Internship project (theme Nanomedicine, January – July 2017)

Regulation of cell deformability during tumor invasion

Background: Tumor cell invasion into tissue is a key step of tumor progression and metastasis. To negotiate space, cell migration through dense ECM requires proteolytic tissue remodeling as well as cell deformation to adjust cell shape to available tissue space (Fig. A, top). As novel, potentially important mechanism of cell-shape regulation, the stiffness of the nucleus is not only regulated by expression of A and B-type lamins, but also by lamin phosphorylation. This project will test whether phosphorylation of nuclear envelope proteins (shown in Fig. A, bottom), i.e. of lamin A/C (at serine 22) or emerin, enhances cell flexibility and invasion into tissue.

In preliminary work we identified lamins A/C and B2 as key regulators of tumor cell migration in a 3D collagen invasion model (Fig. B). Downregulation of lamin A and lamin B2 reduced nuclear stiffness and enhanced migration efficacy, whereas lamin upregulation caused increased stiffness of the nucleus and decreased migration (Fig. C,D). As second option of cell elasticity regulation, this project will test whether dense matrix induces phosphorylation of lamins and related proteins in the nuclear envelope. We hypothesize that phosphorylation-mediated lamin disassembly may help to soften the nucleus and to increase migration. Therefore, the student will characterize lamin phosphorylation in different models outlined below, and inhibit phosphorylation of nuclear envelope proteins to test the consequences on nuclear deformation and migration. Overall, this will help to assess molecular mechanisms of cell deformation during tumor invasion.

Project aims: 1. To establish immunofluorescence (IF) stainings on two tumor cell lines (fibrosarcoma, melanoma) migrating either over glass or within loose collagen (Figs E,F). 2. To detect expression of unphosphorylated and phosphorylated proteins (Fig. D). 3. To detect changes in protein phosphorylation signal in tumor cells migrating in loose versus dense collagen. 4. To quantify local phosphorylation signals at sub-regions of the nucleus by imaging and image analysis (Fig. E). 5. To perform cyclin-dependent kinase inhibitor studies for 1-4. 6. To establish (p)protein stainings in human sarcoma and melanoma histologies (Fig. G).

Techniques: Standard cell culture, three-dimensional cell-collagen tissue culture, western blotting, antibody stainings for immunofluorescence, confocal microscopy, histology, and quantitative image analysis.

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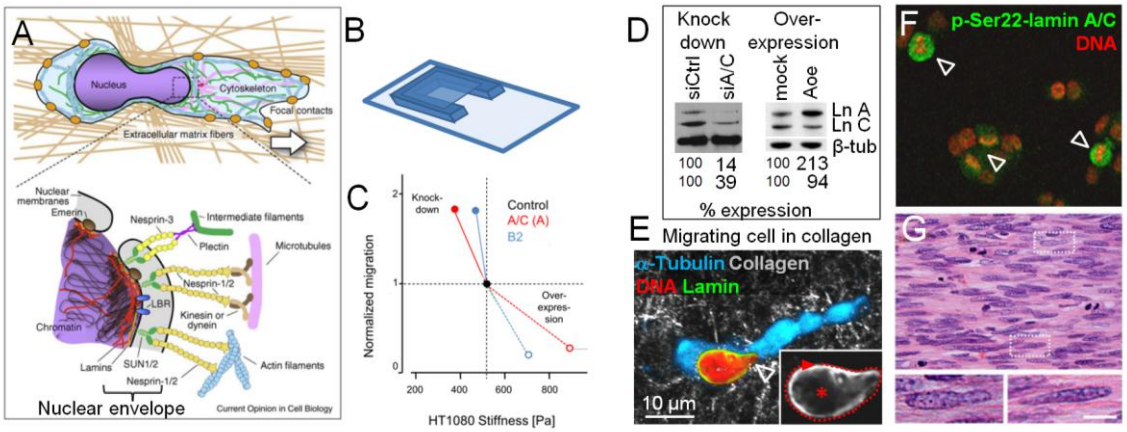

Figure legend. **A**. Top: Cell migrating through 3D matrix of extracellular matrix fibers and encountering a narrow constriction. Bottom: Nuclear envelope-associated proteins contributing to nuclear stiffness and nuclear-cytoskeletal coupling. Lamins underly the inner nuclear membrane (adapted from Friedl, Wolf, Lammerding, *COCB*, 2011). **B**. 3D glass chamber used for investigation of tumor cell migration within 3D collagen lattice. **C**. Interdependence of nuclear stiffness with lamin expression and migration efficacy. **D**. Lamin expression after down-or upregulation of lamin A/C. **E**. Lamin B2 expression of fibrosarcoma cell during migrating in 3D collagen. Inset shows where local lamin expression strength will be quantified: along nuclear rim (dotted curved arrow); area of nuclear interior (*), together with migration-associated nuclear morphology. **F**. Positive p-serine 22 lamin A/C staining on mitotic population (see ∆) of fibrosarcoma cells on glass. **G**. Region of an invasion zone in human fibro-sarcoma exhibiting elongated and partly deformed nuclei (from Friedl, Wolf, Lammerding, *COCB*, 2011).