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Effect of Glioma-Macrophage Interaction on Tumor Sphere Formation

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ABSTRACT

Tumor microenvironment alters the interactions between glioma cells and immune cells. This study shows that immune cells gain new phenotypic properties that are believed to facilitate the proliferation rate and invasiveness of glioma cells.

Key words: Glioma, macrophage, microfluidics, and quantitative data.

GLİOMA-MAKROFAJ ETKİLEŞİMİNİN TÜMÖR KÜTLESİ OLUŞUMUNA ETKİSİ

ÖZET

Tümör mikroçevresi glioma hücreleri ve bağışıklık sistemi hücreleri arasındaki etkileşimi değiştirmektedir. Bu çalışma makrofajların glioma hücrelerine çoğalma ve invazyonu kolaylaştıran yeni fenotipik özellikler kazandırdığını göstermektedir.

Anahtar sözcükler: Glioma, makrofaj, mikroakışkanlar ve niceliksel veriler.

Glioblastoma multiform (GBM) is one of the most deadly tumors , commonly seen in children and adults over the age of fifty (1). The survival of patients could be extended up to 15 months with treatment of chemotherapy, radiation and surgery (2, 3, 4). One of the biggest difficulties during diagnosis , is that the symptoms of the disease are difficult to determine until tumor effects on brain functions are visible. The diagnostic phase is based on magnetic resonance imaging (MRI). However, MRI cannot provide very precise results in many cases; therefore, examination of the biopsy sample is required to determine extent +of the tumor (5). One of the biggest problems in the treatment stage is that cells in the brain are very sensitive to chemotherapeutic reagents. Likewise, the blood-brain barrier prevents effective delivery of nanoparticles and drugs (6). In addition to all these challenges, GBM spreads very aggressively even though a successful surgical removal of the primary tumor is achieved; "secondary tumors" may occur.

Since 1926, researchers have been investigating the intense heterogeneity in GBM and its effects on diagnosis and treatment of GBM (7, 8). Today, recent studies related to GBM heterogeneity have pointed to the interactions between the immune system cells and cancer cells (9, 10, 11). Tumor microenvironment alters the interaction

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of glioma cells and immune system cells, as a result, s the immune cells gain new phenotypic properties that are believed to facilitate the growth and spread of tumor cells (9, 12). Tumor-associated macrophages (TAMs) have been shown to promote malignant glioma growth by creating a local immunosuppressive microenvironment (13), secreting pro-angiogenic factors and enhancing invasion mediated by the production of soluble factors such as colony stimulating factor-1 (CSF-1), transforming growth factor-β (TGF-β), interleukin (IL)-10, vascular endothelial growth factor, and matrix metallopeptidase-9 (14, 15). In glioblastoma, TAMs can comprise up to 40% of all cells in GBM the high-grade gliomas having the higher number of macrophages compared to low-grade gliomas (16, 17, 18). These findings encouraged us to perform glioma-macrophage co-culture experiments using microfluidic devices in order to quantify their interaction in terms of tumor sphere formation capacity.

Materials and methods

Cell culture

The U-87 MG (HTB-14) human glioma and the U937 human monocytes were purchased from ATCC (American Type Culture Collection). 10 ml of the U937 human monocyte cell line was stimulated with 0.5 µL 1 µg/mL phorbol 12-myristate 13-acetate (PMA/Fisher) according to standard protocols for macrophage differentiation. Cells were grown at 37˚C with 5% CO_2. The U-87 cells were detached with Trypsin-EDTA (0.25%) (Gibco, Invitrogen) and maintained in MEM medium (Gibco, Invitrogen), 10% fetal bovine serum (FBS/ATCC). The immune cell lines were cultured in RPMI 1640 medium (Gibco, Invitrogen), 10% FBS (ATCC).

Fluorescent probe staining

U-87 and U937-differentiated macrophages were grown via standard procedure for 4 days. The cells were detached using trypsin, harvested by centrifugation, and their supernatant was removed. The macrophages were resuspended in pre-warmed green Dil lipophilic tracker solution for subsequent identification (Lipophilic Tracers—Dil, DiO, DiD, DiA, and DiR, INVITROGEN). Solution of lipophilic tracers were dissolved in research-grade DMSO (Dimethyl sulfoxide) to a final concentration of 10mM). The final working concentration of cell suspension, including dye was 25 µM in fresh medium. The cells were incubated for 1 hour in a standard tissue incubator. Subsequently, the cells were collected by centrifugation. The dye solution was replaced with fresh medium. This washing step was repeated gently three times with fresh medium. Cell viability, proliferation and functionality were not affected by lipophilic tracker dyes compared to non-fluorescence labeled cells (data not shown).

Imaging and data analysis

Glioma cells, macrophages and their mixture were grown, respectively, in the culture and co-culture wells, imaged with motorized fluorescence microscope (Nikon Eclipse). In the co-culture wells, prior to the experiment, macrophage cells were stained with green live cell tracker dyes (CMFDA, Invitrogen) and FITC channel was merged to phase channel to distinguish them from the glioma tumor cells. A motorized stage (Prior, Proscan III) was used to collect an array of images and stitched them using Elements software (Nikon) in order to visualize the whole microchamber.

Next, the images were used to quantify the number, area and spatial location of the tumor spheres in the microwells. Tumor-sphere analysis was achieved using the Elements image processing software (Nikon), manually the periphery of the tumor spheres were contoured and their area were measured.

The extracted data was compiled and graphics were obtained using Prism 5 software (GraphPad).

Results

In this study, glioma U-87 cells, U937-differentiated macrophages and their co-culture were performed in 6-well plates for 7 days as illustrated in Figure 1. 60 000 glioma cells, 120 000 macrophages and their co-culture with 60 000 glioma cells and 120 000 macrophages were inoculated as explained in the material and methods chapter. The number of macrophages was twice more than the number of glioma cells in the co-culture wells due to the fact that macrophages cannot proliferate. We obtained microscope images of each well everyday for 7 days. After imaging, we replaced half of the medium from the wells with the fresh medium to decrease the waste product of the cells and to provide nutrients to the cells. Then, the 6-well plate was directly placed into the incubator. The growth difference between the glioma alone and glioma-macrophage co-culture well is presented in Figure 2. The tumor spheres were marked with the green line using the microscope software to measure their areas and count their numbers. Figure 2a shows glioma-alone culture, Figure 2b presents glioma-macrophage co-culture. The number of tumor spheres is demonstrated in Figure 3.

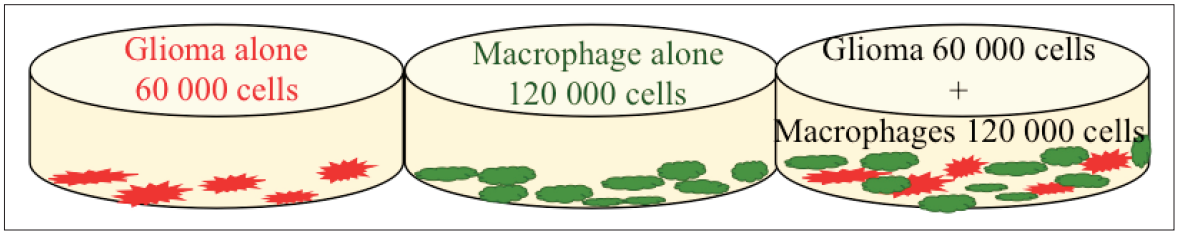

Figure 1. Co-culture experiments for glioma sphere fotmation

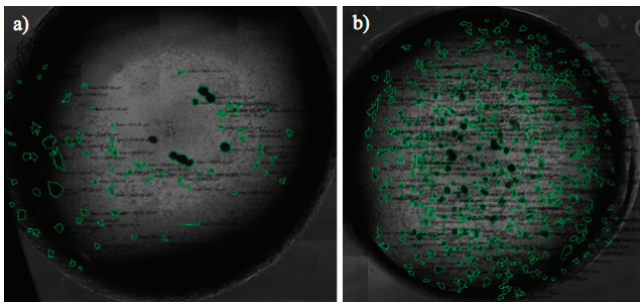

Figure 2. Images of the tumor spheres in the 6 – well plate. a) Glioma alone culture and b) Glioma-macrophage co-culture form tumor spheres. Images of the wells were taken after 7 days of culturing using inverted fluorescent microscope (Nikon). The green-lines show the periphery of tumor spheres when their area was measured using the Elements software (Nikon).

Figure 4 illustrates the area distribution of tumor spheres in glioma alone and glioma-macrophage co-culture wells.

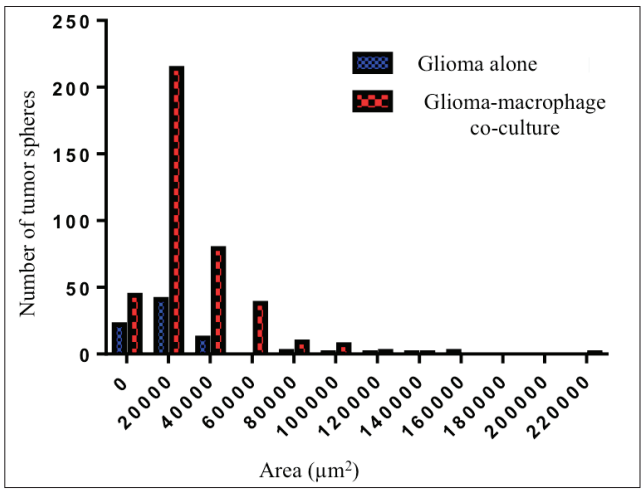

Figure 4. Histogram of the area distribution of tumor-spheres for glioma alone culture and glioma-macrophage co-culture.

Discussion

In this study we investigated glioma-macrophage interaction on tumor sphere formation for glioma tumor cells. Our results clearly showed that when glioma cells were co-cultured with macrophages, the number of formed tumor spheres were higher in these co-cultures compared to glioma-alone cultures. Therefore, this study supports the investigations related to immune cells promoting glioma progession and stimulating invasiveness of glioma cells (13, 14, 15, 18). Our research presented the effect of macrophage-glioma interaction on glioma sphere formation capability. However, the exact underlying interaction mechanism between macrophage and glioma cells remains unsolved.

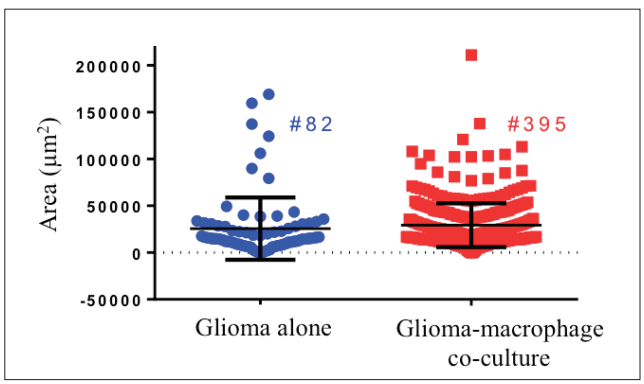

Figure 3. Number of tumor spheres and their area measurements in glioma alone and glioma-macrophage co-culture wells after 7-day culturing.

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