Cancer Immunology Research

The Trojan Horse Tale Revisited: An Eye on Metastatic Spread of Carcinoma Cells

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Abstract

The metastatic spread of carcinoma cells is not fully understood. Here, we compare the peripheral blood mononuclear cells (PBMC) and intraocular metastatic cells in parotid gland carcinoma with the PBMCs of healthy donors by immunohistochemistry and flow cytometry. We found Ber-EP4 tumor marker–positive carcinoma cells in the aqueous humor of the patient's right eye and a CD45 and Ber-EP4–expressing PBMC population in his blood. These Ber-EP4–expressing cells exhibited a monocytic-myeloid phenotype with coexpression of CD11b, CD115, and the macrophage marker CD172a (SIRP-α). Uptake of pHrodogreen revealed their phagocytic activity. Our findings suggest that the tumor cells in the anterior chamber originally derived from cell fusions between tumor cells and myeloid cells in the peripheral blood. Thus, metastases of a solid malignancy could use monocytes–macrophages as the Trojan horse to enter the eye. *Cancer Immunol Res; 4(2); 92–94. ©2015 AACR.*

Introduction

Metastatic spread of carcinoma cells is not fully understood. To leave the primary tumor and enter distant organs, the metastatic cells need to detach from neighboring cells and acquire a migratory phenotype. They then need to move into blood or lymphatics, putatively acquiring the necessary programs through epithelial–mesenchymal transition or by fusion of migratory cells, such as macrophages, with tumor cells (1, 2). Here, we compare peripheral blood mononuclear cells (PBMC) and intraocular metastatic cells in parotid gland carcinoma with PBMCs of healthy donors.

Case Report

A 35-year-old male patient had a painful, red, and rock-solid right eye with blurry vision for 4 days. His best-corrected visual acuity was 20 of 80 in the right eye and 20 of 20 in the left eye. The intraocular pressure of the right eye was elevated (55 mm Hg; normal range, 10–21 mm Hg) and slit-lamp examination revealed conjunctival hyperemia, mild corneal edema, and cells in the anterior chamber, with a hypopyon of 0.5 mm (Fig. 1A and B). Dilated fundus examination revealed a normal-appearing optic disc and retina, and the vitreous body was devoid of cells. Ultrasound (Fig. 1C) showed the hypopyon, but ruled out any solid tumor in the anterior angle, iris, ciliary body, choroid, retina, or optic nerve. The left eye was clinically unremarkable without any cells in the aqueous humor (AH) and vitreous body. The working diagnosis was acute fibrinous anterior uveitis with increased intraocular pressure in the right eye. After 1 week of anti-inflammatory and anti-glaucomatous therapy, the intraocular pressure of the right eye remained elevated (>28 mm Hg), and the intraocular inflammation was virtually unchanged. Furthermore, a systemic work-up by oncologists revealed that the patient had a parotid gland carcinoma with metastases to skin, lung, bone, and cerebrum (the TNM classification was pT4b N2c M1 L1 V1 G3). Histopathology was compatible with an adenocarcinoma. Therefore, we performed an anterior chamber tap of the right eye to obtain AH for cytologic examination (Fig. 1D–F).

Materials and Methods

Under local anesthesia and asepsis, a diagnostic anterior chamber biopsy was obtained by aspiration of 0.2 mL of AH with a 30G needle through the limbal cornea. Cytospin preparations were stained with hematoxilin and eosin, May-Grünwald, and Ber-EP4 (Fig. 1D–F; refs. 3, 4).

Patient and healthy donor ($n = 2$) blood samples were prepared by Ficoll-Hypaque (Sigma-Aldrich) gradient centrifugation. Viability of cells was determined by trypan blue exclusion. PBMCs were stained in three independent experiments with cell surface markers [anti–BerEP4-FITC (DAKO), anti–CD33-APC, anti–CD45-PerCP, anti–CD14-PerCP (BDBioscience), anti–CD11b-APC (Miltenyi Biotec), anti–CD206-APC (ebioscience), anti–CD163-BV421, anti–CD115-PE and anti–CD172a-PE (Biolegend) for 30 minutes at 4°C, washed with PBS, and analyzed by flow cytometry (FACSCantoII; BD Biosciences) using WinMDI 2.8 software (Dr. J. Trotter, Scripps Institute, La Jolla, CA)]. Phagocytosis was assessed through the uptake of *Escherichia coli* particles labeled with a low pH-sensitive dye (pHrodo *E. coli* bioparticles; Invitrogen) into Ber-EP4$^+$ cells (identified with antibody to Ber-EP4 (R&D Systems) and anti–mouse-Alexa Fluor 647 (Cell Signaling Technology) and analyzed by fluorescence microscopy (Axiovert, Zeiss). Informed consent was obtained and the research was performed according to the Declaration of Helsinki.

Results

Ber-EP4 tumor marker–positive carcinoma cells were detected in the AH of the patient's right eye (Fig. 1F). Unlike PBMCs from

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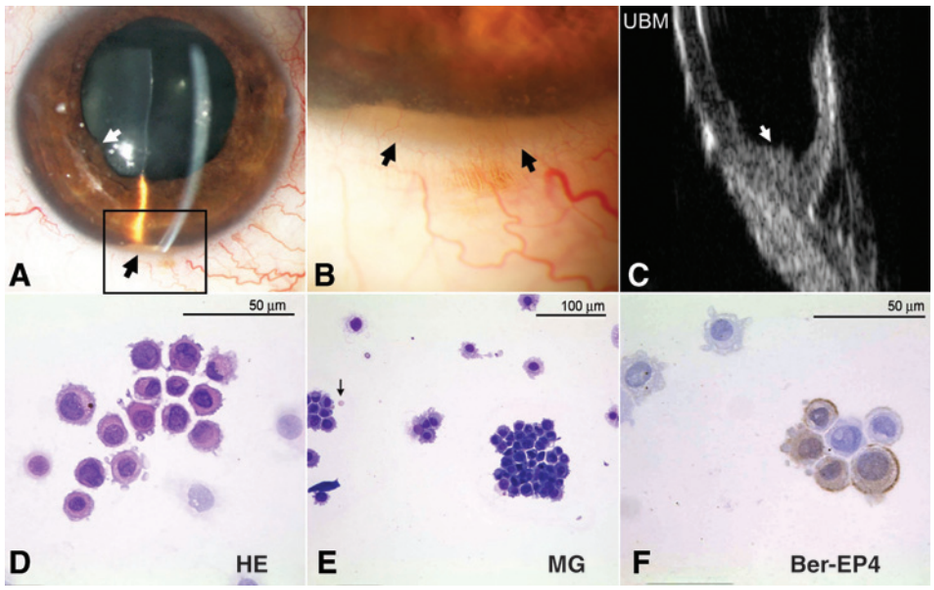

Figure 1.
Carcinoma cells are present in the patient's AH of the eye. A and B, slit-lamp photography of the anterior segment of the right eye showed a pseudohypopyon (black arrows) and an iris-lens-adhesion (posterior synechia) at the 8-o'clock position (white arrow in A) due to a high number of AH cells. C, ultrasound biomicroscopy revealed no adjacent solid tumor (white arrow in C points to pseudohypopyon). D and E, cytospin samples of an AH tap were stained with hematoxylin and eosin (HE; scale bar, 50 μm); and May-Grünwald dye (MG; scale bar, 100 μm) and revealed clusters of carcinoma cells with irregular oval and large hyperchromatic nuclei (arrow, a small rim of cytoplasm, adjacent to a smaller erythrocyte). F, carcinoma cells were stained with Ber-EP4$^+$ (scale bar, 50 μm).

the two healthy donors (Fig. 2A), the patient had a CD45$^+$Ber-EP4$^+$ PBMC population in his blood (Fig. 2B). These Ber-EP4–expressing cells exhibited a monocytic-myeloid phenotype with coexpression of CD11b, CD115, and the macrophage marker CD172a (SIRP-α; Fig. 3A–C). Uptake of phrodogreen revealed their phagocytic activity (Fig. 3D–F).

Discussion

The AH in the anterior chamber of the human eye has to be transparent, and therefore is physiologically devoid of cellular components to preserve vision. AH is cleared by passage through the trabecular meshwork (TMW) and the Schlemm canal and drains through veins leaving the eye and moving into systemic circulation (5). Normally, occasional cells with a maximum size of erythrocytes and lymphocytes can pass the openings of the TMW. Larger cells such as macrophages, especially when in aggregates, are more likely to be trapped in the TMW and cause an increase of intraocular pressure, as observed in the present patient (5).

Systemic tumor diseases (e.g., lymphoma, carcinoma, or melanoma) sometimes manifest themselves inside the eye and most commonly affect the retina, uvea, and the vitreous body. Very

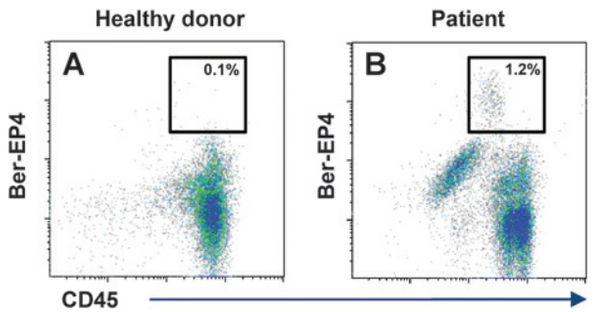

Figure 2.
Ber-EP4–expressing cells are present in the patient's PBMCs. A, flow-cytometry analysis of a healthy donor; and B, of the patient. The density blots show a representative result of three independent experiments.

rarely, individuals with hematologic malignancies such as leukemia or lymphoma may have tumor cells in the anterior chamber, perhaps accompanied by a pseudohypopyon (6, 7). However, solid tumors have usually not been associated with pseudohypopyon formation, except for rare cases associated with extensive infiltration of the choroid or the ciliary body (8, 9).

As we did not find a solid metastasis inside the eye from which these cells may have been seeded, we assume that single metastatic tumor cells got trapped inside the TMW, multiplied by further cell division, and released cells into the AH. This finding of a single-cell suspension of a solid malignancy in the AH of the eye raised the question of how these tumor cells originally got there. The anterior chamber of the immune-privileged eye is devoid of lymphatic vessels (10), making the putative route of entry for Ber-EP4$^+$ carcinoma cells solely hematogenous. Ber-EP4 is expressed by epithelial and adenocarcinoma but not hematopoietic cells (4). Here, we observed a Ber-EP4$^+$ cell population within the PBMCs with phagocytic activity and a monocytic-myeloid phenotype that expresses the macrophage marker SIRP-α that can interact with CD47 on tumor cells to negatively control phagocytosis and to promote cell fusion (11). Tumor-associated macrophages (TAM) can express tumor markers via several mechanisms. Besides phagocytosis, trogocytosis, transdifferentiation, and aberrant expression, cell fusions of macrophages and tumor cells are observed. The "Trojan horse" model suggests that a tumor cell becomes metastatic by fusion to normal cells traveling throughout the body freely, such as macrophages. Macrophages can enter the immune-privileged anterior chamber and cause pathologic conditions such as glaucoma (5). In our patient, the Ber-EP4$^+$ carcinoma cells in the anterior chamber showed cytomorphologic characteristics of macrophages, and their accumulation and persistence there argued for a malignant derivative of the parotid gland carcinoma. Our findings suggest that the tumor cells in the anterior chamber originally derived from cell fusions between tumor cells and myeloid cells in the peripheral blood that continued to divide inside the eye with consecutive elevation of the intraocular pressure. These observations imply that metastases of a solid malignancy could use monocytes–macrophages as their

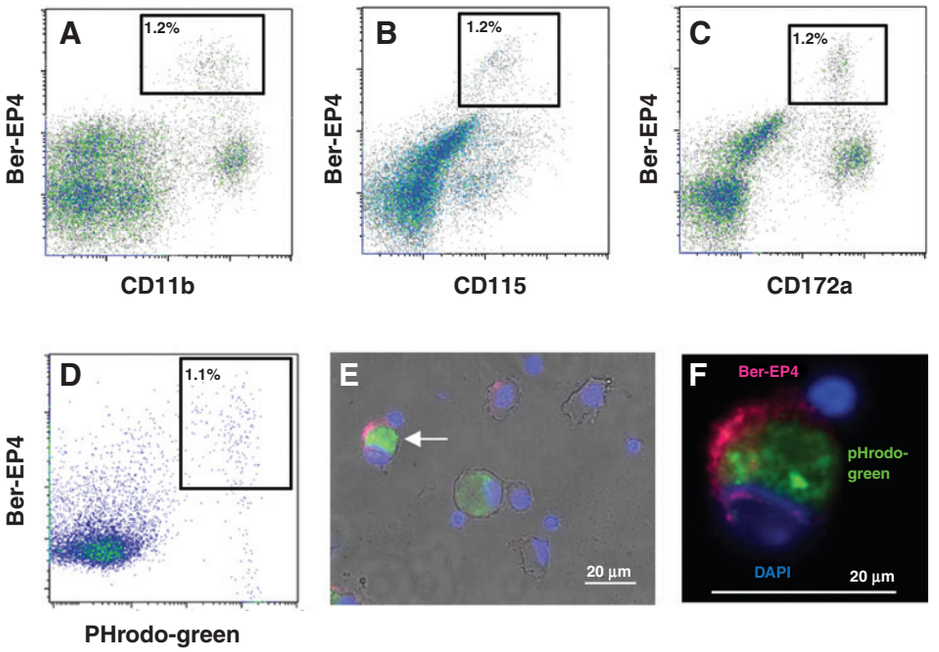

Figure 3.
Ber-EP4–expressing cells in the patient's PBMCs have a monocytic-myeloid phenotype and phagocytic activity. A–C, flow-cytometry analysis of the patient's PBMCs, labeling for Ber-EP4 and myeloid/macrophage markers A, CD11b; B, CD115; and C, CD172a. D–F, phagocytosis of pHrodo-green–labeled *E. coli* particles by Ber-EP4$^+$ cells as detected by D, flow cytometry, and E and F, by immunofluorescence microscopy (white arrow in E points to cell shown in detail in F). DAPI was added for nuclear staining. At least 100 Ber-EP4$^+$ cells were analyzed by fluorescence microscopy (scale bar, 20 μm).

Trojan horse to enter the immune-privileged eye (12) to find a new environment for further tumor growth and metastasis. The limited amount of sample precluded assessment of the abnormal cells in the AH for monocytic markers, so further studies are needed to provide conclusive evidence of the exact nature of these cells inside the eye to demonstrate whether they are carcinoma cells, hybrid cells, or if both are present.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): R.S. Grajewski, J.J. Bosch, H. Bruns, C. Cursiefen, L.M. Heindl
Writing, review, and/or revision of the manuscript: R.S. Grajewski, J.J. Bosch, C. Cursiefen, L.M. Heindl
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R.S. Grajewski, L.M. Heindl
Study supervision: R.S. Grajewski, C. Cursiefen, L.M. Heindl

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