Physics of cancer: mechanotransduction in the adaptive response of glioblastoma cells to matrix stiffness

Khoonkari, M.

DOI:
10.33612/diss.247434776

CHAPTER 6

6

General discussion and future perspectives

Conclusions and perspectives

The journey of this thesis included (1) the introduction of physical traits of cancer, highlighting brain extracellular matrix (ECM) stiffening phenomena; (2) the development of stiffness tunable hydrogels for investigating the Glioblastoma multiforme (GBM) cells adaptive response in presence of mechanical stress; (3) identification of novel functions for protein kinase R-like endoplasmic reticulum kinase (PERK) in regulating mechanosensing and cellular adaptation of GBM cells to matrix stiffness, and (4) the introduction of a new class of gel-like materials with high potential for fabrication of *in vitro* tissue like models. In this chapter I discuss what questions were answered in the thesis and what are the limits and blind spots of the field. This chapter elaborates more on the importance of understanding the physical traits of cancer and its potential to push the boundaries towards development of new therapeutics. Also biomimetic approaches to reconstruct the brain tissue microenvironment are briefly discussed and suggestions are provided for future development. Moreover, in light of the observations on the role of PERK in mechanotransduction, its potential for future studies is highlighted.

Incorporating physics into cancer: lessons from mechanotransduction under stress

During GBM tumor progression, ECM stiffening phenomena are at play within the tumor microenvironment (TME) which, at later stages, expand to the whole brain tissue [1]. ECM stiffening is the outcome of overexpression of chemical components in the ECM, in particular hyaluronic acid (HA), which gradually alters the structure, microenvironment, mechanics, and physicochemical composition of the ECM [2], [3]. As a result, mechanical stress is generated within the brain tissue, which is known to trigger multiscale reprogramming of cancer cells that also includes glioblastoma cells [4]. Upon sensing mechanical stress, malignant cells adapt to maintain a homeostatic state [5], [6]. Upon adaptation, GBM cells become more invasive and proliferative, which directly drives the tumor progression and malignancy. This is extensively reviewed in chapter 2 [1].

Sensing and adaptation to mechanical stress is associated with a series of complex signaling pathways from the ECM to the cytoskeleton and nucleus, known as mechanotransduction [7], [8]. Mechanosensing begins with the formation of focal adhesion complexes at the cell membrane [9]–[11]. In chapter 4, it is reported that the focal adhesion complex consisting of integrin, talin, vinculin, and tensin plays a key role in mechanosensing in GBM stem cells (GSCs). By mimicking the brain ECM stiffening using *in vitro* models, it was observed in chapter 4 that the expression of the focal adhesion proteins mentioned

above was increased with matrix stiffening, which suggests that high matrix stiffness stimulates the formation of mature focal adhesion complexes. In chapter 3, we followed the adaption of the GSCs to matrix stiffening. It was observed that high matrix stiffness stimulates F-Actin and Filamin-A (FLNA) expression and F-Actin network formation. In addition, it triggers cytoskeleton remodeling, cell elongation, high proliferation and migration [12]. We conclude that with the presence of mature focal adhesion complexes bound to the F-Actin network and cytoskeleton proteins, intense signal transmission from the ECM to the cytoskeleton is at play in a bidirectional manner, which leads to cellular adaptation and reprograms the GSCs towards invasive cells.

The findings in this thesis, together with other findings from literature, suggest that physical traits of cancer play a significant role in GBM rapid progression [4]. This means that to suppress GBM, tumor cells should be desensitized to physical traits of cancer such as matrix stiffness. There are already some approaches and drugs to block sensing the physical cues of the matrix in GBM cells, which are briefly discussed in chapter 2 [1], but in chapter 3 we identified the UPR sensor PERK as a novel target for developing functional therapeutics [12].

PERK: a potential key for the development of functional therapeutics for GBM

In this thesis, direct evidence is provided that PERK strongly mediates the maturation of the focal adhesion complex and mediates the adaptation to stiffness through interaction with FLNA, F-Actin, vimentin, and tubulin. It should be emphasized that these observations were made at low stiffness ranges that mimic the situation of normal brain and GBM, and that at higher matrix stiffness other mechanisms appeared to regulate F-Actin remodeling. One of the aspects that might give PERK such an important role is its ability to mediate F-Actin polymerization through FLNA, which was previously demonstrated [12], [13]. F-Actin, with a dominant presence in the cytoskeleton, plays an important role in numerous cellular signaling pathways and orchestrates crosstalk among many signal transmission mechanisms [14], [15]. It is directly involved in the formation and maturation of the focal adhesion complexes [16], [17], cytoskeleton remodeling mechanisms, cell proliferation, cell migration, cell-ECM, and cell-cell interactions [14], [15], [18]. F-Actin links membrane receptors to cytoskeleton proteins and even connects with lamin-A and YAP in the nucleus of the cell [19]–[21]. Thus, PERK showing such a decisive role in mechanotransduction is perhaps not surprising considering its close relation with F-Actin remodeling through FLNA. PERK itself is well studied in the UPR signaling pathways and ER stress context [12], [22], [23], which adds several more roles

to its function. It is also interesting to investigate the effect of mechanical stress on the endoplasmic reticulum itself, especially to monitor its translocation within the cytosol as the matrix gets stiffer. PERK might also be connected to YAP, where it mediates nuclei deformation.

Our findings, along with other studies in recent years, suggest that PERK could be a target to develop functional therapeutics. PERK deficient GSCs are less sensitive to mechanical stress and this impaired cellular adaptation may overall lead to reduced cellular plasticity. This can open new doors in understanding the role of PERK in cellular plasticity and may stimulate further the development of PERK targeted drugs that should also include drugs that block the scaffold function of PERK. For this, the PERK domains required for FLNA binding should be further determined and examined for their role in mechanotransduction during cell-ECM interactions. Such studies could be conducted using in vitro models where physical traits of ECM matrix could be mimicked for better evaluation of drug/inhibitor efficacy. Currently, two chemical inhibitors GSK414 and AMG-PERK-44 have been tested in mouse models to target PERK kinase activity and promising results have been reported, showing that down regulation of PERK in GBM mouse models resulted in reduced tumor progression and cell invasion suppression. Such drugs/inhibitors could find a way to clinical studies in future where new therapeutics can be developed based on the novel PERK functions.

In vitro tissue-like models: strategies for reconstructing the brain tissue microenvironment

Tissue mechanics matters when studying cancer cells, especially GBM, as GSCs are strongly affected by the physical traits of cancer [24]–[26]. It is essential to investigate GSCs cell and cell-ECM interaction within an environment recapitulating the brain tissue mechanics and physicochemical composition. In recent years, hydrogels, 3D polymeric networks with high water contents, have been widely used as scaffolds for cell culture [27], [28]. Since hydrogels are easily tunable in mechanical properties and highly biocompatible, they gradually developed into complex tissue-like *in vitro* models to study the adaptive cellular response to different conditions. In this thesis, we introduced two different gels, one based on human blood plasma and alginate (chapter 3) and the other a product of complex coacervation between hyaluronic acid and chitosan (chapter 5). These gels showed high biocompatibility coupled with easily tunable mechanical properties. The gels introduced in chapters 3 and 5 provide many positive features, but still are not fully transparent and degrade too fast for long-term cell culture. Yet, there is a great potential for improvements of these two systems with optimizing the formulation and improving

the crosslinking and gel fabrication methods. For example, the fibronectin content of the human blood plasma-alginate gel could be tuned to prevent fast degradation of the gel, the concentration of the calcium chloride used for crosslinking could be optimized for more homogeneous crosslinking and finally the bilirubin within the blood plasma could be filtered to fabricate a transparent hydrogel. Also, the complex coacervate gel introduced in chapter 5, could be improved by using other types of natural or synthetic polymers to yield higher coacervation efficiently.

Nevertheless, there is still lots of room for improvement before a good mimic of the brain tissue structure and composition is obtained. To successfully mimic the brain tissue, key ECM components such as hyaluronic acid (HA), proteoglycans, collagen, fibronectin, and laminin should be present followed by an efficient crosslinking method to tune the mechanical properties of the matrix [29], [30]. However, such a matrix is difficult to make, as the interactions of these materials with each other are not fully understood and crosslinking and finetuning the mechanical properties will be a great challenge. Recently, several types of gels have been developed based on decellularized brain tissue to ensure a base material resected from the native environment [31]–[33]. In this case, there are significant challenges as well. The crosslinking of such material is complex, and it is reported that the gel has poor stability as it degrades fast. Also, such gels and many other types of gels are not fully transparent, making microscopy imaging a big challenge. The porosity of the gel is also very important for active nutrient supply to the cells through the culture medium and preventing the formation of hypoxic areas.

Moving beyond the boundaries: physics of cancer on a chip

In this thesis, GSCs cell-ECM interaction was only studied with a focus on matrix stiffness. During GBM tumor progression, all physical traits of cancer stand united to actively reprogram the GBM tumor and tumor cells adaptive responses [4]. Thus, to better understand the role of physical traits of cancer on GBM tumor progression and associative cellular signaling pathways, it is crucial to combine physical traits of cancer within *in vitro* models. Especially in recent years there is a growing interest on the effect of compression on GSCs to understand how GSCs adapt to compression when navigating through confined spaces within the brain ECM [34]–[36]. Such a question asks for development of a platform where tissue stiffness is combined with compression. Due to the current limitations in hydrogel systems, organ-on-chip (OOC) technology has emerged, offering the possibility to combine different physical cues of tissues. OOC is a chip fabricated by photolithography or 3D printing using polycarbonate (PC), polydimethylsiloxane

(PDMS), or other suitable types of plastics [37], [38]. The chip enables the introduction of active flow of the culture medium, which resembles the shear flow. OOCs seems to be an excellent match to mimic all physical traits of cancer, at once, united within a chip. For example, special brain-on-chip models could be developed with an array of micro-pillars or micro-beads in the chip, to generate confined spaces where cells have to push through. The channels can be filled with hydrogels to mimic both the tissue stiffness and chemical composition. Flow of cell culture medium through the channels can resemble the shear flow. Incorporating compression or confined spaces is crucial when studying physical traits of cancer and especially adaptive GBM cellular signaling. The effect of compression on cytoskeleton remodeling and adaptation to the matrix physical/mechanical traits is as important as stiffness and it might even play a bolder role in reprogramming the GBM cell feedback response and tumor progression [35], [39], [40]. For future developments and to better study the physical traits of cancer, brain-on-chip models would provide the best opportunity as stiffness, compression, architecture, and fluid pressure within the brain all at once could be mimicked within a chip coupled with performing cell culture underflow. Such an environment offers a close mimic to the actual condition and could open a new door to study the adaptive response of GSCs.

References

[1] M. Khoonkari, D. Liang, M. Kamperman, F. A. E. Kruyt, and P. Van Rijn, "Physics of Brain Cancer : Multiscale Alterations of Glioblastoma Cells under Extracellular Matrix Stiffening," 2022.

[2] K. J. Wolf, J. Chen, J. D. Coombes, M. K. Aghi, and S. Kumar, "Dissecting and rebuilding the glioblastoma microenvironment with engineered materials," *Nat. Rev. Mater.*, vol. 4, no. 10, pp. 651–668, 2019, doi: 10.1038/s41578-019-0135-y.

[3] J. W. E. Chen, S. Pedron, P. Shyu, Y. Hu, J. N. Sarkaria, and B. A. C. Harley, "Influence of hyaluronic acid transitions in tumor microenvironment on glioblastoma malignancy and invasive behavior," *Front. Mater.*, vol. 5, no. June, pp. 1–11, 2018, doi: 10.3389/fmats.2018.00039.

[4] H. T. Nia, L. L. Munn, and R. K. Jain, "Physical traits of cancer," *Science (80-.).*, vol. 370, no. 6516, 2020, doi: 10.1126/SCIENCE.AAZ0868.

[5] H. Huang, R. D. Kamm, and R. T. Lee, "Cell mechanics and mechanotransduction: Pathways, probes, and physiology," *Am. J. Physiol. - Cell Physiol.*, vol. 287, no. 1 56-1, pp. 1–11, 2004, doi: 10.1152/ajpcell.00559.2003.

[6] N. Belaadi, J. Aureille, and C. Guilluy, "Under Pressure: Mechanical Stress Management in the Nucleus," *Cells*, vol. 5, no. 2, p. 27, 2016, doi: 10.3390/cells5020027.

[7] X. Li and J. Wang, "Mechanical tumor microenvironment and transduction : cytoskeleton mediates cancer cell invasion and metastasis," vol. 16, 2020, doi: 10.7150/ijbs.44943.

[8] F. Martino, A. R. Perestrelo, V. Vinarský, S. Pagliari, and G. Forte, "Cellular mechanotransduction: From tension to function," *Front. Physiol.*, vol. 9, no. JUL, pp. 1–21, 2018, doi: 10.3389/fphys.2018.00824.

[9] D. W. Dumbauld *et al.*, "How vinculin regulates force transmission," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 110, no. 24, pp. 9788–9793, 2013, doi: 10.1073/pnas.1216209110.

[10] S. Chakraborty, S. Banerjee, M. Raina, and S. Haldar, "Force-Directed 'mechanointeractome' of Talin-Integrin," *Biochemistry*, vol. 58, no. 47, pp. 4677–4695, 2019, doi: 10.1021/acs.biochem.9b00442.

[11] A. Zuidema, W. Wang, and A. Sonnenberg, "Crosstalk between Cell Adhesion Complexes in Regulation of Mechanotransduction," *BioEssays*, vol. 42, no. 11, pp. 1–11, 2020, doi: 10.1002/bies.202000119.

[12] D. Liang, M. Khoonkari, T. Avril, E. Chevet, and F. A. E. Kruyt, "The unfolded protein response as regulator of cancer stemness and differentiation: Mechanisms and implications for cancer therapy," *Biochem. Pharmacol.*, vol. 192, no. August, p. 114737, 2021, doi: 10.1016/j.bcp.2021.114737.

[13] A. R. van Vliet *et al.*, "The ER Stress Sensor PERK Coordinates ER-Plasma Membrane Contact Site Formation through Interaction with Filamin-A and F-Actin Remodeling," *Mol. Cell*, vol. 65, no. 5, pp. 885-899.e6, 2017, doi: 10.1016/j.molcel.2017.01.020.

[14] Q. Zhang, Y. Yu, and H. Zhao, "The effect of matrix stiffness on biomechanical properties of chondrocytes," *Acta Biochim. Biophys. Sin. (Shanghai).*, vol. 48, no. 10, pp. 958–965, 2016, doi: 10.1093/abbs/gmw087.

[15] J. Stricker, T. Falzone, and M. L. Gardel, "Mechanics of the F-actin cytoskeleton," *J. Biomech.*, vol. 43, no. 1, pp. 9–14, 2010, doi: 10.1016/j.jbiomech.2009.09.003.

[16] A. F. Pegoraro, P. Janmey, and D. A. Weitz, "Mechanical properties of the cytoskeleton and cells," *Cold Spring Harb. Perspect. Biol.*, vol. 9, no. 11, 2017, doi: 10.1101/cshperspect.a022038.

[17] M. L. Gardel, I. C. Schneider, Y. Aratyn-Schaus, and C. M. Waterman, "Mechanical integration of actin and adhesion dynamics in cell migration," *Annu. Rev. Cell Dev. Biol.*, vol. 26, pp. 315–333, 2010, doi: 10.1146/annurev.cellbio.011209.122036.

[18] K. Bhadriraju and L. K. Hansen, "Extracellular matrix- and cytoskeleton-dependent changes in cell shape and stiffness," *Exp. Cell Res.*, vol. 278, no. 1, pp. 92–100, 2002, doi: 10.1006/excr.2002.5557.

[19] R. Abylkassov and Y. Xie, "Role of yes-associated protein in cancer: An update (Review)," *Oncol. Lett.*, vol. 12, no. 4, pp. 2277–2282, 2016, doi: 10.3892/ol.2016.4955.

[20] N. Koushki *et al.*, "Lamin A redistribution mediated by nuclear deformation determines dynamic localization of YAP," pp. 1–32, 2020, doi: 10.1101/2020.03.19.998708.

[21] S. Dupont *et al.*, "Role of YAP/TAZ in mechanotransduction," *Nature*, vol. 474, no. 7350, pp. 179–184, 2011, doi: 10.1038/nature10137.

[22] N. M. Peñaranda-Fajardo *et al.*, "ER stress and UPR activation in glioblastoma: identification of a noncanonical PERK mechanism regulating GBM stem cells through SOX2 modulation," *Cell Death Dis.*, vol. 10, no. 10, pp. 1–16, 2019, doi: 10.1038/s41419-019-1934-1.

[23] N. M. Peñaranda Fajardo, C. Meijer, and F. A. E. Kruyt, "The endoplasmic reticulum stress/unfolded protein response in gliomagenesis, tumor progression and as a therapeutic target in glioblastoma," *Biochem. Pharmacol.*, vol. 118, pp. 1–8, 2016, doi: 10.1016/j.bcp.2016.04.008.

[24] V. Umesh, A. D. Rape, T. A. Ulrich, and S. Kumar, "Microenvironmental stiffness enhances glioma cell proliferation by stimulating epidermal growth factor receptor signaling," *PLoS One*, vol. 9, no. 7, pp. 1–8, 2014, doi: 10.1371/journal.pone.0101771.

[25] W. L. K. Chen and C. A. Simmons, "Lessons from (patho)physiological tissue stiffness and their implications for drug screening, drug delivery and regenerative medicine," *Adv. Drug Deliv. Rev.*, vol. 63, no. 4, pp. 269–276, 2011, doi: 10.1016/j.addr.2011.01.004.

[26] W. Xi, T. B. Saw, D. Delacour, C. T. Lim, and B. Ladoux, "Material approaches to active tissue mechanics," *Nat. Rev. Mater.*, vol. 4, no. 1, pp. 23–44, 2019, doi: 10.1038/s41578-018-0066-z.

[27] S. Jekhmane *et al.*, "Design Parameters of Tissue-Engineering Scaffolds at the Atomic Scale," *Angew. Chemie - Int. Ed.*, vol. 58, no. 47, pp. 16943–16951, 2019, doi: 10.1002/anie.201907880.

[28] E. Caló and V. V. Khutoryanskiy, "Biomedical applications of hydrogels: A review of patents and commercial products," *Eur. Polym. J.*, vol. 65, pp. 252–267, 2015, doi: 10.1016/j.eurpolymj.2014.11.024.

[29] J. Cha and P. Kim, "Biomimetic strategies for the glioblastoma microenvironment," *Front. Mater.*, vol. 4, no. December, pp. 1–8, 2017, doi: 10.3389/fmats.2017.00045.

[30] A. Belousov *et al.*, "The Extracellular Matrix and Biocompatible Materials in Glioblastoma Treatment," *Front. Bioeng. Biotechnol.*, vol. 7, no. November, 2019, doi: 10.3389/fbioe.2019.00341.

[31] D. Lam *et al.*, "Tissue-specific extracellular matrix accelerates the formation of neural networks and communities in a neuron-glia co-culture on a multi-electrode array," *Sci. Rep.*, vol. 9, no. 1, pp. 1–15, 2019, doi: 10.1038/s41598-019-40128-1.

[32] K. P. Robb, A. Shridhar, and L. E. Flynn, "Decellularized Matrices As Cell-Instructive Scaffolds to Guide Tissue-Specific Regeneration," *ACS Biomater. Sci. Eng.*, vol. 4, no. 11, pp. 3627–3643, 2018, doi: 10.1021/acsbiomaterials.7b00619.

[33] L. P. Ferreira, V. M. Gaspar, and J. F. Mano, "Decellularized Extracellular Matrix for Bioengineering Physiomimetic 3D in Vitro Tumor Models," *Trends Biotechnol.*, vol. 38, no. 12, pp. 1397–1414, 2020, doi: 10.1016/j.tibtech.2020.04.006.

[34] C. Rianna, M. Radmacher, and S. Kumar, "Direct evidence that tumor cells soften when navigating confined spaces," *Mol. Biol. Cell*, vol. 31, no. 16, pp. 1726–1734, 2020, doi: 10.1091/mbc.E19-10-0588.

[35] G. Seano *et al.*, "Solid stress in brain tumours causes neuronal loss and neurological dysfunction and can be reversed by lithium," *Nat. Biomed. Eng.*, vol. 3, no. 3, pp. 230–245, 2019, doi: 10.1038/s41551-018-0334-7.

[36] H. T. Nia *et al.*, "In vivo compression and imaging in mouse brain to measure the effects of solid stress," *Nat. Protoc.*, vol. 15, no. 8, pp. 2321–2340, 2020, doi: 10.1038/s41596-020-0328-2.

[37] P. Nikolakopoulou, R. Rauti, D. Voulgaris, I. Shlomy, B. M. Maoz, and A. Herland, "Recent progress in translational engineered in vitro models of the central nervous system," *Brain*, 2020, doi: 10.1093/brain/awaa268.

[38] D. E. Ingber, "Human organs-on-chips for disease modelling, drug development and personalized medicine," *Nat. Rev. Genet.*, vol. 0123456789, 2022, doi: 10.1038/s41576-022-00466-9.

[39] H. T. Nia *et al.*, "Solid stress and elastic energy as measures of tumour mechanopathology," *Nat. Biomed. Eng.*, vol. 1, no. 1, pp. 1–11, 2017, doi: 10.1038/s41551-016-0004.

[40] K. Pogoda *et al.*, "Compression stiffening of brain and its effect on mechanosensing by glioma cells," *New J. Phys.*, vol. 16, pp. 1–17, 2014, doi: 10.1088/1367-2630/16/7/075002.