



**HAL**  
open science

## $\alpha v \beta 3$ integrin expression increases elasticity in human melanoma cells

Leda Lacaria, Janina R Lange, Wolfgang H Goldmann, Felix Rico, José Luis Alonso

► **To cite this version:**

Leda Lacaria, Janina R Lange, Wolfgang H Goldmann, Felix Rico, José Luis Alonso.  $\alpha v \beta 3$  integrin expression increases elasticity in human melanoma cells. *Biochemical and Biophysical Research Communications*, inPress, 10.1016/j.bbrc.2020.02.156 . hal-02505792

**HAL Id: hal-02505792**

**<https://hal.science/hal-02505792>**

Submitted on 12 Mar 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## $\alpha v \beta 3$ integrin expression increases elasticity in human melanoma cells

Leda Lacaria, Janina Lange, Wolfgang Goldmann, Felix Rico, José Luis Alonso

► **To cite this version:**

Leda Lacaria, Janina Lange, Wolfgang Goldmann, Felix Rico, José Luis Alonso.  $\alpha v \beta 3$  integrin expression increases elasticity in human melanoma cells. Biochemical and Biophysical Research Communications, Elsevier, 2020, 10.1016/j.bbrc.2020.02.156 . hal-02505792

**HAL Id: hal-02505792**

**<https://hal.archives-ouvertes.fr/hal-02505792>**

Submitted on 12 Mar 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# $\alpha v\beta 3$ integrin expression increases elasticity in human melanoma cells

Leda Lacaria <sup>a</sup>, Janina R. Lange <sup>b</sup>, Wolfgang H. Goldmann <sup>b, \*</sup>, Felix Rico <sup>a</sup>, José Luis Alonso <sup>c</sup>

<sup>a</sup> Aix Marseille Univ, CNRS, INSERM, LAI, Marseille, France

<sup>b</sup> Dept. of Biophysics, Friedrich-Alexander-University, 91052, Erlangen, Germany

<sup>c</sup> Dept. of Medicine, Mass General Hospital/Harvard Medical School, Charlestown, MA, 02129, USA

## ARTICLE INFO

### Article history:

Received 22 February 2020

Accepted 26 February 2020

Available online xxx

### Keywords:

Integrin  $\alpha v\beta 3$

$\alpha 5\beta 1$

and  $\alpha 11\beta 3$

M21 human melanoma cell

Atomic force microscopy

Micro-constrictions

Cell elasticity

## ABSTRACT

Living cells interact with the extracellular matrix (ECM) transducing biochemical signals into mechanical cues and vice versa. Thanks to this mechano-transduction process, cells modify their internal organization and upregulate their physiological functions differently. In this complex mechanism integrins play a fundamental role, connecting the extracellular matrix with the cytoskeleton. Cytoskeletal rearrangements, such as the increase of the overall contractility, impact cell mechanical properties, the entire cell stiffness, and cell deformability. How cell mechanics is influenced via different integrins and their interaction with ECM in health and disease is still unclear. Here, we investigated the influence of  $\alpha v\beta 3$  integrin expression on the mechanics of human melanoma M21 cells using atomic force microscopy and micro-constriction. Evidence is provided that (i)  $\alpha v\beta 3$  integrin expression in human melanoma cells increases cell stiffness in both adherent and non-adherent conditions; (ii) replacing  $\alpha v\beta 3$  with  $\alpha 11\beta 3$  integrin in melanoma cells, cell stiffness is increased under adherent, while decreased under non-adherent conditions; (iii)  $\alpha v\beta 3$  integrin cell stiffening is also maintained when cells adhere to fibronectin, but this phenomenon does not strongly depend on the fibronectin concentration. In all, this study sheds light on the role of  $\alpha v\beta 3$  in regulating cellular mechanics.

## 1. Introduction

There is growing evidence that the mechanical properties of the extracellular matrix (ECM) regulate cellular decision-making processes via mechano-transduction [1]. The current understanding of adhesion-mediated environmental sensing is still fragmentary, and several design principles have emerged from experiments. For example, surface biochemistry, namely the presence of diverse matrix proteins, has a strong effect on the selection of specific integrin receptors, and consequently on the initial assembly of integrin complexes. The mechanical properties of the extracellular environment play a more important role in cellular behavior than originally thought. It has been shown that cells (i) upregulate the cytoskeleton and cell-matrix adhesion on stiffer substrates more strongly, and (ii) locomote in favor of stiffer or strained substrates, and that (iii) extracellular mechanical properties and cellular decision-making are connected to the internal force developing at cell-matrix contacts due to cellular acto-myosin contractility [2,3].

Integrins are a superfamily of transmembrane receptors composed of two non-covalently associated distinct subunits  $\alpha$  and

$\beta$  [4]. Each subunit is composed of a large extracellular *N*-terminal domain that allows specific ligand binding, a single transmembrane-spanning helix and a short *C*-terminal cytoplasmic sequence that provides docking for signaling, adaptors, and cytoskeletal-associated proteins. In mammalian tissues, subunits  $\alpha$  and  $\beta$  combine in a restricted mode forming 24 different integrins, which are widely distributed. They integrate the extra- and intracellular environment through bidirectional signaling, and consequently are highly dynamic modulators of cell function, including cell adhesion, shape, motility, proliferation, differentiation, and survival. Through these processes, integrins are involved in many physiological aspects, including wound healing, immune response, hemostasis as well as in pathological dysfunctions, i.e. fibrosis, inflammatory disease, bleeding, and thrombosis. On top of that, integrins are key elements for the modulation of the mechanics involved in the interaction between cells and the surrounding ECM. Importantly, expression of specific integrin types may have a different impact on the mechanical properties of the cell.

Integrins such as  $\alpha 5\beta 1$ ,  $\alpha 11\beta 3$ , and  $\alpha v\beta 3$  are major fibronectin (FN) receptors that bind in an RGD-dependent manner [5]. Redundancy, however, exists with respect to certain integrin-ECM interactions, as some integrins bind the same extracellular ligands, albeit at different affinities and conversely, some ligands are

\* Corresponding author.

E-mail address: [wolfgang.goldmann@fau.de](mailto:wolfgang.goldmann@fau.de) (W.H. Goldmann).

recognized by different integrins. For example, recent studies have shown that  $\alpha v\beta 3$  integrin promotes melanoma tumor survival *in vivo* and that the interaction with the ECM seems to play a critical role in this process [6]. However, its mechanisms are still unknown. The aim of this work was to explore the influence of integrins  $\alpha v\beta 3$ ,  $\alpha 5\beta 1$ , and  $\alpha IIb\beta 3$  on the mechanical properties of human melanoma cell lines.

We used the following model cells derived from the same cell line: M21, expressing  $\alpha v\beta 3$  and  $\alpha 5\beta 1$ ; M21L, expressing only  $\alpha 5\beta 1$ ; and M21GPIIb, expressing  $\alpha IIb\beta 3$  and  $\alpha 5\beta 1$ , but not  $\alpha v\beta 3$  [7]. Cells were grown on surfaces without specific coating and/or with fibronectin, and the elasticity was probed using atomic force microscopy to answer the following questions:

- (i) Does the expression of  $\alpha v\beta 3$  integrin in M21 cells influence cellular stiffness?
- (ii) Do possible differences in cellular stiffness prevail under adherent and non-adherent conditions?
- (iii) Does the binding to fibronectin influence the mechanics of M21 cells? Does it influence the mechanics of M21 cells differently when  $\alpha v\beta 3$  integrin is expressed? Does it depend on fibronectin concentration?

The results from these measurements were compared with previous stiffness measurements of the same cell lines using a microfluidic device under non-adherent cellular conditions [8].

## 2. Materials and methods

### 2.1. Cell culture

Human melanoma cells M21, M21L [9,10], and M21GPIIb [11] were grown in RPMI-1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% fetal calf serum (FCS) from Gibco Laboratories, 100 U/ml of penicillin, and 100  $\mu$ g/ml of streptomycin in 5% CO<sub>2</sub> within a fully humidified incubator at 37 °C.

### 2.2. Antibodies and reagents

Mouse monoclonal antibodies against the  $\alpha v\beta 3$  heterodimer

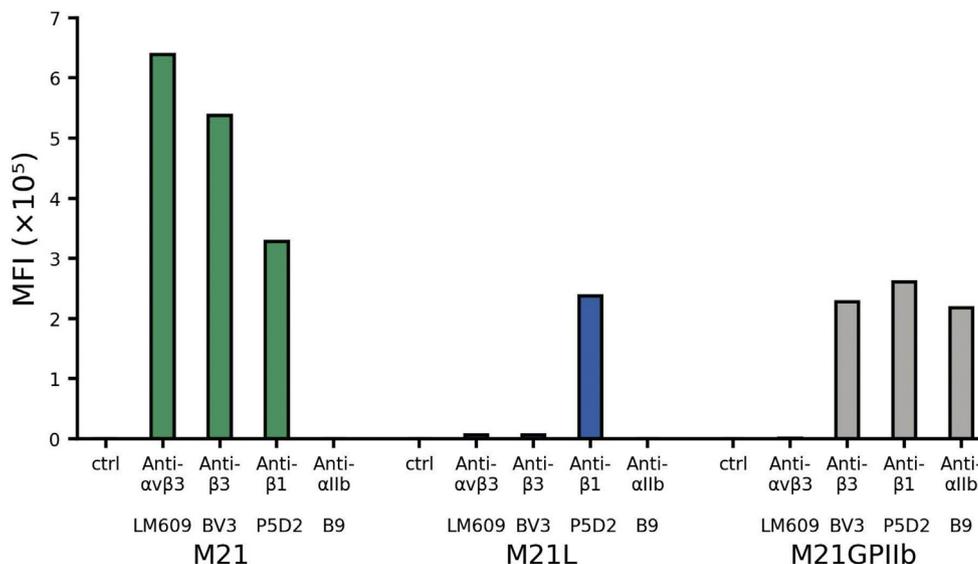
(LM609) and  $\beta 1$  subunit (P5D2) were purchased from Millipore-Sigma. Specific mouse monoclonal antibodies for  $\beta 3$  (BV3) and  $\alpha IIb$  (B9) subunits were bought at Origene (Rockville, MD) and Santa Cruz Biotechnology (Dallas, TX), respectively. The APC-labeled goat anti-mouse IgG-specific secondary antibody was obtained from Jackson Immuno-Research (West Grove, PA).

### 2.3. Flow cytometry

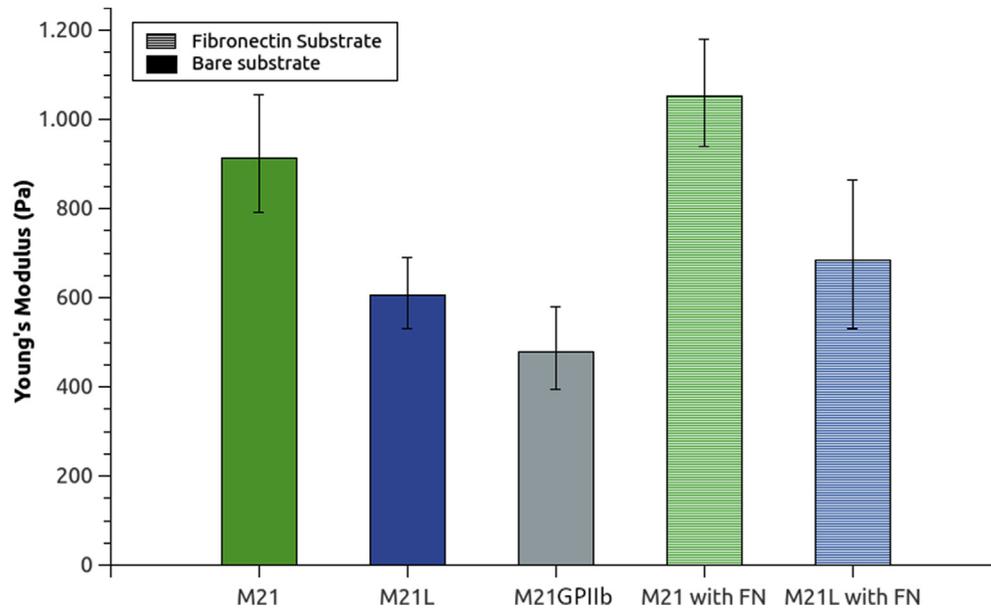
M21, M21L, and M21GPIIb cells were detached from the tissue culture dishes by mild trypsinization and washed in 20 mM HEPES buffered saline (HBS) at pH 7.4 containing 1% bovine serum albumin (BSA). Cells ( $0.5 \times 10^6$ ) in 100  $\mu$ l of the same buffer were incubated for 30 min at 4 °C with 20  $\mu$ g/ml mouse mono-clonal antibodies against the  $\alpha v\beta 3$  heterodimer (LM609),  $\beta 3$  (BV3),  $\beta 1$  (P5D2), and  $\alpha IIb$  (B9) subunits. Cells were then washed with HBS by centrifugation at 500g for 5 min. APC-labeled goat anti-mouse IgG-specific antibody at 20  $\mu$ g/ml was added to the cells for an additional 30 min at 4 °C. The cells were subsequently washed, fixed in 1% paraformaldehyde, and analysed by flow cytometry using a FACSCalibur flow cytometer. The antibody binding level was expressed as mean fluorescence intensity using the FlowJo software.

### 2.4. Fibronectin coating of petri dishes

We performed two sets of experiments to determine the Young's modulus of M21, M21L, and M21GPIIb cells adherent to (i) cell culture dishes without fibronectin coating and (ii) coated with fibronectin. To assess the effect of fibronectin coating, two different coating conditions were tested. The first condition tested the effect of FN at a high concentration (1 mg/ml), keeping the remaining bare surfaces available for FCS proteins. Thus, the surface of cell culture dishes (Falcon, REF 353004) was coated with a solution of 1 mg/ml fibronectin from bovine plasma (F1141 Sigma-Aldrich) and allowed to air-dry for at least 45 min at room temperature. The second condition tested the effect of FN-only binding, using different fibronectin concentrations, blocking remaining bare surfaces with bovine serum albumin. The surface of cell culture dishes was divided into three separate regions using two



**Fig. 1.** Flow cytometric analysis shows the surface expression of integrins expressed on the melanoma cell panel used in this study. After the cells were detached, they were stained with specific antibodies against the  $\alpha v\beta 3$  heterodimer,  $\beta 3$ ,  $\beta 1$ , and  $\alpha IIb$  subunits. The histogram shows the integrin surface expression level (mean fluorescence intensity, MFI) analysed by flow cytometry.



**Fig. 2.** Young's modulus of M21, M21L, and M21GPIIb cells grown on non-coated (left) and M21 and M21L grown on fibronectin-coated dishes (right). The values represent mean and standard error of the mean of the log-transformed data.

**Table 1**

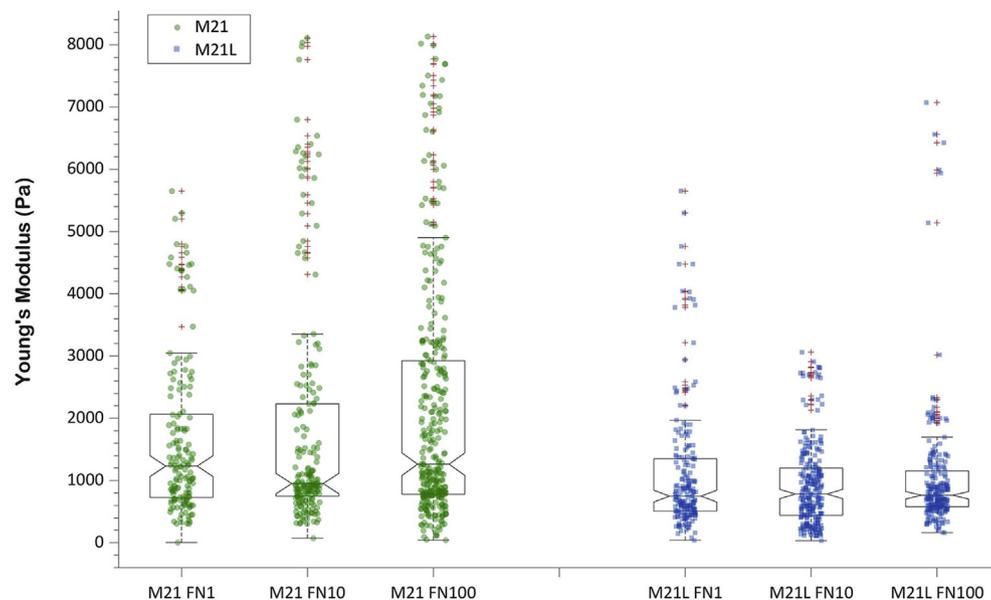
Average Young's moduli obtained for the cell lines M21, M21L, and M21GPIIb cultured on the fibronectin-coated and non-coated petri-dishes.

| Cell line | Bare Substrate   |        |     |     |
|-----------|------------------|--------|-----|-----|
|           | ln(YM[Pa])       | YM[Pa] | +   | -   |
| M21       | 6.82 ± 0.14      | 913    | 142 | 123 |
| M21L      | 6.41 ± 0.13      | 606    | 85  | 74  |
| M21GPIIb  | 6.17 ± 0.19      | 478    | 102 | 84  |
| Cell line | Fibronectin (FN) |        |     |     |
|           | ln(YM[Pa])       | YM[Pa] | +   | -   |
| M21       | 6.96 ± 0.11      | 1054   | 128 | 114 |
| M21L      | 6.53 ± 0.25      | 685    | 180 | 155 |

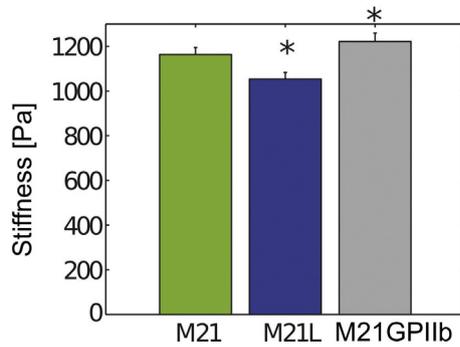
**polydimethylsiloxane (PDMS)** pools to coat the surface with three different fibronectin concentrations (1, 10, and 100  $\mu\text{g/ml}$ ). The surface was incubated for at least 45 min, then the excess solution was removed by aspiration, and finally the surface was blocked by BSA (100  $\mu\text{g/ml}$ ) for 1 h at room temperature to saturate non-specific cellular interactions.

### 2.5. Atomic force microscopy (AFM)

All AFM measurements were performed on a NanoWizard IV Atomic Force Microscope (JPK, Bruker, Santa Barbara, CA, USA) coupled with an inverted optical microscope Ti-Eclipse (Nikon, Japan). During the first experiments, a Novascan V-shaped



**Fig. 3.** Young's modulus of M21 and M21L cells growing on FN-only coated substrates at different concentrations (FN1, FN10, and FN100 stand for 1  $\mu\text{g/ml}$ , 10  $\mu\text{g/ml}$ , and 100  $\mu\text{g/ml}$ , respectively). The values represent mean and standard error of the mean of the log-transformed data.



**Fig. 4.** Influence of  $\alpha$ IIb $\beta$ 3 on cell mechanics by micro-constriction analysis. The stiffness of M21L and M21GPIIb cells compared to the control population M21 were calculated from the power-law fitting routine [15].

cantilever was used with a spherical silica bead of 1  $\mu$ m radius as a tip with a spring constant of 0.03 N/m. During the second experiment, a V-shaped cantilever (MLCT-D from Bruker, Santa Barbara, CA, USA) was used, and a silica bead of 10  $\mu$ m diameter with a spring constant of 0.042 N/m. The cantilever spring constant was calibrated according to the Sader method in air, and the optical lever sensitivity was determined from the thermal spectrum in liquid [12–14], using a custom algorithm based on Matlab (Mathworks, Natick, MA). Force curves were generated in force mapping mode using a square array of 8  $\times$  8 or 5  $\times$  5 points across the perinuclear region of the cells. The size of the grid was chosen according to the dimension of each cell (between 10  $\times$  10 and 20  $\times$  20  $\mu$ m<sup>2</sup>), and 5 curves per point were recorded. To minimize the acquisition time, the approach velocity was set to 15  $\mu$ m/s. A set point of 0.4 nN was defined to obtain an indentation of at least 200 nm.

### 2.6. Micro-constriction analysis

Cell mechanical properties of M21 and M21L cells were also assessed by the micro-constriction method [15] with a constriction width of 7.7  $\mu$ m and a device height of 18.9  $\mu$ m. M21 cells were cultured as shown in Ref. [8] and harvested at a confluency of approx. 80%.

### 2.7. Data processing and statistics

The Hertz model was used, avoiding the substrate bottom effect. A reference force curve was acquired on the substrate near every cell to estimate the cell thickness at all points of the force curve map. The thickness of the cell at each point of the force curve map ( $h$ ) was determined from the cantilever vertical position at the point of contact with the cell, and at the point of contact with the substrate, which was used as reference ( $h = 0$ ). To avoid the effect of the underlying substrate, only data points fulfilling an indentation of  $<0.1h$  were considered to determine the Young's modulus [16]. The geometric mean of all values, from each tested cell line, was used.

## 3. Results

### 3.1. Integrin cell surface level

The histogram in Fig. 1 shows the expression level of integrins on the surface of human melanoma cells, M21 using specific antibodies [7].

### 3.2. AFM measurements

We compared the average Young's modulus (cell stiffness) of ( $n = 15$ ) M21, M21L, and M21GPIIb cells cultured on treated, non-coated FN petri dishes and the same number of M21 and M21L cells on FN-coated petri dishes. As shown in Fig. 2 and Table 1, the Young's moduli of M21 cells on FN-coated and non-coated dishes were ~50% higher compared to M21L cells. The mechanical properties (elasticity) of M21GPIIb cells, expressing  $\alpha$ 5 $\beta$ 1 and  $\alpha$ IIb $\beta$ 3 instead of  $\alpha$ v $\beta$ 3 integrins, were compared to M21 ( $\alpha$ v $\beta$ 3 and  $\alpha$ 5 $\beta$ 1) and M21L (only  $\alpha$ 5 $\beta$ 1) cells (Fig. 2). It was hypothesized that the replacement of  $\alpha$ v $\beta$ 3 by  $\alpha$ IIb $\beta$ 3 integrin might restore the mechanical properties of M21 control cells, as both integrins have a similar molecular structure; however, the expectation could not be verified by these measurements; M21GPII showed a lower Young's modulus than M21 and M21L cells.

FN-coated surfaces at high concentration (1 mg/ml) led to an increase of ~15% of the Young's modulus on both M21 and M21L cells (Fig. 2). The Young's modulus of cells growing on fibronectin-only surfaces at different concentrations is shown in Fig. 3. As reflected, fibronectin-only binding did not lead to major changes on cell stiffness when varying the concentration. The results suggest that integrin  $\alpha$ v $\beta$ 3 influences the mechanical properties of M21 cells, while the interaction between this integrin and fibronectin has a moderate effect on the cell's Young's modulus.

### 3.3. Micro-constriction measurements

Two independent measurements were conducted, and the results were pooled. Using the power-law fitting routine of the scatter ( $t_{\text{entry}}$  vs.  $\epsilon_{\text{max}}/\Delta p$ ), a cell stiffness of  $1,215 \pm 17$  Pa for M21 cells expressing  $\alpha$ v $\beta$ 3 and  $\alpha$ 5 $\beta$ 1 integrins was calculated. M21L cells, which only express  $\alpha$ 5 $\beta$ 1 integrin, showed a significant reduction in stiffness to  $1,065 \pm 17$  Pa. The power-law exponents were inversely correlated and markedly different ( $0.07 \pm 0.01$  for M21 and  $0.09 \pm 0.01$  for M21L cells). The expression of  $\alpha$ v $\beta$ 3 integrin was correlated with increased stiffness and a decreased power-law exponent in both cell lines.

Moreover, the mechanical properties of M21GPIIb cells, expressing  $\alpha$ 5 $\beta$ 1 and  $\alpha$ IIb $\beta$ 3 instead of  $\alpha$ v $\beta$ 3 integrins, were calculated in comparison to M21 ( $\alpha$ v $\beta$ 3 and  $\alpha$ 5 $\beta$ 1) and M21L (only  $\alpha$ 5 $\beta$ 1) cells (Fig. 4). In contrast to AFM measurements, expression of  $\alpha$ IIb $\beta$ 3 led to an increase in the stiffness of M21GPIIb cells in suspension.

## 4. Discussion

In this study, we show that individual expression of different integrins, even those with specificity for the same ligand, are influential to the mechanical status of the cell probed by two different technologies, AFM and micro-constrictions.

A systematic approach, comparing the cell mechanical properties under suspended or adherent conditions, provides insight into the effect adhesion may have on cell mechanics. We have chosen a panel of three M21 melanoma cells expressing the fibronectin receptor  $\alpha$ 5 $\beta$ 1 alone (M21L), in conjunction with  $\alpha$ v $\beta$ 3 (M21), or with  $\alpha$ IIb $\beta$ 3 (M21GPIIb). In summary, a systematic effect of  $\alpha$ v $\beta$ 3 integrin expression on the cellular mechanics was found. In M21 cells, an increase in cell stiffness is correlated with the expression of  $\alpha$ v $\beta$ 3 integrin. This could be due to its association with the actin cytoskeleton, namely via a link between integrin, focal adhesion-associated proteins, and actin cytoskeleton that leads to higher connectivity inside the cell and/or to increased actin polymerization [17–19]. Support for this notion comes from a decreased actin content measured by flow cytometry in M21L compared to

M21 cells and from the finding that M21 cells expressing  $\alpha v\beta 3$  integrin show a higher cell volume (results not shown). In the case of M21GPIIb, which expressed  $\alpha IIb\beta 3$  instead of  $\alpha v\beta 3$ , the stiffness of cells could not be restored, having a different effect on the stiffness on attached (Fig. 2) and suspended (Fig. 4) cells [8]. These results might be due to a less deformable nucleus in M21GPIIb cells or to a softer perinuclear area of the cell with a weaker adhesion to the substrate; this points in the direction that micro-constriction experiments are more sensitive to the nuclear deformability, while the AFM measurements are probing the perinuclear area.

Our AFM data on FN-only coated substrates, blocked with BSA (Fig. 2), showed that M21 cells are only slightly affected by FN. In contrast, data shown in Fig. 3, on FN substrates not blocked with BSA, to which fetal calf serum (FCS) proteins may adhere, such as vitronectin and fibrinogen, M21 cells appear stiffer. It suggests that M21 cells require ligands other than FN to reinforce their mechanical properties via integrin  $\alpha v\beta 3$  binding. This is in agreement with previous work indicating that  $\alpha 5\beta 1$  mainly determines adhesion strength, while  $\alpha v\beta 3$  would control adhesion reinforcement and mechano-transduction [20]. Thus,  $\alpha v\beta 3$  binding to FN-only seems to be insufficient to regulate cellular mechanics.

Future work entailing gene expression knock-down and a more detailed analysis using cell populations expressing different levels of only  $\alpha v\beta 3$ ,  $\alpha IIb\beta 3$ , or  $\alpha 5\beta 1$  will be required to finally assess the differential contribution of these integrins to integrin-mediated cellular stiffness. Moreover, experiments using other ligands such as vitronectin and/or fibrinogen, specifically targeting only some of the integrins, will be required to obtain a more complete picture of integrin regulation on cell mechanics.

#### Declaration of competing interest

This is to confirm that the publication of this manuscript does not conflict with any financial or any other interests.

#### Acknowledgements

We thank Celia Marshall (MA) for proofreading the manuscript, the cell culture platform PCC TPR2-Luminy for technical assistance, and Mr Delf Kah for re-drawing Figs. 1 and 4. This project was funded by the European Union's Horizon 2020 Research and Innovation Program under the Marie Skłodowska-Curie grant agreement Nos. 713750 and 812772. Further, research was carried out with the financial help of the Regional Council of Provence-Alpes-Côte d'Azur and with support from A\*MIDEX (n° ANR-11-IDEX-0001-02), funded by the Investissements d'Avenir project of the French Government and managed by the French National Research Agency (ANR).

#### References

- [1] W.H. Goldmann, Mechanosensation, A basis cellular process, in: Engler and Kumar (Ed.), *Progress in Molecular Biology and Translational Science*, vol. 126, 2014, pp. 75–102.
- [2] M. Dembo, Y.L. Wang, Stresses at the cell-to-substrate interface during locomotion of fibroblasts, *Biophys. J.* 76 (1999) 2307–2316.
- [3] A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification, *Cell* 126 (2006) 677–689.
- [4] R.O. Hynes, Integrins: bidirectional, allosteric signaling machines, *Cell* 110 (2002) 673–687.
- [5] A. van der Flier, A. Sonnenberg, Function and interactions of integrins, *Cell Tissue Res.* 305 (2001) 285–298.
- [6] L.W. Contois, A. Akalu, J.M. Caron, E. Tweedie, A. Cretu, T. Henderson, L. Liaw, R. Friesel, C. Vary, P.C. Brooks, Inhibition of tumor-associated  $\alpha v\beta 3$  integrin regulates the angiogenic switch by enhancing expression of IGFBP-4 leading to reduced melanoma growth and angiogenesis in vivo, *Angiogenesis* 18 (2015) 31–46.
- [7] B.D. Adair, J.L. Alonso, J. van Agthoven, V. Hayes, H.S. Ahn, I.S. Yu, S.W. Lin, J.P. Xiong, M. Poncz, M.A. Arnaout, Structure-guided design of pure orthosteric inhibitors of  $\alpha IIb\beta 3$  that prevent thrombosis but preserve hemostasis, *Nat. Commun.* 11 (2020) 398.
- [8] J.R. Lange, W.H. Goldmann, J.L. Alonso, Influence of  $\alpha v\beta 3$  integrin on the mechanical properties and the morphology of M21 and K562 cells, *Biochem. Biophys. Res. Commun.* 478 (2016) 1280–1285.
- [9] D.A. Cheresh, R.C. Spiro, Biosynthetic and functional properties of an Arg-Gly-Asp-Directed receptor involved in human melanoma cell attachment to vitronectin, fibrinogen, and von Willebrand factor, *J. Biol. Chem.* 262 (1987) 17703–17711.
- [10] B. Felding-Habermann, B.M. Mueller, C.A. Romerdahl, D.A. Cheresh, Involvement of Integrin  $\alpha v$  gene expression in human melanoma tumorigenicity, *J. Clin. Invest.* 89 (1992) 2018–2022.
- [11] N. Kieffer, L.A. Fitzgerald, D. Wolf, D.A. Cheresh, D.R. Phillips, Adhesive properties of the beta 3 integrins: comparison of GP IIb-IIIa and the vitronectin receptor individually expressed in human melanoma cells, *J. Cell Biol.* 113 (1991) 451–461.
- [12] J.E. Sader, J.W.M. Chon, P. Mulvaney, Calibration of rectangular atomic force microscope cantilevers, *Rev. Sci. Instrum.* 70 (1999) 3967.
- [13] M.J. Higgins, R. Proksch, J.E. Sader, M. Polcik, S. McEndoo, J.P. Cleveland, S.P. Jarvis, Non-invasive determination of optical lever sensitivity in atomic force microscopy, *Rev. Sci. Instrum.* 77 (2006), 013701-1-013701-5.
- [14] H. Schillers, C. Rianna, J. Schäpe, F. Rico, et al., Standardized nanomechanical atomic force microscopy procedure (SNAP) for measuring soft and biological samples, *Sci. Rep.* 7 (2017) 5117.
- [15] J.R. Lange, J. Steinwachs, T. Kolb, L.A. Lautscham, I. Harder, G. Whyte, B. Fabry, Micro-constriction arrays for high-throughput quantitative measurements of cell mechanical properties, *Biophys. J.* 109 (2015) 26–34.
- [16] E.K. Dimitriadis, F. Horkay, J. Maresca, B. Kachar, R.S. Chadwick, Determination of elastic moduli of thin layers of soft material using the atomic force microscope, *Biophys. J.* 82 (2002) 2798–2810.
- [17] W.H. Goldmann, Mechanotransduction in cells, *Cell Biol. Int.* 36 (2012) 567–570.
- [18] W.H. Goldmann, Mechanotransduction and focal adhesions, *Cell Biol. Int.* 36 (2012) 649–652.
- [19] J.L. Alonso, W.H. Goldmann, Cellular mechanotransduction, *AIMS Biophysics: Cell Surface receptors.* 3 (2016) 50–62.
- [20] P. Roca-Cusachs, N.C. Gauthier, A. del Rio, M.P. Sheetz, Clustering of  $\alpha 5\beta 1$  integrins determines adhesion strength whereas  $\alpha v\beta 3$  and talin enable mechanotransduction, *Proc. Natl. Acad. Sci. U.S.A.* 106 (2009) 16245–16250.