# On forces involved in cancer cell migration and invasion; a hypothesis

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**Abstract**

The development of new methods to measure tractions of single cells during migration in connective tissue and test whether cancer cell invasion is correlated with higher force generation are of prime interest. Therefore a better understanding of the role of forces may therefore help to explain tumor-specific differences of invasiveness, tissue preferences and metastasis formation. The question therefore is whether forces generated by tumour cells are influenced by stiffness of the stroma surrounding it, adhesive ligands, mesh size and other factors in the surrounding environment.

## Mechanics of cancer cell migration

Rampant cell growth, which is described in general terms as cancer, is a deadly disease primarily due to the cells' ability to form secondary tumors in distant organs by metastasis. It entails numerous steps: (*i*) separation of single cancer cells from the primary tumor, (*ii*) invasion of tissue and the cellular matrix by these cells, and (*iii*) endothelial transmigration into and out of blood and lymph vessels (Lange and Fabry, 2013; Wolf et al., 2013). In the past, cancer research has focused largely on the gene regulation and signaling that underlie uncontrolled cell growth; however, recent crucial advances in cell biology and biophysics have provided great knowledge to examine links between the mechanics of cellular structures and cell function such as mitosis, signaling, mechano-transduction, vesicle transport, and cell locomotion and motility (Wolf et al., 2013). A significant outcome of this has been the availability of techniques to determine the forces of living cells through a variety of sophisticated biomechanical assays (Mierke, 2011; Goldmann et al. 2013; Lang et al., 2015; Lautscham et al., 2015). Mechanical measurements in combination with chemical, biological, and genetic pathways, as well as with the characteristics of the actin cytoskeleton, intermediate filaments, and microtubules, offer now a unique perspective through which the highly complex mechanistic connection between human health and disease at the molecular and cellular levels may be better understood. A huge number of experimental methods to assess the biophysical properties of cancer cells are now available (Unal et al., 2014; Lange et al., 2015). But

does force generation of tumor cells and cell invasion depend on matric stiffness, adhesive ligands, mesh size, and surrounding environment?

To date, all we know about cell migration, mechanical tensions, and forces were deduced from studies of cells cultured on planar substrates. Pelham and Wang (1997) developed a method to measure the forces during cell migration in 2-D culture systems by traction microscopy, which was later refined by Butler et al. (2002). This form of microscopy provided abundant information about the mechano-biology of cells, for example, how cells respond to extracellular matrix stiffness by dynamic regulation of integrin adhesion receptor clustering, focal adhesion complex formation, and cytoskeletal architecture remodeling (Discher et al., 2005; Mierke et al., 2010; 2011; Licup et al., 2015). As a result, contractile force generation and cell migration are strongly influenced by the mechanical properties of the matrix. How such a force feedback mechanism works in a 3-D environment is currently the topic of much research (Koch et al, 2012; Steinwachs et al., 2015).

**Physical regulation of 3-D cell invasion**

Irrespective of the cell types, 3-D cell migration is controlled by a number of biophysical processes (Wolf et al., 2013; Lange and Fabry, 2013) such as cell adhesion, force generation, detachment, i.e. de-adhesion and cytoskeletal rearrangement, and matrix remodeling. During these processes, cell adhesion and detachment are the only important parameters that influence migration speed, while the forces needed to overcome the viscous drag imposed by the environment are negligible. In contrast, the resisting forces of a stiff 3-D connective tissue to cell migration might be larger than the traction forces a cell can produce, and if the cell is not secreting matrix-degrading enzymes (e.g., MMPs), the migration could come to a standstill (Zaman et al., 2005; Pathak and Kumar, 2011). Numerous fundamental questions are still unanswered today: (*i*) Do cells push against the tissue to propel themselves forward or do they grab tissue matrix in front of them and then pull? (*ii*) How hard do they push or pull? (*iii*) How strongly do they adhere onto the matrix? (*iv*)To what extend do cancer cells need to degrade the extracellular matrix? (*v*) Through what size hole can they squeeze, and what are the forces during amoeboid versus mesenchymal invasion strategies?

**Complexity of force measurements and calculations of invading cells**

In numerous studies, the forces that cells exert in a 3-D environment have been determined using gel contraction assays (Lang et al., 2013; Lautscham et al., 2015). In brief, cells are mixed with collagen prior to gelation onto a disk. The gel disk has free boundaries and shrinks when the cells exert contractile forces (Jonas et al., 2011). From the gel shrinkage, a qualitative estimate of the contractile forces can be

obtained (Steinwachs et al., 2015). Theoretically, a quantitative estimate of the average forces generated by the cells inside the gels can be obtained if the cell number and the viscoelastic gel properties are known and if the spatial cell density distribution and cell orientation is homogenous and isotropic throughout the gel (Lang et al., 2015). In practice, however, these prerequisites are not satisfied. Moreover, cells can remodel their extracellular matrix by compacting the matrix, by secretion of matrix degrading enzymes or by secretion of new matrix proteins. As a consequence, the local and global viscoelastic properties of the gels can significantly change with time. Matrix remodeling, for instance, can be continuously monitored by twisting micrometer-scale ferrimagnetic beads embedded in the gel and measuring the angular bead rotation. However, those measurements do not give the sensitivity and spatial resolution needed for a quantitative estimate of cell forces. To complicate matters, cells modify their biomechanical properties in response to rheological properties such as environmental stiffness, structural properties such as mesh size and fiber orientation, and biochemical properties such as adhesive ligands of the extracellular matrix (Discher at al., 2005).

Finally, different cell types employ different migrating strategies (Fig. 1). It is assumed that the more invasive tumor cells there are, the more adhesion receptors and proteolytic enzymes are expressed; but exceptions from this rule are possible (Rolli et al., 2003). For example, even the same cell can change its migration strategy from a mesenchymal to an amoeboid mode after blocking pericellular proteolysis (Wolf et al., 2003). Hence, no single factor by itself can explain the large differences observed in the invasive behavior between different cancer cell types or between individual cancer cells of the same type (Friedl and Wolf, 2006). Therefore, differences in mechanical properties could be taken as an inherent marker for cancer diagnosis and treatment.

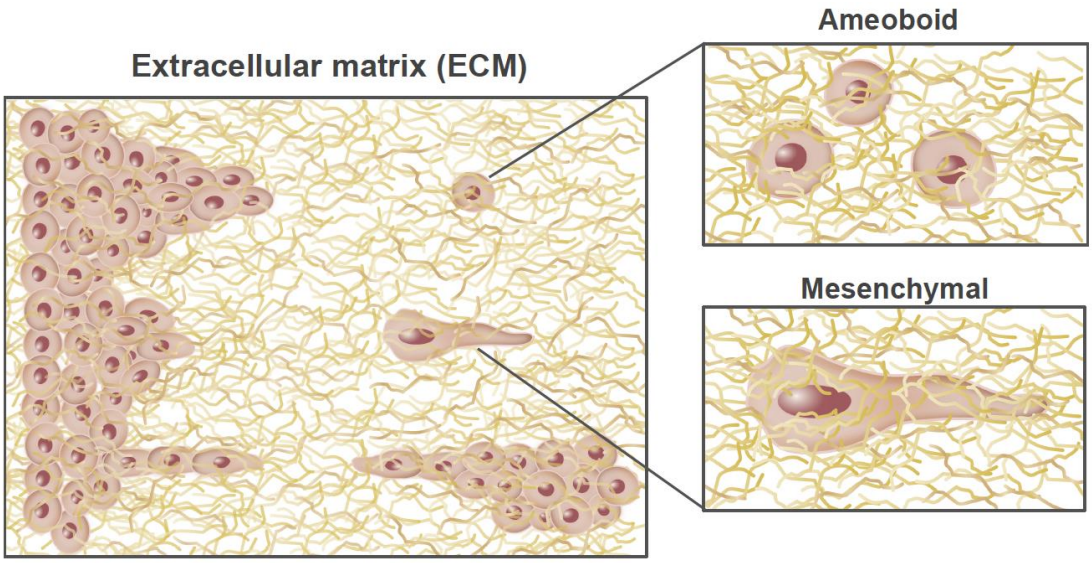


**Figure 1.** 3-D cancer cell invasion: (left) collective invasion, (right, top) single cell amoeboid migration, and (right, bottom) mesenchymal migration. The different phenotypes depend on the tumor type, the state of the stromal ECM, and the cellular composition of the microenvironment. Invasive cells can switch between these phenotypes to migrate efficiently through ECM matrices (taken and modified with permission from *Trends in Cell Biology*).

**Future work**

Cancer cell behaviors such as adhesion, migration, and division were studied under different mechanically induced stimulations, which helped towards understanding metastatic mechanisms (Friedl and Wolf, 2006; Wolf et al., 2013; Lautscham et al., 2015). Quantifying the interplay between cancer cell mechanics and the underlying chemistry could be very advantageous. For instance, observations of the biology of cancer cells and of the role of actin microfilaments, intermediate filaments, and microtubule components influencing cell mechanics, locomotion, differentiation, and neoplastic transformation are most urgent. Further, information on biomechanical parameters such as adhesion/de-adhesion, contractile forces, cellular stiffness, cytoskeletal remodeling dynamics (cellular fluidity/plasticity) and matrix degradation through secreted enzymes, which determine the migration speed and the invasiveness of cancer cells, as well as on the microenvironment such as the composition of the connective tissue are also needed (Wolf and Friedl, 2006; Suresh, 2007; Jonietz, 2012; Lange and Fabry, 2013).

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