx and poor prognosis in patients with breast cancers. The pan-macrophage marker CD68 is now generally utilized to identify TAMs in diagnostic biopsy samples, and some other TAM-related biomarkers are also utilized in prognosis prediction, including CD163, vascular endothelial growth factor (VEGF), hypoxia-inducible factors (HIFs), proliferating cellular nuclear antigen (PCNA), ferritin light chain (FTL) and C–C motif chemokine ligand 18 (CCL18). In this review, we highlight the recent progress made in understanding the relationship between TAMs and clinicopathological parameters in human breast cancer and address the potential value of TAMs as diagnostic and prognostic biomarkers.

1. Introduction

Breast cancer is the most prevalent malignant disease of women in the developed world, excluding the non-melanoma skin cancers, and is one of the leading causes of death among women. Approximately, 1 in 8 women in the United States are diagnosed with breast cancer at some time in their lives. After slowly increasing for many years (0.4% annually from 1975 to 1990), breast cancer mortality decreased by 2.2% per year from 1990 to 2007 [1]. Declines in breast cancer mortality have been attributed to both improvements in treatment (adjuvant chemotherapy as well as radiation, hormonal and targeted therapies) and early detection (better characterization of diagnostic and prognostic factors) [2]. However, not all populations have benefited from those advances, and the morbidity and mortality is still high. Therefore, there are a number of hard nuts to crack before we could defeat breast cancers completely. On one hand, it is required to explore novel drugs and strategies for the treatment of breast cancer. On the other hand, new adjuvant diagnostic and prognostic biomarkers in combination with current parameters are required to make better treatment decisions.

As technologies advance, we are rapidly approaching an integrated understanding of breast cancer, from the origin and genetic alternations that license uncontrolled cell proliferation, to the unique contribution of the tumor microenvironments that support or reduce malignancy [3]. Genetically, current genome-wide association studies (GWAS) have identified amount of single nucleotide polymorphisms (SNPs) in women with breast cancer [4–7]. The functions of some SNPs have been uncovered [6], and the assessment of those SNPs could be used for the identification of individuals with higher risk of developing breast cancer. On the other hand, the tumor microenvironment has been increasingly considered as a therapeutic target or biomarker for diagnosis and prognosis. The microenvironment of breast cancer is populated by many cells including the adipocytes, fibroblasts, a wide range of hematopoietic cells, as well as newly formed blood and lymphatic vessels and their associated cells [8], most of which participate as abettors or double-edged swords in breast carcinogenesis. Among those cells, the tumor-associated macrophages (TAMs) represent a predominant component of the tumor mass in breast carcinoma, and appear to be a pivotal orchestrator.

Abbreviations: TAM, tumor-associated macrophage; HR, hormone receptor; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2; VEGF, vascular endothelial growth factor; EGFR, epidermal growth factor receptor; HIF, hypoxia-inducible factor; TP, thymidine phosphorylase; PCNA, proliferating cellular nuclear antigen; FTL, ferritin light chain; FTH, ferritin heavy chain; CCL, C–C motif chemokine ligand; MMP, matrix metalloproteinase.

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http://dx.doi.org/10.1016/j.canlet.2013.01.024

Macrophages have remarkable plasticity that allows them to efficiently respond to environmental signals and change their phenotype, and their physiology can be markedly altered by both innate and adaptive immune responses [9]. In an effort to emulate the T-cell literature, macrophages have been classified along what could be viewed as linear scale, on which classically activated macrophages (M1) represent one extreme and alternatively activated macrophages (M2) represent the other [9,10]. M1 macrophages are pro-inflammatory and characterized by high expression of pro-inflammatory factors, such as interleukin (IL)-12, nitric oxide synthase 2 (NOS2), tumor necrosis factor (TNF)-α, and by high microbicidal and tumoricidal activity [11]. Conversely, M2 macrophages are immunosuppressive and produce high levels of anti-inflammatory cytokines, such as IL-10, transforming growth factor (TGF)-β, and low levels of pro-inflammatory cytokines. Nevertheless, this dichotomy may not be so clear cut *in vivo*, as some populations exhibit both the M1 and M2 behaviors, depending on the certain physiological and pathological conditions and the microenvironment. Investigators have implicated that macrophages participate in a range of physiologic and pathological processes, including homeostasis, inflammation, repair, metabolic functions, and malignancy [10–13]. Biswas and Mantovani currently reviewed the functions and mechanisms of M1 and M2 macrophages [14].

Macrophages continuously infiltrate into the tumor microenvironment, where breast cancer cells and other infiltrates release factors that induce macrophages to TAMs. TAMs are a heterogeneous population, both in human and mouse, whereby distinct subsets perform distinct functions [15–17]. Among TAM subpopulations in tumor sites, some populations are highly M2-activated, CD163 and CD206-positive, and express amount of tumor-promoting cytokines and growth factors [17,18]. Once activated, M2-like TAMs facilitate breast tumor growth, angiogenesis, metastasis, matrix remodelling and immune evasion by releasing a variety of cytokines, including chemokines, inflammatory and growth factors [8,19–21]. In contrast, M1-like macrophages have the potential to contribute to the earliest stages of neoplasia, primarily because the free radicals that they produce can lead to the DNA damage; this causes mutations that can predispose host cells to transform [9]. Nevertheless, as tumors progress and grow, the tumor microenvironment markedly influences TAMs, the majority of which exhibit M2-like pro-tumoral properties, whereas the inflammatory M1-like macrophages behave as scavengers. TAMs act as a double-edged sword but a pivotal orchestrator in breast cancer development. As a matter of fact, TAMs have now been considered as potential targets for adjuvant therapy [22]. Either depletion of TAMs or reversion of their pro-tumoral properties (M2 to M1) has been demonstrated to reject tumor progression in mouse models of breast cancer [23–27].

More importantly, clinical evidences show that TAMs significantly correlate with micro-vessel density, lymph-node, hormone receptor (HR) status and tumor grades, and predict the efficacy of treatment and the survival in breast cancer patients. Therefore, TAMs is a novel candidate for targeted cancer therapy and prognostic biomarker of response and clinical benefit. Here in this article, we will review the available information on TAMs in diagnosis and prognosis for breast cancers.

2. TAMs as a prognostic marker in breast cancers

2.1. CD68

Early in the late 1970s, Wood and Gollahon [28] have observed the presence of macrophages (as measured by presence of the Fc [IgG] receptor) in breast tumor microenvironment that determined the risk of disease progression and therapeutic resistance. Further, Steele et al. [29] confirmed the existence of macrophages using four surface markers: the receptors for the Fc portion of IgG and for C3, HLA-DR antigen, and a macrophage-associated antigen (defined by the mouse monoclonal antibody VEP-7). In the late 1980s, Kelly et al. [30] quantified the macrophages in benign and malignant breast tissues using the mouse monoclonal antibody EBM/11, which has high cellular specificity for human macrophages. Now, CD68, the human homolog of macrosialin, has been widely used as a pan-macrophage marker, although some limitations have currently been found [31,32]. Several monoclonal antibodies (mAbs) that recognize CD68 are grouped together on the basis of pan-macrophage reactivity on tissue sections: Ki-M6, Ki-M7, Y2/131 and Y1/82A, EBM11, KP1, Ki-M1P, and PG-M1 [32]. Among these mAbs, PG-M1 and KP1 are more specific for macrophages than the others and now are widely used as diagnostic mAbs against CD68 [33,34].

Multiple clinical studies support the value of enumerating breast TAMs in pre-treatment biopsies for outcome prediction in human breast cancer (Table 1). Increased macrophage ($CD68^+$) index is associated with high vascularity and nodal metastasis, as well as reduced recurrence-free and overall survival in human breast cancer [35,36]. Patients with higher TAM density have significantly worse disease-free survival [37]. Large cohort studies also evidenced the predictive value of CD68 TAMs. Currently, Mahmoud et al. [38] have confirmed the predictive value of TAMs using a large cohort (1322) of patients with breast cancer. In their univariate survival analysis, higher numbers of $CD68^+$ macrophages predict worse breast cancer-specific survival and shorter disease-free interval. Furthermore, a multi-centric 2004 national PHRC study also suggested that the patients who have lower $CD68^+$ TAMs level gained better metastasis-free survival [39]. Taken together, the $CD68^+$ TAMs index predicts prognosis in human breast cancer.

Breast cancer is one of the few tumor types in which molecular classification has successfully been used for the design of individualized therapies, leading to significant improvements in disease-specific survival [40]. Accumulating data reveals that breast cancer subtypes defined by expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) represent distinct biological entities with distinct clinical profiles. For instance, ER^+ and/or PR^+ breast cancers are associated with the most favorable prognosis, largely for the reason that they positively respond to hormonal therapy [41]. Breast cancers that overexpress HER2 are associated with a favorable prognosis, for the efficacy of anti-HER2 mAbs [41]. Interestingly, Volodko et al. [42] reported that the intensity of macrophage infiltration was strongly associated with ER-negativity, PR-negativity and high mitotic rate. Several independent studies have confirmed the association of TAM index to HR negativity and HER2 positivity [36–38,43–48]. Nevertheless, this does not mean that HER2 mAbs should be given to all breast cancer patients with high TAMs regardless of molecular subtype, because some HER2-negative patients are also infiltrated with high levels of TAMs, but these patients are not suitable to get HER2 mAbs treatment. In addition, we should note here that TAMs associated with poor prognosis do not always correct for HR and HER2 status, which indicates that the association of TAMs and HR/HER2 status should be re-evaluated in a larger cohort using different statistical methods. Mechanically, how TAMs are associated with HR/HER2 status needs to be answered.

TAMs status also predicts the sensitivity to chemotherapy and radiotherapy. When evaluating the leukocytic complexity in breast cancer tissue, Ruffell et al. [31] found in chemotherapy-naïve patients that macrophages were predominately present in nonadjacent normal but not the breast tissues. In contrast, tumors from