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Keywords*:* Cancer cell migration; 3D fibrous medium; Correlation method; Spatio-temporal displacement of collagen fibers.

1. Introduction

Cell migration plays a key role in several disease processes as cancer or immunosuppression. Up to now, migration on a 2D substrate was widely studied whereas in 3D medium much less resullts are available. The aim of our study was to analyze the migration of cancer cell with different invasive capacities in collagen fibrous medium thanks to the reflectance confocal microscopy (Iordan et al. 2010, Wolf et al. 2013) and a new correlation method (Fertin et al., 2019). The reflectance microscopy allows to visualize simultaneously the cell actin and collagen fibers. The method gives the displacement field of collagen following the more intense zone of collagen fiber network.

First, we showed that the morphology of migrated cancer cells varies with the invasive capacity of the cell. Moreover, the more invasive cells induced larger levels of fiber displacements and various mechanism of migration.

2. Methods

2.1 Cell culture and collagen gel preparation

Three epithelial cancer cell lines (RT112, T24 and J82) were used and cultured in RPMI culture medium supplemented with Fetal Bovine Serum and antibiotics (penicillin streptomycin). RT112 cells are characterized by an intermediate grade (grade 2) whereas T24 and J82 cells exhibit a high grade (grade 3). RT112 cells have a smaller metastatic potential compared to J82 cells (Abidine et al., 2018). The cell lines are transfected with the Life-Act GFP plasmid to stain for F-actin (Riedl et al., 2008).

Collagen gels (0.95 mg/ml) were prepared from rat tail collagen solutions (Corning, USA) and mixed with culture medium, then neutralized to reach pH=7.4 at 4°C. Then, cells were added to the collagen. Collagen polymerized at 37°C for 30 min.

2.2 Visualization of cell migration and collagen fibers

We simultaneously visualized cell actin and collagen fibers thanks to the use of the reflection and fluorescence modes of a confocal microscope (Zeiss, LSM 510 model). The acquisition of Z-stacks in the 2 modes allowed to construct a 3D cell image contour and to visualize the spatial organization of collagen fibers. The Z-distance between 2 optical slices was 0.77 µm for all acquisitions. To study the cell migration, we made a recording over 2 hours (with a time interval of 10 minutes between Z-stack acquisitions).

2.3 3D displacement of collagen fibers

The 3D displacement of collagen fibers were determined using an home-made algorithm (Fertin et al., 2020) applied to the reflection images. This algorithm uses a correlation method to determine the positions of fibers that are bright enough. We obtained incremental 3D displacemnts between t and t + Dt . The phase-only correlation (POC) was used :

$$r(\boldsymbol{x}) = \mathcal{F}^{-1}\left(\frac{I_1^*(\omega) * I_2(\omega)}{|I_1^*(\omega) * I_2(\omega)|}\right)$$

where $i_1(x,t)$ is the image intensity at time t and position x, $I_1(\omega)$ the Fourier transform of i_1, F^{-1} the inverse Fourier transform, ω the angular frequency, and $I_1^*(\omega)$ denotes the complex conjugate of $I_1(\omega)$. The same applies for i_2 and I_2 at time t + Dt. The POC function exhibits a unique sharp peak when the signal i_1 is a shifted version of i_2. Using this property, we obtain the required displacement **u** in voxels :

$$\boldsymbol{u} = \arg\max_{x}(r(\boldsymbol{x}))$$

3. Results and discussion

First, we investigated cell morphology of migrating cells by studying their sphericity index. The grade-2 cells (RT112 cells) were found to have a significantly higher value of the sphericity index than grade-3 cells (T24 and J82 cells) (mean value of 0.467 ± 0.006 for RT112 cells, 0.429 ± 0.008 for T24 cells, 0.426 ± 0.006 for J82 cells).

Next, to analyze how cancer cells deform their environment, we determined the 3D displacement of collagen fibers. Figure 1 shows superposition of two successive reflection images obtained at 10 min time interval (left images) with the representation of cell contour (cyan for the time t and yellow for the time t+Dt) for a RT112 cell (first line), a T24 cell (second line) and a J82 cell (third line). The corresponding displacement fields (right images) in µm are shown for each cell.

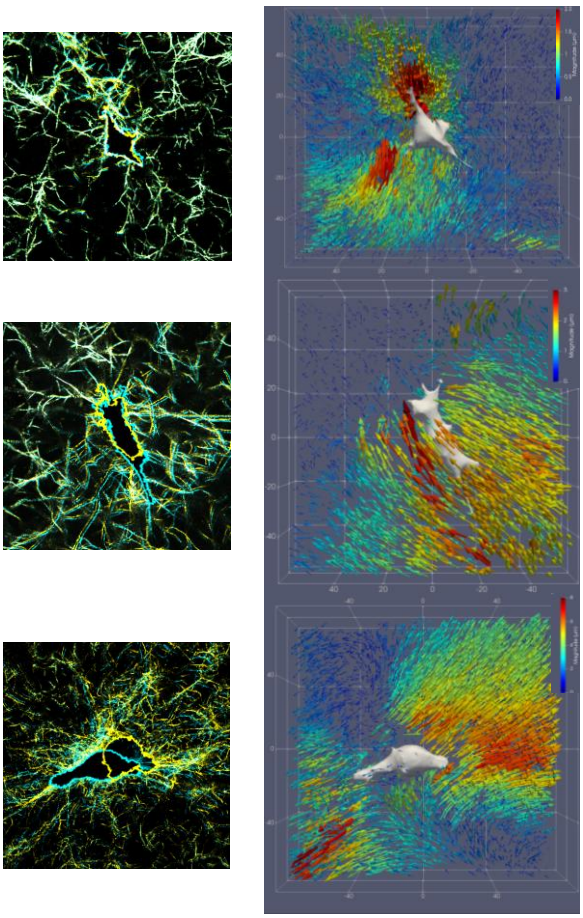

Figure 1: Cell contour (respectively from top to bottom) of RT112, T24 and J82 cells inside collagen matrix at one Z-slice level (left column; first position in cyan and second position in yellow, 10 min time interval). Corresponding 3D-displacement fields (right column), the initial cell shape is shown in grey level. The vector length and color indicate the magnitude of the displacement in µm.

Grade-3 cells (T24 and J82 cells) showed an elongated shape compared to RT112 cells. The deformation of the collagen network induced by the migration of the J82 cells is significantly higher compared to the T24 and RT112 cells : the displacement magnitude reaches a maximum value of 8 µm for the case of the J82 cell, 3 µm for the T24 cell, 2 µm for the RT112 cell. Furthermore, we observed that cancer cells are able to push and/or pull on collagen fibers. The main difference in the migration strategies developped by these cells are as follows:

- RT112 cells motion resemble that of amoeboid cells,
- T24 and J82 cells pull/push more on the matrix and undergo a mesenchymal type of motion (Mierke et al., 2015; Friedl and Wolf, 2010).

4. Conclusions

Here, we compared morphologies and migration of cancer cells with an increasing level of metastatic potential (RT112 cells < T24 cells < J82 cells) in collagen matrices. We found that grade-3 cells (T24 and J82 cells) are characterized by a more elongated shape compared to grade-2 cells. (RT112 cells). Furthermore, these cells pull/push more on the matrix and undergo a mesenchymal type of motion whereas the RT112 cell migration resemble that of amoeboid cells. Our results also revealed that cells with the higher level of metastatic potential (J82 cells) induced significantly higher level of collagen fiber displacements.

Acknowledgements

We thank the ”Agence Nationale de la Recherche” (grant # 12-BS09-020-01, TRANSMIG). C.V. and V.M.L. are members of the LabeX Tec 21 (Investissements d’Avenir: grant agreement No. ANR-11-LABX-0030).

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