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Abstract: Cancer metastasis is still a major society issue with some limited knowledge of the formation of tumors and their growth. In addition the formation of metastases is still very difficult to understand, as tumor cells escape from an initial tumor, travel through the vasculature and finally escape through the vessel wall. This involves very complex physical mechanisms such as cellular interactions and cell rheology, which are flow-dependent. The previous parameters have been recently investigated using sophisticated techniques such as flow chambers, microfluidics, traction force microscopy (TFM) or other mechanical tools such as optical manipulators or Atomic Force Microscopy (AFM), combined with physical modelling. Here we summarize recent results and raise the question of the best possible ways to investigate the precise mechanisms used by cancer cells to undergo transendothelial migration.

Introduction

Cancer arises as tumors are formed within the body and grow in size because cells behave abnormally and divide rapidly. Tumors can be localized due the pressure exerted on the surrounding medium (Deptuła *et al.*, 2020), and can possibly be destroyed using chemo- or radiotherapy. Unfortunately, before the operation or after, cancer cells manage to escape from the initial tumor and penetrate into the blood stream where they can be transported far away, until they reach a distant organ (colon, breast, skin, bladder), i.e. a soil (Fidler, 2003). At this precise location, cancer cells (CCs) interact with the vessels walls covered by endothelial cells (ECs) as shown in Fig. 1. It is known from other works on leukocytes that a possible rolling motion (Alon *et al.*, 1997) can first occur due to the presence of weak interactions between ligands located on the ECs (selectins for instance) and leukocytes or CC receptors. After rolling has taken place,

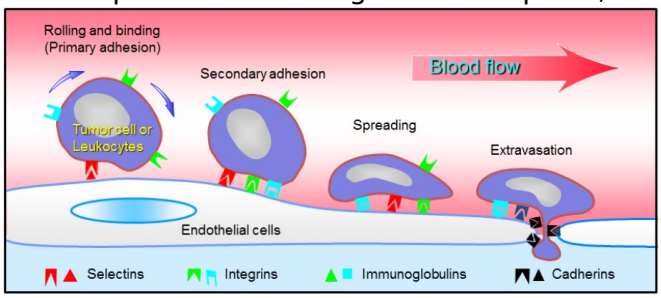

FIGURE 1. Extravasation process. Different steps used by leukocytes or tumor cells to interact with the endothelium. Sketch of the possible molecules involved.

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the next step is secondary adhesion when stronger forces are generated to balance the flow forces. At this time, new bonds are formed involving integrins, immunoglobulins (Orr *et al.*, 2000; Laurent *et al.*, 2014) located on CCs and ECs, that can lead to larger forces or create catch bonds (Kong *et al.*, 2009). The activation of these adhesion proteins can sometimes take time, up to hours (Haddad *et al.*, 2010). One of the important questions is to determine which molecules are involved in such processes and whether they are common in all cancers. Also it is relevant to quantify precisely the forces necessary to create strong bonds (Zhu *et al.*, 2005). The final two steps are CC migration down to the endothelial junction, and transmigration (also called extravasation) through the gap. This process involves both chemical signaling and mechanical effects (Mierke, 2014; Arefi *et al.*, 2020), but is not so well understood. Due to the interest of biophysicists, new physical tools are now available to quantify precisely interactions and forces involved in these dynamic processes (Michor *et al.*, 2011), as well as to measure cell mechanical properties (Gück *et al.*, 2005; Cross *et al.*, 2008; Lekka *et al.*, 2012; Rianna *et al.*, 2017). The viewpoint is organized as follows. Recent results concerning new techniques developped for the investigation of transendothelial migration are presented in the next part, and further researches are proposed, in particular promising methodologies to be enhanced, in relation with essential biological needs. Finally, conclusions will be drawn.

Recent developments

As discussed above, it seems essential to understand what mechanisms are used by cancer cells to **a)** resist the flow in order to adhere to the endothelium; **b)** to form strong bonds i.e. receptor-ligand ones; **c)** to migrate

along the soft endothelium; **d)** to be able to deform in order to pass through tight junctions, in other words to change their rheological properties rapidly.

Flow chambers and microfluidics

Flow chambers have been designed in the 80's in order to study cell interactions between the endothelium and circulating cells such as leukocyte, or cancer cells. The role of flow has been shown to be important for the binding of cells at low shear rates, but for high shear rates, the lift force detaches cells and they are unable to adhere to the endothelium (Lawrence *et al.*, 1987; Couzon *et al.*, 2009). Another important aspect is the alignment of endothelial cells under flow. Usually, after 12 to 24 hours, ECs align in the direction of flow, depending on the shear stress (typically 0.2 to 2 Pa) and the actin cytoskeleton follows this trend (Chien, *2006*). But it has been shown that the signaling pathway involving CCM proteins and β1-integrins can actually produce an opposite effect with ECs not aligned along the flow direction (Jilkova *et al.*, 2014). Regarding cancer cells, the role of higher flow rate is determinant to enhance axial spreading of cancer cells within the endothelium, as compared to radial spreading (Chotard-Ghodsnia *et al.*, 2007). Finally, flow affects the overexpression of cellular adhesion molecules (CAMs) like E-selectins, ICAM-1 and VCAM-1, through the NFκB pathway, but this effect is ruled out at higher shear stresses (Haddad *et al.*, 2010).

Cell-cell interactions using AFM

To analyze cell-substrate or cell-cell interactions directly, AFM in liquid environment is a good tool to probe the presence of receptor-ligand interactions, it also enables to test detachment forces vs. loading rate, in other words to determine how force rates can affect the dissociation of bonds, for example between LFA-1 and ICAMs (Wojcikiewicz *et al.*, 2006). More precisely, in the case of adhesion of tumor cells to the endothelium, the expression of ICAM-1 on CCs has been confirmed (Laurent et al., 2014) and the role of ligands has been explored, in particular CD43 and MUC1 (Rajan et al., 2017). It appears that more invasive bladder cancer cells use the latter CAMs simultaneously in order to bind more efficiently and a reduction of around 70% of cancer cell adhesion has been obtained when blocking these two molecules with antibodies. Morover, CD43 and MUC1 are associated with ICAM-1 with a stronger connexion with the cytoskeleton in the case of CD43, whereas MUC1 is more likely to form tethers when detaching. However other molecules are involved in CC adhesion to the endothelium, so no general trend can be proposed. Ultimately, as CCs transmigrate through the endothelium, they find Extra-Cellular Matrix (ECM) proteins that bind to other CAMs such as β1 integrins or P-selectins, to migrate further (Mierke *et al.*, 2011; Reeves *et al.*, 2013; Le Cigne *et al.*, 2016).

Traction Force Microscopy and cell migration

Another possible way to explore the physics of cancer is to find how invasive cells can exert forces on the surrounding medium. Such methods called Traction Force Microscopy (TFM) have been developped in the years 2000 on two-dimensional substrates using the displacement of beads embedded in elastic gels onto which cells adhere, then an inverse problem is solved to determine traction stresses (Butler *et al.*, 2002; Schwarz *et al.*, 2002; Ambrosi *et al.*, 2009). This method allowed to show, for example, that invasive cancer cells migrate differently than non invasive cells and exert less stress in order to move faster (Peschetola et al., 2013). This technique also proved to be quite efficient to determine the forces exerted by cancer cells as they transmigrate through an endothelium layer (grown as a circular patch on a 10 kPa gel, see Fig. 2A-B). In such a case, the horizontal (shear) forces exerted by CCs do not seem to be very strong as compared to other ones at the edges of the patch (Fig. 2C-D). This reveals that forces necessary for transmigration are vertical ones, necessary to pull the cell through the junction. They can be related to the strength of bonds between CAMs located at the cell invadopodium (intense green levels in Fig. 2A, Rajan, 2016) and ECM proteins on the gel surface below (fibronectin or collagen for instance).

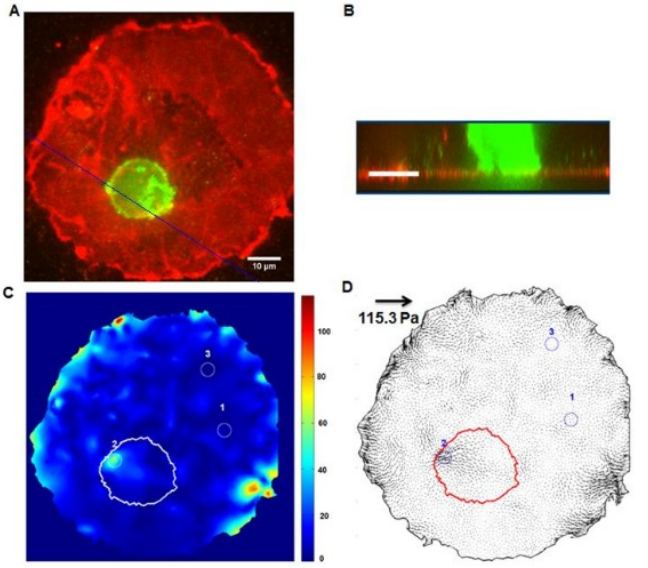

FIGURE 2. Traction Force Microscopy performed when a cancer cell interacts with the EC monolayer. A) Fluorescence image of ECs (red) and CC(green). B) Confocal side view, taken along the blue line in A. C) Stresses (Pa) due to CC, white line is cell contour. D) Stress vectors with maximum value indicated. Cell contour in red. Scale bar = 10µm (Rajan, 2016).

Therefore, it is important to continue in this direction and explore this process using 3D TFM as used in recent studies (Legant *et al.*, 2013; Jorge-Peñas *et al.*, 2017; Fertin *et al.*, 2019).

Cell deformability using AFM

The ability of cancer cells to extravasate through the tight endothelial junctions depends on crosstalk between CCs and ECs during contact, implies β-catenins and E-cadherins, and is mediated by reactive oxygen species (Haidari *et al.*, 2013). But it depends on the ability of CCs to deform a lot, a property well known because cells are viscoelastic materials (Canetta *et al.*, 2005) and can change shape (Cross *et al.*, 2008; Lekka *et al.*, 2012). On the other hand, it seems necessary for cells to present a rigid enough leading edge to push through the junctions. In order to verify this idea, it is necessary to carry out precise local microrheology measurements of CCs in contact with various substrates, and this can be done using AFM in force modulation mode at different frequencies (Abidine *et al.*, 2015). Interesting results have been obtained showing the adaptation of CC stiffness when plated on different elastic gels: cells usually

spread more and their elasticity increases (Solon *et al.*, 2007). In addition, it was shown that viscoelastic effects are also enhanced as cells spread on more elastic substrates but also the typical crossover frequency (between G' the elastic modulus, and G'' the loss modulus) is reduced for low elasticity substrates or when in contact with an endothelium (Abidine *et al.*, 2018). This demonstrates how biological environments (i.e. the endothelium) influence the cell response leading to a glassy-like response. This property of cancer cells to modify their rheology quite rapidly is a key mechanism (see Fig. 2A) where CCs relocalize rigid actin-rich domains right at the endothelial junction to push through this barrier. Therefore, local stiffening is important, but global soft stiffness is needed later, as CCs deform a lot to pass through the gap.

Modeling cell rheology processes

Modeling cell mechanical processes has been a source on interest within the physics community for a very long time so only a few features will be addressed here. There is a large number of cellular models, going from vesicles (Biben *et al.*, 2011), composite or deformable beads (Jadhav *et al.*, 2005), tensegrity models (Ingber, 1993), active drops (Joanny, 2013) that can be used to model cells depending on the problem studied. Flow effects can also be included (Verdier *et al.*, 2009) and cell interactions are usually based on the stochastic behavior of cell bonds that can form or break based on previous theories (Kramers, 1940; Evans *et al.*, 1997). This results in a force vs. loading rate relationship, being able to explain AFM data as well as flow effects. Finally cell-cell interactions involving the contact of cells and deformations like in the extravasation process have been proposed (Arefi *et al.*, 2020) but have not been developped so much, since they involve key mechanical effects. This could indeed lead to a vast number of parameters to be determined or adjusted, and this is still a challenge. Future models and simulations could use deep learning to try and identify the model parameters effects in order to build a smaller parameter landscape and get a better understanding of the transmigration process.

Conclusion

New physical tools have been developped in the past twenty years and promise to give a better understanding of the mechanisms at play during cancer cell transmigration. At present, the major results concern the quantification of forces developped during cell interactions in a complex media. Still more *in vitro* experimental data are necessary, and need to be collected in view of models adapted to a 3D cell environment. Such models have reached a state of sophistication that should help select the relevant parameters sometimes hidden within the vast biological pool data.

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Author Contribution

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

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Conflicts of Interest

The author declare that he has no conflicts of interest to report regarding the present study.

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