2 **Biophysical properties of cells for cancer diagnosis**

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Abstract: Biophysical properties associated with the microenvironment of a tumour has been recognised as an important modulator for cell behaviour and function. Particularly, tissue rigidity is important during tumour carcinogenesis as it affects the tumour's ability to metastasis. Multiple downstream pathways are affected with a difference in rigidity of the extra cellular matrix. The insight into tumour mechanosignalling represents a promising field that may lead to novel approaches for cancer diagnostics. Measurement of rigidity of the extracellular matrix or the tissue is a potential diagnostics approach for cancer detection. Altered extracellular matrix states persist for a long period of time and have lower heterogeneity compared to protein or genetic markers, therefore are more reliable as biomarkers. On the other hand, measurement of different kinase associated proteins or transcripts provide an early insight into potential transition of cells towards metastasis. Co-localization of transcriptional factors like YAP/TAZ provide an insight to determine if the cells is undergoing metastatic changes. This review explains the unique biophysical properties of the tumour microenvironment that present the potential targets for the diagnosis of cancer.

Keywords: mechanotransduction, mechanical stress, cancer diagnosis, cancer cell, cancer stem cells, YAP/TAZ

30 1. Introduction

31 Cancer is the second leading cause of mortality in the world and is expected to overtake cardiovascular
32 diseases soon. Due to difficulties with early clinical and histological diagnosis, and the lack of suitable
33 molecular markers for accurate treatments, the death rate from cancer has been increasing (Ferlay J, 2013).
34 Only few patients die directly from the primary tumours. However, the cancer becomes very difficult to
35 manage once it has metastasised. About 90% of metastatic cancers are terminal. Metastasis is caused by the
36 migration of cancer cells into a new tissue. The process may occur either through nearby tissues through
37 extracellular matrix (ECM) or far via blood or lymph to distant tissues (Fidler, 1975) like, bones, brain, liver,
38 and lungs (Martin et al., 2000-2013). The secondary tumour in the new location is invariably different in both
39 molecular and genetic structure in comparison to the primary tumour source. The metastatic cells are also
40 known as circulating tumour cells (CTCs). CTCs hold information about the tumour type, its evolution and
41 heterogeneity and hence can serve as potential diagnostic markers (Krebs et al., 2016). On the other hand, cells
42 migrating through neighbouring tissues provide critical information on mechanobiology. During the
43 migration process, these cells change the morphology and exert pressure to the surrounding cells. The stiffness
44 of the cells and the tumour's microenvironment can regulate and potentiate its invasiveness (Kurniawan et
45 al., 2016; Quail and Joyce, 2013).

46 Over the last few decades, cancer research has placed a significant focus on extracellular and intracellular
47 biochemical signals in proliferation, invasion, and metastasis of cancer cells. In recent years, tumour mechanics
48 and especially tissue rigidity has been considered as a crucial aspect of tumour progression and metastasis
49 (Chaudhuri et al., 2018; Jaalouk and Lammerding, 2009; Wei and Yang, 2016; Yousafzai et al., 2017). To
50 measure the stiffness of the cancer cell, Young's modulus gives the quantitative measure of elastic properties
51 and shows the positive correlation between cell deformability and cell malignancy (Seyedpour et al., 2015; Sun
52 et al., 2016; Xu et al., 2012). Recent study by Zhou et al had reported that the nanoindentation measures
53 stiffness could be biomarker for cancer (Zhou et al., 2017). Tumours are often stiffer than neighbouring tissue.
54 In soft tissues such as breast and abdomen, the stiffness of tumours is the base of clinical diagnosis and also
55 act as a foundation for detection with imaging techniques such as MRI (Fass, 2008). The tissue of breast cancer
56 tumour can be ten times stiffer than the normal breast tissue, and increased breast density is seen in 30% of
57 breast tumors (Lu et al., 2012). The stiffness-sensing capability of cancer and stromal cells influence cell
58 survival and proliferation and provide new insights, so that researchers are able to develop diagnostic
59 methods for cancer formation (Zhang et al., 2018). These diagnostic techniques have the advantage of
60 explaining both mechanical and molecular mechanisms.

61 Cells sense the changes in their surrounding and transduce physical to biochemical signals, which helps
62 to control the function, biochemical changes and gene expression (Chin et al., 2016). The physical changes in
63 tumour tissue correlate with the biological process inside the cancerous cells (Bao and Suresh, 2003). Increasing

cell stiffness activates the integrins, which are the transmembrane proteins serving as adhesive in ECM. The activated integrins trigger a biological chain of reactions that decrease the cell contractibility (Aoudjit and Vuori, 2012; Desgrosellier and Cheresch, 2010). This process in turn provides a feedback mechanism for increasing ECM stiffness. The autocrine loop of ECM stiffness and integrin activation provides an interaction between the mechanical force on the cell and its biological processes (Huang and Ingber, 2005; Ross et al., 2013).

Cellular mechanotransduction is a process that converts mechanical signals into biochemically relevant information through a series of cellular processes (Alonso and Goldmann, 2016). The mechanical properties of the cell environment affect the cell differentiation, growth, locomotion and development (Engler et al., 2006; Rajagopalan and Saif, 2011; Yeung et al., 2005). Cells sense and exert forces on their environment, which comprise of the ECM and the basement membranes, as well as other neighbouring cells. Several generated forces are concentrated at the adhesion points between cells and the ECM, or at homotypic cell–cell adhesion. Thus, the conversion of physical stimuli into intracellular biochemical signalling or mechanotransduction should occur within the multi-protein complexes at these adhesion sites (J.H.-C.Wang and Thampatty, 2006). The possible mechanosensors include integrins, mechanosensitive ion channels, and stretch sensitive focal adhesions proteins.

The difference in the cellular mechanical properties provides an indicator of the existence and state of the disease. Changes in cancer cell from the point of initiation of a tumour to its spread to other parts of the body are observed as promising biomarker for detection of metastatic potential of these cancer cells. Also, altered ECM condition such as increased stiffness persist for a long period of time and have lower heterogeneity compared to protein or genetic markers, therefore being more reliable biomarkers. Current available technologies that can detect the cancer and metastasis based on biophysical cues are magnetic resonance elastography, ultrasound elastography, cell based sensors and atomic force microscopy (Carlsen et al., 2015; Darling et al., 2007; Pepin et al., 2015) . Although the stiffness of tissue is utilized as a basis of differentiation for tools like MRI, there are no evidences that the biochemical signals during the biophysical process being utilized for diagnostics.

This review aims to provide insights into the role of mechanical stress in cancer and the interplay between mechanical and biological processes in tumour environment as well as mechanobiology signalling. The paper also aims to provide a better insight into the biomechanical changes that occur between a healthy cell and a cancer cell. Finally, a perspective is given for the potential use of cues from biophysical processes as diagnostic markers.

2. Cellular biology background

2.1 Extracellular matrix (ECM) and Integrins

97 Tissues and organs are formed by cells embedded in an extracellular matrix. Cells within a tissue establish
98 the ECM, maintain its health, alter it during adaptations and also repair it in response to disease and wound
99 (Humphrey et al., 2014). ECM not only contributes to binding cells together, but also provides the physical
100 scaffold for their growth, influencing their migration, differentiation, survival, homeostasis and
101 morphogenesis (Bonnans et al., 2014). Different types of proteins and polysaccharides that are locally secreted
102 form the matrix by organizing into a meshwork within the cell surface. The ECM consists of polysaccharide
103 chains of repeating disaccharide units termed of glycosaminoglycans (GAGs). These are generally found to be
104 linked to proteoglycans by covalent bonding and structural and specialized proteins such as collagen,
105 fibrillins, elastin, fibronectin, and laminin (Mouw et al., 2014). Cells reacts to the mechanical and biochemical
106 changes in ECM through the crosstalk between integrins and the actin cytoskeleton, to form the ECM
107 architecture (Kim et al., 2011).

108 The close connection between cells and their supporting matrix is operated by the cell-matrix receptors.
109 The cells organize the matrix and the matrix in turn influence the cell fate through the exchange of mechanical
110 signals from the matrix to the cytoskeleton and vice versa. The interacting point between cells and their ECM
111 is called the focal adhesion point. Cell sense their environment using specific receptors and respond to
112 mechanical cues and induce biochemical signals from the ECM. This sensing process is a combination of
113 mechanical and chemical systems like adhesion via integrins, focal points, molecular motors and the
114 cytoskeletal network. Therefore, when a disease such as cancer occurs, the ECM is strongly involved (Clause
115 and Barker, 2013; Ingber, 2008; Kumar and Weaver, 2009; Rozario and DeSimone, 2010).

116 Integrins are the family of adhesion proteins that regulates adhesion, progression and invasion of cancer
117 cells. Integrins bind directly to the focal adhesion points in ECM providing the traction for motility and
118 invasion of cancer (Figure 2). Integrin bridges two neighbouring cells and triggers intracellular signalling. Two
119 common receptors activated by integrin are the cytokine receptors and the growth factor receptors. The
120 communication between the growth factor receptors and the integrin leads to the hypothesis that the crosstalk
121 between these receptors is necessary for tumour growth and invasion. They are also involved on tumour cell
122 invasion by regulating the localisation and function of matrix- degrading proteases, for example,
123 metalloprotease 2 and urokinase- type plasminogen activator (Ata and Antonescu, 2017; Eliceiri, 2001).

124 After the binding of ligands, integrins form clusters on the cell surface at focal adhesion points and serve
125 as the origin for signal transduction from the ECM to intracellular signalling pathways. Although integrins do
126 not possess any kinase activity, they are capable of recruiting various kinases, such as focal adhesion kinases
127 (FAKs), integrin linked kinase (ILK) and Src family kinases (SFKs). These proteins play an active role in
128 processes such as migration of cells, activation of Rho-GTPases, cross talk of integrins with growth factor and
129 overturning the cellular adhesion (Srichai and Zent, 2010; Wozniak et al., 2004). The Rho GTPase contribute
130 to many steps in cancer progression including invasion, proliferation, dodging apoptosis, and metastasis. In

131 cell mechanics, Rho stimulate cellular contractility by activating Rho-associated kinase (ROCK) (Parri and
132 Chiarugi, 2010; Totsukawa et al., 2000).

133 Rho regulates the secretion of soluble matrix metalloproteinases (MMPs) like lysophosphatidic acid and
134 transforms growth factor beta (TGF- β) in the tumour cells, which in turn facilitate matrix remodelling (Kumar
135 and Weaver, 2009; Parri and Chiarugi, 2010). A typical example is the formation of cancer-associated
136 fibroblasts (CAFs) where secreted MMPs promotes remodelling of stromal fibroblast. The isometric tension
137 exerted on these fibroblasts enhances matrix stiffening, that triggers the formation of stress fibre and integrin
138 mediated activation of SFK at focal points. The increase of Src signalling then promotes the nuclear
139 accumulation of the Yes-associated protein YAP and the transcriptional co-activator TAZ, which in turn
140 increases expression of actin modulating proteins and stabilizing actinomyosin proteins. This “continuous
141 positive feedback loop” promotes ECM stiffening that in turn facilitates the conversion of stromal fibroblast
142 to cancer associated fibroblasts (Calvo et al., 2013; Katsumi et al., 2004; Ross et al., 2013).

143 *2.2 Cell stiffness in cancer*

144 The spread of cells is determined by the change in the stiffness of the cell. Cellular stiffness as biomarker of
145 relative metastatic potential has been demonstrated in ovarian cancer (Xu et al., 2012), breast cancer (Parikh et
146 al., 2018) and prostate cancer (Reid et al., 2017). When a cell transforms from a normal mature cell to an
147 immortal cancer cell, the cytoskeleton transforms into more irregular state from a rigid structure. The changes
148 on the cytoskeleton are evident as malignant cells replicate more and are very motile; which is usually
149 inhibited by a rigid cytoskeleton. Considering this fact, the changes in the cytoskeleton would generally reflect
150 the overall mechanical properties of the cell. Thus, measuring the cellular rigidity provides information to
151 differentiate as a normal or a cancerous cell, and is potentially a new biological marker. Differential sorting of
152 these cells has also demonstrated the response to therapy where stiff cells are more sensitive to chemotherapy
153 compared to softer cells (Islam et al., 2018).

154 Multiple techniques have been used to probe the mechanical properties of cancer cells. Most cell stretching
155 approaches include the use of laser/ optical tweezers, mechanical microplate stretcher, microfluidic platform
156 and micropipette aspiration (Bao and Suresh, 2003; Suresh, 2007). A microfluidic cell stretching platform was
157 used for studying the effects of exerted stress on prostatic normal tissue-associated fibroblasts, where the
158 authors noted that the mechanical force leads to the alignment of fibronectin secreted from stretched normal
159 tissue-associated fibroblasts (Berrueta et al., 2018). Furthermore, Swaminathan et al. used a magnetic tweezers
160 to demonstrate an inverse power-law relationship between cancer cell invasion and cell stiffness correlation
161 between mechanical properties of cancer cells and their metastatic potential. As cancer cells become more
162 invasive, they show softer mechanical characteristics which cause cell deformation and shape changes suitable
163 for a metastatic population (Swaminathan et al., 2011). Zhang et al. have reported a novel method to measure

the mechanical properties of cancer cells, which could be one of the criteria that help for determining whether the tissue contains lesions at the single cell level (Zhang et al., 2016). This technique could potentially be important for early prevention and accurate diagnosis of diseases.

2.3 Hippo pathway and role of YAP/TAZ in cancer

The Hippo signal pathway (Figure 2) has been identified for the first time in *Drosophila melanogaster*, controlling the size of organ through mechanisms like cell proliferation and apoptosis (Gateff, 1978; Xu et al., 1995). This signal pathway plays a vital role in the behaviour of stem cells; controlling the growth, the suppression and the regeneration of organ, and of potentially carcinogenesis (Gaspar and Tapon, 2014). The core of the mammalian hippo pathway consists of a kinase cascade, transcription coactivators, and their DNA-binding partners. The two kinases, namely mammalian Ste20-like kinases (MST) and large tumour suppressor kinase (LATS), control the activity of these two closely related transcriptional co-activators, the YAP and TAZ through phosphorylation at a specific site, which is Serine residue in the amino-terminal region of YAP-TAZ.

The transcriptional co-activators YAP/TAZ are responsible for biomechanical answer to cell shape and ECM elasticity (Totaro et al., 2018). YAP and TAZ nuclear localization are regulated by mechanical cues that include cell-cell adhesion, and cell mechanosensing of ECM physical properties or stretching. The function of YAP/TAZ is necessary for the regulation of cell proliferation, differentiation, stem cell function and organ size. Current interest in molecular biology of YAP/TAZ has gained momentum through the realization that YAP/TAZ is highly active within human tumours (A. Warren et al., 2018). Some mouse models have indicated that the inactivation of YAP/TAZ in adult organs such as breast (Chen et al., 2014), intestine (Cai et al., 2010) and pancreas (Zhang et al., 2014) increase the emergence or progression of tumours (Azzolin et al., 2014; Harvey et al., 2013; Zanconato et al., 2016a).

3. Cellular Mechanotransduction

Direct interactions between cells themselves, and cells and the ECM, are critical to the development and function of multicellular organisms (Khalili and Ahmad, 2015). The key cell surface receptors involved during the attachment of cells to the ECM are integrins. Integrins are the main receptors connecting the cytoskeleton to ECM have a close relation to external force and transmit generated mechanical stresses across the plasma membrane. Apart from providing the structural stability, integrins also serve as receptors for intercellular signalling pathways. Many actions like cell adhesion, gene expression and response to triggers are mediated by integrins. (Geiger and Yamada, 2011). As mentioned earlier, the nonreceptor protein- tyrosine kinase called FAK plays an important role in integrin signalling. Along with FAK, nonreceptor protein-tyrosine kinases are also involved in signaling via integrin and focal adhesion points. (Matsui et al., 2012). They simultaneously

196 act for signalling via integrin receptors (Jansen et al., 2015). As integrins also regulate the signalling pathways,
197 they are capable of signal transduction from physical signals to chemical signals (Katsumi et al., 2004).
198 Invasion by tumour cells is mediated by integrins and ECM attachment (Ganguly et al., 2013).

199 Integrins are the primary receptors connecting the cytoskeleton with the ECM, making them the bridge
200 to transmit forces and mechanical stresses across the plasma membrane (DuFort et al., 2011). As integrins also
201 regulate signalling pathways, they are positioned to transduce physical forces into chemical signals. Integrin
202 mediated tumour invasion is regulated by localization and activity of matrix-degrading proteases, such as
203 matrix metalloprotease 2 (MMP2) and urokinase-type plasminogen activator (uPA). Integrin-mediated
204 migration generally requires FAK and SFK signalling. Mechanical stimuli can open ion channels, alter proteins
205 binding in focal adhesion and cause changes in cell morphology. Unfolding or stretching of molecules and the
206 opening of ion channels under mechanical forces transduce a signal to downstream-signalling pathways.
207 Some studies demonstrate a direct link between Rho/ Rock and TRP mediated signalling that illustrate that
208 cross talk exists between mechanical signalling via integrins and mechanosensitive ion channels.

209

210 3.1 Mechanotransduction in cancer

211 Mechanotransduction of physical signals to initiate intracellular signalling pathways are recognized in
212 numerous cancer types through a broad range of mechanisms for example, opening of ion channels, gene
213 modulation and protein production. Glentis *et al.* emphasized the central role that mechanosensing plays
214 throughout the tumour metastasis (Glentis et al., 2017). Cancer associated fibroblasts (CAFs) are the
215 furthestmost abundant stromal cells surrounding the tumour and play a role in tumour formation, progression
216 and metastasis (Labernadie et al., 2017). Calvo *et al.* showed that YAP function is critical for the establishment
217 and maintenance of CAFs. YAP is important for many pro-tumorigenic functions of CAFs, including matrix
218 stiffening, and invasion. (Calvo et al., 2013). Glentis *et al.* also demonstrated that CAFs isolated from colon
219 cancer patients promote cancer cell invasion by interacting physically and remodelling basement membrane
220 using mechanical forces such as pulling, stretching and softening the membrane (Glentis et al., 2017).

221 Cancer cells express mechanoreceptors such as integrins for increased sensitivity of ECM stiffness. Usual
222 proteins in the adhesion complex activate the Rho GTPase for cytoskeletal reorganization. This process on the
223 other hand leads to triggering YAP/TAZ for production of cancer stem cells. The various steps proposed for
224 the production of these CSCs are proliferation, metastasis, survival, and additional stiffness of the ECM.
225 Another proposed mechanism is the involvement of CAFs, which directly transmit mechanical tension to
226 cancer cells through N- and E-cadherin heterophilic junctions for promoting invasion. Increased stiffness in
227 the ECM activates YAP inside the CAFs, which then promote the stiffness of the ECM on a continuous

autocrine feedback loop. The third proposed mechanism is the process, where mechanosensitive molecules like Polycystins 1(PC1) and 2 (PC2) activate the mTOR pathway and promote proliferation, EMT, and metastasis. In fact, PC1-CTT (carboxy-terminal tail) translocates along with TAZ to the nucleus (Gargalionis et al., 2018b; Gargalionis et al., 2015; Gargalionis et al., 2016). The above three mechanisms indicate the clear link between mechanical signals and formation of drug resistant cancer stem cells, which require further studies.

During cell differentiation, a subpopulation of cells differentiates into CSCs primarily due to oncogenic hit or initiation of oncogenesis (Figure 3). CSC are created from normal stem cells or precursor cells within the tissues after mutations take place and are usually resistant to conventional treatments (Papaccio et al., 2017). It is hypothesised that these CSCs replicate indefinitely (Hao et al., 2013), initiate tumour formation and are resistant to chemotherapy (Zanconato et al., 2016b). Recent studies have shown that the mechanical properties of ECM and cell rigidity affect normal stem cell and CSC fate (Bissell and LaBarge, 2005; Gilbert et al., 2010). Stem cells including CSCs follow FAK/ phosphatidylinositol-3 kinase (PI3K)/Akt (also known as protein kinase B) pathway for cell signalling. The stem cells use contractile forces, that result in tensile stresses in the cytoskeleton. Downstream signalling via the FAK/PI3K/Akt pathway then enhances cytoskeletal reorganization in CSCs, and affects the cell shape and focal point interactions (Kallergi et al., 2007).

The involvement of FAK/PI3K/Akt pathway can be selective to certain types of cancer. For example, chemokine receptor 1 (CXCR1), which is one of the key upstream mediators of FAK/PI3K/Akt, can be selectively targeted in vitro (Ginestier et al., 2010) by phosphorylation of the FAK. This selectivity results in activation of PI3K/Akt, which plays an vital role in mechanotransduction (J.J.Waugh and Wilson, 2008). YAP/TAZ activation leads to induction of CSC properties in a relatively wide range of human cancers. Aberrant activation of YAP/TAZ via dysregulation of the Hippo pathway results in tumorigenesis and confers cancer stem cell traits that lead to anoikis resistance, epithelial mesenchymal transition, drug resistance, and metastasis. Notably, drawbacks of current CSC markers makes it difficult to isolate CSCs from bulk heterogeneous cancer cells. Consequently, identifying the CSC-specific oncogenic signalling network of YAP/TAZ is an attractive direction to eliminate CSC and treat cancer progression (Park et al., 2018).

254

255 4. Conclusion and Perspectives

Cell stiffness is an important change that occurs in metastatic cells. The changes to the ECM, integrins and overall cell mechanical process define the survivability of the cell. Studies have shown that measurement of cell stiffness is a potential biomarker for possible metastasis of cells. Thus, stiffness could be a possible early marker for cancer development. Measurement of cell stiffness may predict whether a cell is likely to undergo

260 a metastatic process of not. Therefore, biomechanical methods to measure cell rigidity may become an
261 important diagnostic method to predict cancer formation.

262 The earliest use of mechanobiological property for cancer diagnostics is the method Magnetic Resonance
263 Elastography (MRE) (Pepin et al., 2015). This technique utilizes the difference in rigidity between normal and
264 tumour cells. Multiple cancer types including breast, brain, liver, prostate, uterus are scanned for contrast in
265 the elasticity of the tumour or its microenvironment. In this technique, stress is applied to the tumour by
266 mechanical waves. Images are taken during subsequent deformation process. The difference in the
267 corresponding mechanical property is correlated back to the difference in underlying tissue architecture.
268 Despite the innate limitation of spatial resolution and the inability of MRE to resolve small tumours, this
269 technique is one of the biggest examples, where mechanical changes of tissue are imaged for diagnostics
270 application.

271 Another potential markers for cellular stiffness are the YAP/TAZ proteins (Gargalionis et al., 2018a;
272 Panciera et al., 2017). Cancer cells sense cell rigidity with the help of integrins. Activation of integrins triggers
273 the HIPPO pathway initiating the nuclear internalization of YAP/TAZ proteins. Tracking this internalisation
274 may predict the potential cancer formation in cells. Tracking the YAP/TAZ internalization would be similar
275 to an immunohistochemistry process. Sample collected with fine needle aspiration could be probed with
276 YAP/TAZ specific antibodies and tested for their co-localization with the nucleus. A similar approach could
277 be followed for samples collected for histopathological examination post-surgery. Internalization of YAP/TAZ
278 could indicate the contrast between cancerous or benign tumors.

279 Circulating endothelial cells (CECs) have been reported as markers of cancer (Goon et al., 2006). However,
280 CECs are not always specific to cancer but also represent vascular damage (Uta Erdbruegger et al., 2010). CECs
281 (Rahbari et al., 2017) may have more prognostic significance in cancer than CTCs as they are phenotypically
282 different (Cima et al., 2016). Measurement of the difference in cell rigidity would possibly tell that if the cells
283 will undergo metastatic transformation. Measurement of rigidity could possibility be performed using cell
284 stretching devices providing external stress (Kamble et al., 2017). Co-localization of YAP/TAZ or estimation
285 of MMPs (Wirtz et al., 2011) levels are also potential options for differentiating normal cells with cancer cells.
286 Proteomic or transcriptomic estimation of Rho, MMPs or Rac1 provide information on epithelial-to-
287 mesenchymal transition (EMT) of cells. Rho is upregulated in epithelial cells whereas MMPs and Rac1 are
288 upregulated in mesenchymal cells (Friedl and Alexander, 2011).

289 Recently, polycystins have also been reported as mechano-transducer molecules in cancer. Their role is
290 largely undefined. However, their co-internalization with TAZ has been reported (Gargalionis et al., 2016).
291 The interaction of these molecules with both oncogenes and/or tumour suppressor genes has been reported.

292 Further investigation with these molecules may determine if they could serve as an early marker for cancer
293 diagnostics.

294 Understanding the overall mechanosensing properties of cancer cells can also leads to better drug
295 discovery or regulate patient's response to anti-cancer treatment. Involvement of YAP/TAZ in various aspects
296 of cancer mechanobiology makes them exciting targets for cancer drug development. Moreover, the
297 involvement of the Hippo pathway in the proliferative potential, drug resistance and metastasis of cancer cells
298 should also be explored. The main component of the Hippo pathway YAP has recently been shown to function
299 as both a protooncogene and a tumour suppressor (Broders-Bondon et al., 2018; Moroishi et al., 2015). There
300 is potentially a correlation between mechanotransduction pathway and CSCs. Mechanical stimulation
301 influences cancer growth and the biomechanical pathway (FAK/PI3K/Akt) pathway influences CSCs activity,
302 potentially elucidating newer pathways for the treatment of drug resistance cancer (Ginestier et al., 2010; Vara
303 et al., 2004).

304 In conclusion, understanding cell stiffness and mechanosensing molecules in cells and ECM are essential
305 for discovering possible biomarkers of cancer. The added benefit of these markers is their predictive role as
306 compared to diagnostic or prognostic markers currently employed clinically. We have previously described
307 the different methods to induce strain to cells (Kamble et al., 2016a; Kamble et al., 2016b; Kamble et al., 2017).
308 However, further research to measure the rigidity of cells in relation to cancer would lead new predictive
309 biomarkers for cancer. In addition to diagnostic applications, measurement of cell rigidity and
310 mechanobiological parameters may indicate their likelihood for differentiation into cancer. Measurement of
311 these parameters may also predict if the cancer cells will become drug resistant. In this respect, our cell
312 stretching technology may also be used as a tool for drug development.

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316 **Conflict of Interest**

317 The authors declare no conflict of interest.

318 **References**

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538 Fig 1: A graphical representation of the overall concept of the review article.

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548 **Figure 2.** The Extra Cellular Matrix is composed of proteoglycan complex that is interwoven with
 549 collagen fibers. This mesh is connected to fibronectins, which is then connected to the integrins. Integrins
 550 bind directly to the focal adhesion points in ECM. Integrins help in signal transduction from the ECM to
 551 intracellular signalling pathways. Hippo-YAP signal pathway is regulated by extracellular forces.
 552 Extracellular stress activates the Rho GTPases, which inhibit Lats1/2 kinase activity. Activated YAP/TAZ
 553 migrate inside nucleus whereas the inactive YAP/TAZ goes to proteolytic digestion. GPCRs, cellular
 554 junction and cell polarity modulate the Hippo pathway.

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Figure 3. Cancer stem cells (CSCs) are derived during normal cell division process due to oncogenic hits on the way. Stem cells undergo cell differentiation process for production of normal cells. Sometimes these stem cells get an oncogenic hit and they stay as CSCs. Chemotherapy acts on all the other cancer cells however, the CSCs are resistant to chemotherapy and hence retain their property to proliferate and develop into cancer again.