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Original contribution

Inflammatory cell infiltrates in advanced metastatic uveal melanoma☆,☆☆

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Summary Current treatments for metastatic uveal melanoma (mUM) are limited and rarely prolong patient survival. Immunotherapy trials for mUM are few and to date have demonstrated only marginal success. High densities of tumor-associated macrophages (TAMs) and infiltrating T lymphocytes (TILs) in primary UM are associated with poor prognosis. Little is known about the immune microenvironment of mUM. Our aim was to examine the presence and distribution of TAMs and TILs in mUM within the liver. Whole-tissue sections of liver mUM (n = 35) were examined by immunohistochemistry. For TAMs, monoclonal antibodies against CD68 and CD163 were used. Macrophage density and morphology were scored using previous established systems. Density and spatial distribution of TILs were highlighted using antibodies against CD3 (pan-lymphocyte marker), CD4 (T-helper cells), and CD8 (T-cytotoxic cells). CD68+ and CD163+ TAMs were seen within the tumor in all 35 specimens; their density was "moderate" in 50% of cases and "few" in 43%, and the majority showed an "indeterminate" phenotype. CD3+ TILs were noted both within mUMs and surrounding the tumor. Of these, CD8+ TILs were "few" in number within mUM but were predominantly seen peritumorally at the tumor/normal liver interface, whereas CD4+ TILs showed a high perivascular density within mUM. CD68+ and CD163+ TAMs of "indeterminate" morphology were observed in mUM, suggesting a tendency toward the protumorigenic M2 phenotype. CD4+ TILs were seen within the mUM, whereas CD8+ TILs tended to be peritumoral. The biological and functional roles of inflammatory cells in mUM require further investigation to determine if they represent potential targets for future therapies in mUM.

1. Introduction

Uveal melanoma (UM) is an aggressive intraocular malignancy, with up to 50% of UM patients developing metastatic disease, usually involving the liver, even several years after the primary treatment [1-4]. Current treatments for metastatic UM (mUM) to the liver include metastectomy, liver resection, radio- and/or chemotherapy, and radiofrequency ablation; all of these therapies are limited, only being suitable for certain patients and rarely prolonging patient survival [3-6].

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Consequently, there is an urgent need to improve current treatments for established metastatic disease alongside adjuvant therapy.

Chronic inflammation is recognized as a hallmark of cancer and is thought to be a key mediator in all steps of tumorigenesis—from initiation through to progression and metastasis [7-11]. This has recently led to the development of new treatment strategies using immunotherapies, such as ipilimumab, nivolumab, and pembrolizumab, which have been successful in subsets of patients with metastatic cutaneous melanoma. However, ipilimumab has only shown marginal success in mUM to date [4,12-18].

It is known that high densities of tumor-associated macrophages (TAMs) and infiltrating T lymphocytes (TILs) in primary UM are found in tumors with a high risk of metastasizing. These include UM with a large basal diameter, predominantly of epithelioid cell type, high microvascular density, and monosomy 3 [2-4,19-24]. Macrophages and T cells are central to the general regulation of the immune response. TAMs have been implicated in tumorigenesis by promoting angiogenesis, tumor cell migration and invasion, and tumor growth [7-11,13,14]. T cells, in contrast, orchestrate the immune response to cancer through recognition, priming, and attacking cancer cells [25-29]. Very little is known about the immunomodulatory microenvironment of mUM, and studying this is difficult because mUM specimens are usually difficult to acquire and often are small percutaneous biopsies.

In this study, we examined the density and spatial distribution of TAMs and TILs in advanced cases of hepatic mUM.

2. Materials and methods

The study was approved by the Health Research Authority (REC Ref 11/NW/0759) and conducted in accordance with the Declaration of Helsinki. All samples were provided by the Ocular Oncology Biobank (REC Ref. 16/NW/0380) and the Liverpool Bio-Innovation Hub following approval from their Biobank.

2.1. Specimens

Archival formalin-fixed, paraffin-embedded specimens of mUM were obtained from (1) 19 patients who had undergone local resection of their liver metastases and 7 patients who had percutaneous fine needle biopsies at Aintree University Hospital between 2005 and 2016 and (2) dissection of the liver metastases during the autopsy of 9 patients (Queen Alexandra Hospital Portsmouth). All of the samples were assessed by a Senior Consultant Histopathologist (S. E. C.), and the hepatic metastases were classified for the following: staging, dominant cell type, degree of pigmentation, presence of necrosis, and growth pattern.

2.2. Immunohistochemistry

Sections were cut at 4 μm from the formalin-fixed, paraffin-embedded blocks and processed for immunohistochemistry as previously described [30]. Briefly, antigen retrieval and immunohistochemistry were performed using the Dako PT Link and Autostainer Plus with the EnVision FLEX+ detection system according to the manufacturers' recommendations (Dako UK Ltd, Cambridgeshire, UK).

Primary antibodies (Abs) against CD68 (mouse anti-human PG-M1, M0876; Dako) at 1:200 and CD163 (mouse anti-human, NCL-L-CD163; Leica Biosystems, Newcastle Upon Tyne, UK) at 1:400 were used to identify the macrophages. Immunostaining of T cells was undertaken using the following primary Abs: pan T–cell marker CD3 (polyclonal rabbit anti-human, ready to use, IR503; Dako), helper T-cell marker CD4 (monoclonal mouse anti-human, NCL-L-CD4–368; Leica Biosystems) at 1:20, and cytotoxic T-cell marker CD8 (monoclonal mouse anti-human, M7103; Dako) at 1:200. Positive and negative controls for each of the primary Abs were run for each assay.

Positive staining was detected with either 3-amino-9-ethylcarbazole (AEC peroxidase substrate, SK-4200; Vector Laboratories Ltd, Peterborough, UK, for 30 minutes) or 3,3′-diaminobenzidine (DAB+ chromogen, K3467; Dako; for 20 minutes). Slides were counterstained with Mayer hematoxylin and mounted with an aqueous or resin-based mountant, respectively.

2.3. Scoring of TAMs and TILs within the liver metastases

Following the grading system previously described by Mäkitie et al [22], TAM and TIL density was qualitatively scored as "few," "moderate," or "many." For TAMs, their morphology was also assessed, and the predominant cell type was recorded as "round," "dendritic," or "indeterminate" (in between the dendritic and round morphologies).

Slides were subsequently scored by 3 independent observers (Y. K., C. M., and H. K.), and the number, spatial distribution, and/or morphology were recorded for TAMs and TILs within the liver metastases. The entire specimen was evaluated using a high-power field (×40). Immunopositive cells in areas of necrosis or normal liver tissue distant from the mUM were not included in the scoring. Discrepancies in scoring were resolved by consensus between all members involved during reanalysis.

3. Results

3.1. Patients and samples

Detailed clinical, pathological, and genetic data for liver metastases cases 1 to 7 have been previously published by our group [30]. The growth patterns of mUM to the liver from sections obtained postmortem (cases 8-16) have also been described [31]. For the current study, these 16 specimens from