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RAPID COMMUNICATION

Influence of matrix-metalloproteinase inhibitor on the interaction between cancer cells and matrigel*

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Various behaviors of cancer cells are strongly influenced by their interaction with extracellular matrices (ECM). We investigate how this interaction may be influenced if the cancer cells' ability of secreting matrix metalloproteinases (MMPs) to degrade ECM is inhibited by adding the MMP inhibitor. We use MDA-MB-231-GFP cells as model cells and use matrigel to mimic ECM. It is found that the added MMP inhibitor significantly reduces the migration speed of cancer cells covered by matrigel but has little influence on the migration persistence and shape factor of the cells and that with the MMP inhibitor added the presence of matrigel on the top has no influence on the migration speed of the cells but increases the cells' shape factor and migration persistence.

Keywords: cancer cells, matrigel, matrix metalloproteinase (MMP) inhibitor, migration

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1. Introduction

Most behaviors of tumor cells are strongly influenced by the tumor microenvironment, which are composed of stromal cells (including endothelial cells,[1] fibroblasts,[2] and immune cells[3]), extracellular matrix (ECM), cytokines, inhibitors, nutrients, and so on.[4–6] In these components, the ECM, which is a macromolecular fiber network, plays an important role in regulating tumors' invasiveness and formation.[7–10] Matrigel, an extract derived from EHS tumor cells of mice,[11] has been widely used in *in-vitro* experiments to model the ECM and *in-vivo* microenvironment.[12] It has been shown evidently that matrigel can influence cancer cells' adhesion, proliferation, and the expression of invasion-related mRNAs[13] and improve the growth of tumor spheroids in three-dimensional models.[14] In addition, it has recently been shown that matrigel can greatly facilitate single cancer cells' migration and shape deformation.[15]

Matrix metalloproteinases (MMPs) play an important role in regulating the interaction between cancer cells and matrigel. MMPs are proteolytic enzymes, which can degrade various kinds of proteins in the extracellular matrix. Animal-research results have shown that MMPs are active contributors in tumor progression.[16] It has also been shown that the expression level and activity of MMPs are enhanced in human breast cancers, which may be related with the metastasis and increased invasion of malignant cells.[17–19] In this article, we investigate how the interaction between single cancer cells and matrigel may be influenced if the cells' ability of secreting MMPs is inhibited.

2. Materials and methods

The MDA-MB-231-GFP cells we used in the experiments were obtained from H. Lee Moffitt Cancer Center (in Tampa, Florida, USA) and the magrigel was purchased from the Corning BioCoat company.

2.1. Sample preparation

The cell-culture medium consisted of Dulbecco's modified Eagle's medium (DMEM, Corning), 10% fetal bovine serum (FBS, Gibco), 100 units/ml penicillin (Corning), and 100 μg/ml streptomycin (Corning). The cells were added into the medium and cultured in an incubator with 5% CO_2 at a temperature of 37 °C. After being trypsinised with trypsin-EDTA (Corning), centrifuged, and further re-suspended by the culture medium, the cells were then counted under a microscope (TS-100, Nikon) and seeded onto a 96-well plate (at a density of 1000 cells per well). After being incubated for a whole night, the cells were then covered with matrigel (Corning BioCoat, standard version) and further incubated at 37 °C for 30 min in order to solidify the matrigel. The culture

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medium (200 μl) was then added on the matrigel. In the control experiments having no matrigel, 200 μl culture medium was directly added on the adherent cells, after over-night incubation.

The MMP inhibitor (Batimastat, Millipore) was dissolved in DMSO at concentration of 10 mmol/L, and the DMSO solution was mixed with the culture medium at a concentration of 2 μl/ml.

2.2. Data analysis

After the sample preparation, the MDA-MB-231-GFP cells were tracked under an inverted microscope (Ti-ECLIPSE, Nikon) for eight hours (by using a $\times 10$ objective lens), with one fluorescent image taken every 5 min. The images were then used to calculate the mean squared displacement (MSD), the speed, and the shape factor of the cells. In the MSD calculation, the cell trajectories were obtained by using the CellTracker plugin of Matlab 2014a,[20] and the coordinates of 200 single cells' 8-hour tracking data were then utilized to plot the MSD curves.[21]

The calculation of the shape factor was also based on the fluorescent brightness of cells. By using ImageJ, we first calculated the areas (between 300 μm^2 and 2500 μm^2) and the perimeters of the cells. Given the area and perimeter, the shape factor, which is equal to the ratio between the cell perimeter and the square root of the cell area, can then be obtained. The data of each group resulted from the measurements of 4000 single cells.

3. Results

We investigate the influences of the MMP inhibitor on cell migration and shape in matrigel environment (see Fig. 1 for the illustration of the experimental setup). The MMP inhibitor inhibits several kinds of matrix metalloproteinase secreted by MDA-MB-231 cells, thereby reducing the degradation of matrigel by cancer cells. Considering that DMSO is the solvent for the MMP inhibitor, we start with experiments in which only DMSO (with no MMP inhibitor) is added, and then proceed to investigate the influence of the MMP inhibitor. We also perform experiments that have no matrigel in order to better reveal the mechanism of the cancer cell-matrigel interaction (see Fig. 1).

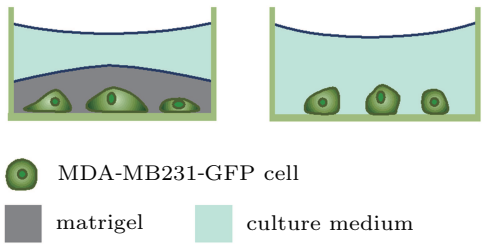


Fig. 1. Illustration of the experimental setup.

We focus on three physical quantities: the mean squared displacement (MSD), the migration speed, and the shape factor, with the former two characterizing the migration ability of cancer cells and the last one, which is defined as the ratio between the cell perimeter and the square root of the cell area, characterizing the deformation of the cells.[22,23] For circular cells, their shape factor is about 3.54; the larger the shape factor is, the more irregular the shape is. The variation of MSD with time is usually presented in log-log plots: a line with the slope equal to one indicates the migration of cells is random; the larger the slope is, the more persistent the migration is.[24,25]

3.1. Influences of MMP inhibitor and DMSO in the presence of matrigel

We start with experiments in which the cells are covered by 100% matrigel. We first added only DMSO into the system. Our results show that DMSO has almost no influence on the MSD [see Fig. 2(a)]; the slope of the MSD curves in the log-log plot is about 1.61, indicating that the cells perform persistent (rather than completely random) migration due to the existence of matrigel. After adding DMSO, the speed and shape-factor averages are only slightly increased (from 0.80 μm/min to 0.84 μm/min and from 4.92 to 5.11, respectively) [see Figs. 2(b) and 2(c)], and the speed and shape-factor distributions remain almost unchanged [see Figs. 2(d), 2(e), 2(g), and 2(h)].

We then added the DMSO solution containing the MMP inhibitor. The cells' average speed now decreases significantly [Fig. 2(b)] (from 0.84 μm/min to 0.57 μm/min); the speed distribution also shows that the probability of cells having low speed (in 0–0.6 μm/min) increases dramatically [Fig. 2(f)]. We thus conclude that the MMP inhibitor significantly reduces the cells' interaction with matrigel and thus weakens migration of MMPs. However, note that the MMP inhibitor has little influence on the slope of the MSD curve [Fig. 2(a)], and induces only a slight increase of the shape factor, as shown in Figs. 2(c) and 2(i).

3.2. Influences of DMSO and MMP inhibitor in the absence of matrigel

We now investigate the influence of DMSO and the MMP inhibitor on cells' behaviors in the case of no matrigel. As shown in Fig. 3, after the addition of DMSO, the MSD, speed, and shape factor of the cells are all increased, indicating that DMSO itself can enhance the migration and deformation capabilities of MDA-MB-231-GFP cancer cells. However, the MMP inhibitor appears to have almost no effect, in addition to inducing a slight decrease in the average speed and average shape factor (see Fig. 3).

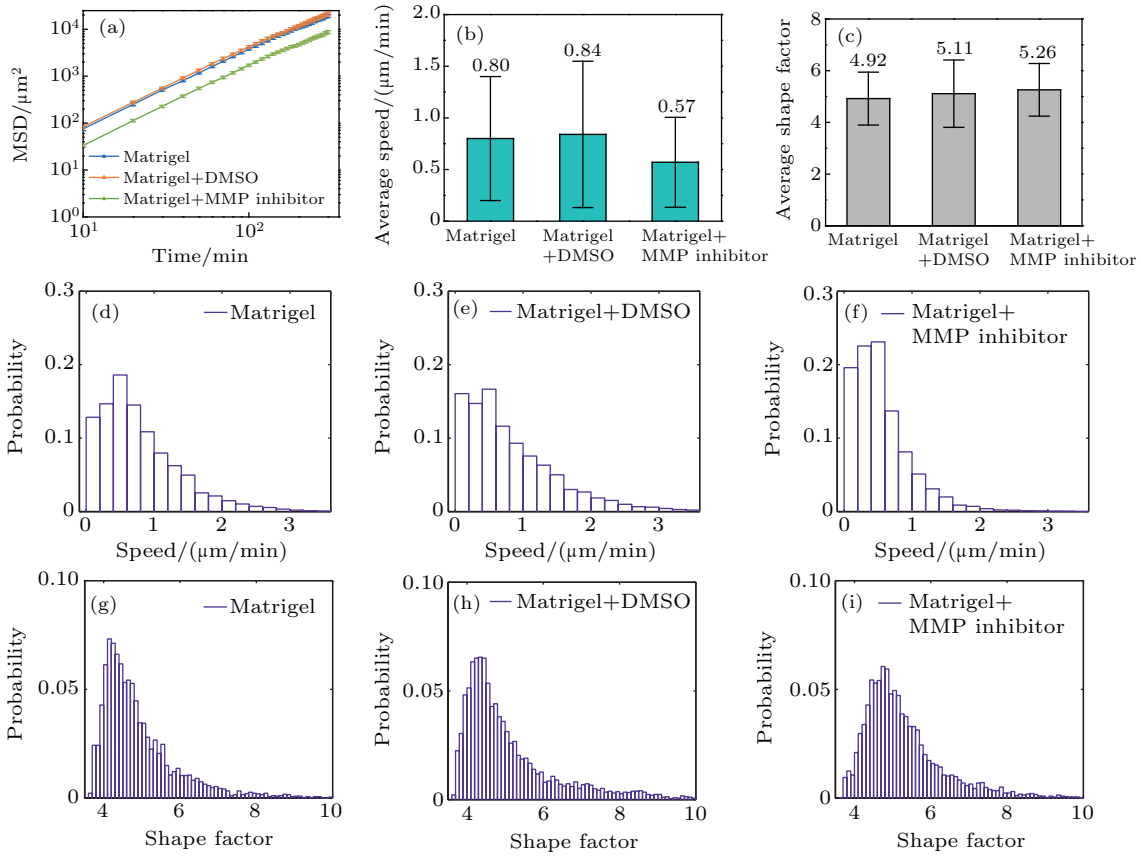


Fig. 2. Comparison of MSD (a), average speed (b), average shape factor (c), and distributions of speed (d)–(f) and shape factor (g)–(i) of cells treated with MMP inhibitor or DMSO, in the matrigel environment. Blank-control data are also shown for comparison.

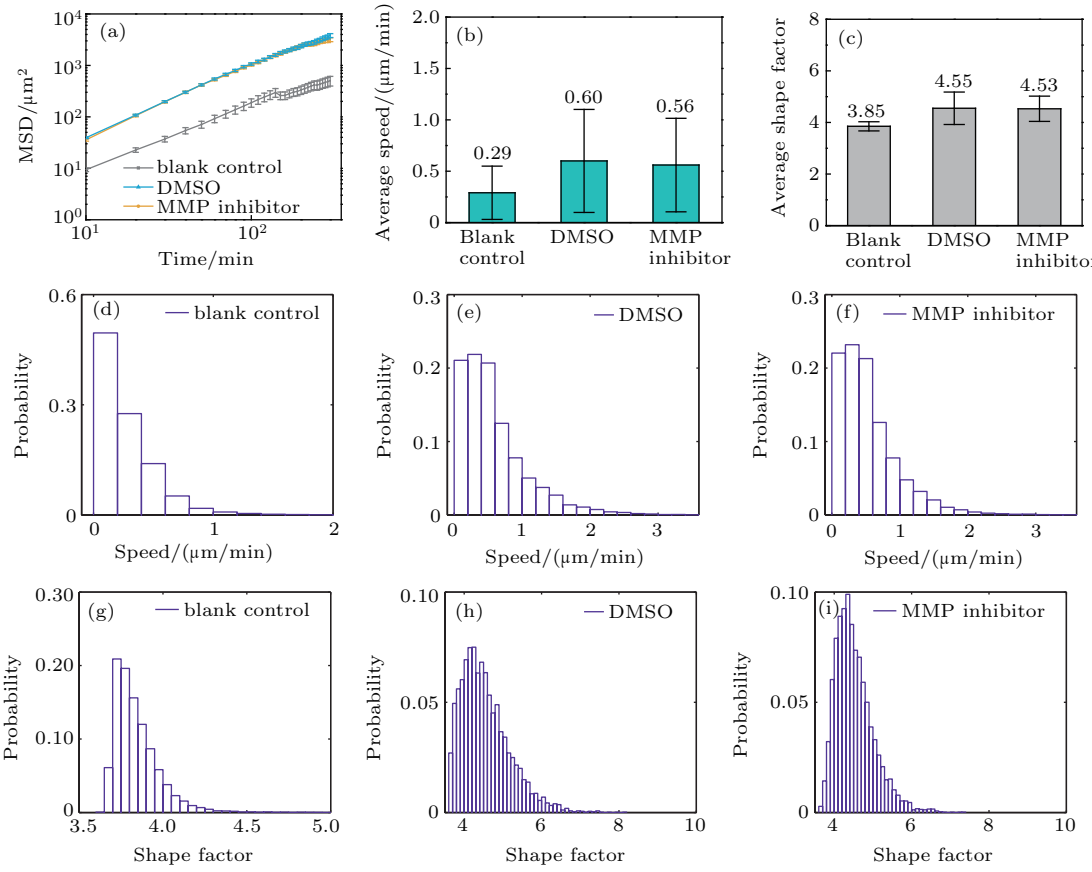


Fig. 3. (a) MSD, (b) average speed, (c) average shape factor, and (d)–(i) the corresponding speed and shape-factor distributions of cells treated with MMP inhibitor or DMSO, in the absence of matrigel. Blank-control data are also shown for comparison.

It is important to note that the average speed of the cells treated by the MMP inhibitor is 0.56 μm/min [Fig. 3(b)], which is almost the same as that of the cells covered by matrigel and treated by the MMP inhibitor (0.57 μm/min) [Fig. 2(b)]. The speed distributions in these two cases are also very close to each other [see Figs. 2(f) and 3(f)]. In other words, the speed of cells treated by the MMP inhibitor is insensitive to the presence of matrigel. However, as shown in Figs. 2 and 3, matrigel on the top does affect MSD and the shape factor. In the cases of MMPs-inhibitor treatment, the slope of the MSD curve in the log-log plot and the average shape factor are 1.61 and 5.26, respectively, if there is matrigel, and reduce to 1.31 and 4.53, respectively, if there is no matrigel.

4. Discussion and conclusion

We have investigated the MMP inhibitor's influence on the interaction between cancer cells and matrigel and the potential influence from the solvent DMSO. Our results show that, with the addition of the MMP inhibitor and the inhibition of the degradation of matrigel by cancer cells, the cancer cells' migration speed decreases significantly, whereas the shape factor increases slightly because of the influence of DMSO. In the presence of the MMP inhibitor, matrigel covering on the top increases the cells' shape factor and MSD (or migration persistence) but has no influence on their migration speed. Thus, our results imply that the increase of cells' shape factor does not necessarily induce the increase of their migration speed although it may enhance the migration persistence.

The aforementioned decorrelation between the migration speed and the shape factor can be attributed to the complex interaction between matrigel and cancer cells. With matrigel covered on top, focal adhesions form not only between the cells and the substrate but also between the matrigel and the cells, and the cells' migration speed is thus greatly enhanced. With the further addition of the MMP inhibitor, the focal adhesions between the cells and the matrigel are probably destroyed, and the cells' migration is driven only by the focal adhesions between the cells and the substrate and thus possesses the same speed as that of the blank control group. However, probably due to the pressure of the matrigel, the cells' shape factor increases. We will investigate in detail the microscopic mechanism underlying this decorrelation in near future. Our current results provide useful information for the development of microscopic models on the interaction between cancer cells and matrigel.[26]

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