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Original contribution

Tumor-associated B cells in cutaneous primary melanoma and improved clinical outcome☆,☆☆

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Summary B cells often infiltrate the microenvironment of human tumors. B cells can both positively and negatively regulate antitumor immune responses. In several human cancers, higher numbers of $CD20^+$ TAB are associated with a favorable prognosis, whereas in human primary melanomas, this association is contentious. In this study, we determined the association of TAB numbers in cutaneous primary melanoma tissue samples and patients' overall survival. The CD20 immunohistochemistry on archival nonmetastasized and metastasized cutaneous primary melanoma tissues from 2 independent patient cohorts was performed. One cohort was used in class comparison for metastasis, the most important prognostic factor for overall survival, and the other cohort for a subsequent survival analysis. Survival association was further validated with RNA data from a third independent cohort. Whole tissue sections were read automatically via quantitative digital imaging and analysis. Survival data were analyzed by Cox proportional hazard modeling. We discovered that cutaneous primary melanomas without metastasis contain significantly more TAB than primary melanomas that had metastasized. At time of first diagnosis, a higher number of TAB is associated with a significantly better overall survival in patients with cutaneous primary melanomas of > 1 mm Breslow depth. Also, higher CD20/CD19 tumor mRNA levels are correlated with a significantly better overall survival. Thus, our data support TAB numbers as a prognostic biomarker in cutaneous primary melanoma patients with a tumor of > 1 mm Breslow depth. For a survey in larger studies, whole tissue section analysis seems to be key to accurate assessment of TAB numbers.

Abbreviation: TAB, tumor-associated B cells.

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1. Introduction

In the last decades, T cells, NK cells, and their subtypes have been extensively evaluated for their role as inducers, effectors, or biomarkers of antitumor immunity; the biological significance of TAB, however, is not well defined. TAB form a significant part of the tumor microenvironment in prostate, breast, and ovarian cancer as reviewed by Nelson [1].

As the central component of the humoral immune system, TAB can support antitumor immune responses by secretion of antibodies and immunostimulatory cytokines, by secretion of lymphotoxin to support generation of tertiary lymphoid structures in tumors, or by acting as antigen-presenting cells providing help for the differentiation of antitumor T cells. Consistently, the presence and number of TAB have been reported to be associated with a favorable prognosis in patients with breast and ovarian cancer as reviewed by Linnebacher and Maletzki [2]. In addition to induction of classical adaptive immune responses, B cells are also able to dampen antitumor immune responses through secretion of cytokines such as interleukin (IL)-4, IL-10, or transforming growth factor–β. In mouse models of squamous cell and prostate carcinoma, additional growth-promoting and therapy resistance mechanisms have been identified. Here, TAB secreted immunoglobulins that bound Fcγ-receptors to induce myeloid-derived cells with tumor-supporting activity [3], lymphotoxin-β leading to activation of IKKα and STAT3 signaling in castration-resistant prostate cancer cells [4], or IL-10 along with expression of the immunosuppressive cell surface molecule PD-L1 [5].

TAB are also present in primary and metastatic lesions of cutaneous melanoma [6–12]; however, their role in antitumor immunity or as prognostic biomarker for melanoma patients awaits further clarification. In syngeneic mouse models, some authors have shown B-cell deficiency or depletion of mature $CD20^+$ B cells to promote melanoma growth [13], whereas others reported a delayed growth in B-cell–deficient mice [14,15]. Similar contradictory results were obtained for TAB numbers in human primary melanoma and their association with prognosis. Whereas early studies reported low, if any, numbers of TAB [9,16], recent articles evaluating larger sample cohorts reported a higher TAB number in melanoma lesions as compared with normal skin [10] and a high number of TAB to be associated with a significant survival advantage [11]. In contrast, a percentage of more than 15% of TAB in tumor-infiltrating lymphocytes has been associated with a worse prognosis [12], and TAB have been reported as part of a 7-marker signature with negative prediction of overall and recurrence-free survival [17]. Similarly, nodular melanomas, known to have a worse prognosis than superficial spreading ones, have been reported to contain higher peritumoral TAB numbers as compared with superficial spreading melanomas [6]. Together, these reports strongly support an accumulation of TAB at melanoma sites but significantly differ in the potential clinical implications from this observation, particularly the correlation of TAB numbers with prognosis of patients with primary melanoma.

In this study, we used 3 independent patient cohorts to examine the correlation of TAB numbers with overall survival in patients with cutaneous primary melanoma.

2. Material and methods

2.1. Patient samples

Immunohistochemical analyses were performed in 2 independent cohorts including Caucasian patients with cutaneous primary melanomas who underwent surgery between 2002 and 2014 at the Cantonal Hospital Baselland, Liestal, and between 1991 and 2005 at the Department of Dermatology, Medical University of Graz. All tumor samples were obtained with informed consent. Archival whole tissue samples were retrieved from the pathology files as approved by the respective Ethics Committees. Histological diagnoses were made by 4 authors of this study, 2 board-certified pathologists (K. D. M. and N. W., cohort 1) and 2 board-certified dermatologists (I. W. and S. N. W., cohort 2), respectively. A third cohort comprised public data sets from cutaneous melanoma samples of The Cancer Genome Atlas (TCGA). We followed the criteria of the REporting recommendations for tumor MARKer prognostic studies [18]. Desmoplastic melanomas, which have a distinct clinical behavior [19], were not present in patient cohorts 1 and 2; in patient cohort 3, this subtype was not reported.

2.2. Patient cohort 1

This cohort included 57 patients with primary cutaneous melanoma, aged between 31 and 89 years at the time of first diagnosis. Of these, 43 patients developed no metastasis within a follow-up of 12 to 144 months (median, 35), determined as the interval from first diagnosis until last clinical visit. In 14 patients, the tumor metastasized; none died of a melanoma-unrelated cause. None of the 43 patients received antitumor treatment before surgery. Patients and tumor characteristics are given in Table 1 and Supplementary Table 1.

2.3. Patient cohort 2

This cohort included 41 patients with primary cutaneous melanoma, aged between 25 and 78 years at the time of first diagnosis. In 16 patients, the disease metastasized later; 25 patients developed no metastasis. The interval from the date of first diagnosis until the date of either death or last clinical visit ranged from 28 to 253 months (median, 160). The progression-free survival of patients with occurrence of metastasis ranged from 1 to 220 months (median, 66); the 5-year survival was 56.25%. In patients without metastasis, the progression-free survival was 59 to 253 months (median, 164); the 5-year survival was 95.83%. No patient received