## Supplementary Information

# Biophysical and biochemical characteristics as complementary indicators of melanoma progression 

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## Note 1. The Young's modulus determination

To quantify the indentation depth in the AFM-based elasticity measurements, in parallel to the measured sample, force-distant curves are recorded on stiff, non-deformable reference surface like glass or silicon. In such a case, the deflection of the cantilever registered after the probing tip touches the surface is linear, since the deformation caused by the AFM tip does not occur. By subtracting the reference force-distance curve from that recorded on sample surface, the force-indentation curves can be determined.
The analysis of the force-indentation curves using the Hertz-Sneddon model leads to the determination of the Young's modulus (i.e. elasticity modulus). Based on the Hertz contact mechanics, Sneddon derived a model assuming a rigid, axisymmetric probe indenting a soft, elastic, flat surface. As the AFM tip is frequently a four-sided pyramid, its shape is often assumed to be a cone. In such a case, the Hertz-Sneddon model delivers the following relation between applied load force $F$ and indentation depth $\delta$ :

$$
\begin{equation*}
F(\delta)=\frac{2 \tan \alpha}{\pi} E^{\prime} \delta^{2} \tag{1}
\end{equation*}
$$

Where $\alpha$ is the open angle of the cone $\left[{ }^{0}\right], E$ ' is the reduced Young's modulus that depends on elastic properties of the cantilever and the sample, as described by the following equation:

$$
\begin{equation*}
\frac{1}{E^{\prime}}=\frac{1-\mu_{\text {cantilever }}^{2}}{E_{\text {cantilever }}}+\frac{1-\mu_{\text {sample }}^{2}}{E_{\text {sample }}} \tag{2}
\end{equation*}
$$

where $\mu_{\text {cantilever }}$ and $\mu_{\text {sample }}$ represent the Poisson ratios of the cantilever and a sample. In the case of living cells, $E_{\text {cell }} \ll E_{\text {cantilever }}$, and thus the reduced Young's modulus can be re-written as:

$$
\begin{equation*}
E^{\prime}=\frac{E_{\text {cell }}}{1-\mu_{\text {cell }}^{2}} \tag{3}
\end{equation*}
$$

It is difficult to determine the exact value of $\mu_{\text {cell }}$, thus it is often assumed to be 0.5 , bearing in mind that a cell which is mainly composed of water, which is an incompressible material.

Finally, the relation between applied load force $F$ and indentation depth $\delta$ can be written as follows:

$$
\begin{equation*}
F=\frac{2 \tan \alpha}{\pi} \frac{E_{\text {cell }}}{1-\mu_{\text {cell }}^{2}} \delta^{2} \tag{4}
\end{equation*}
$$

Using this equation, the Young's modulus for all studied cells was calculated. Force curves were analysed with the aim to determine the Young's modulus using Origin 8.0 and self-made software ${ }^{1,2}$.

In Figure S1 the results of Young's modulus distribution for each melanoma cell line is presented.

Figure S1. Elastic properties of melanocytes and melanoma cells obtained based on AFM measurements.


Figure S1. The Young's modulus of melanocytes and all studied melanoma cells, calculated from more than 50 cells measured for each cell line. The indentation depth 600 nm , model cone.

Table 1 presents mean ( $\pm \log$ standard error) and median determined from lognormal distribution together with Mann-Whitney test verifying statistical significance of the differences among cell lines.

Table S1. Summary of elastic properties of melanoma cells in regards to melanocytes. a) mean, median and left \& right standard deviation (SD) calculated from log SD, b) Mann-Whitney test verifying statistically the differences among cell lines.
a)

| Cell line | Mean <br> $(\mathrm{kPa})$ | left SD <br> $(\mathrm{kPa})$ | right SD <br> $(\mathrm{kPa})$ | Median <br> $(\mathrm{kPa})$ |
| :---: | :---: | :---: | :---: | :---: |
| HEMa-PL | 14.28 | 6.11 | 11.44 | 12.0 |
| $W M 793$ | 10.18 | 3.71 | 6.51 | 9.9 |
| $W M 115$ | 9.68 | 3.47 | 6.00 | 9.2 |
| $W M 239$ | 6.95 | 2.71 | 5.03 | 6.6 |
| $W M 266-4$ | 8.84 | 3.15 | 5.41 | 8.7 |
| $1205 L u$ | 7.29 | 3.11 | 6.24 | 7.1 |
| A375P | 5.00 | 1.81 | 3.13 | 4.9 |



## Note 2. Details of PCA applied to ToF SIMS mass spectra.

As mass spectra of biological materials are very complex there is a need to use statistical analysis in order to look for alterations between investigated samples. PCA is one of the established methods to analyse large data sets ${ }^{3,4}$. The principle of PCA is to reduce the dimensionality of a data set, while retaining as much as possible the variation present in the original predictor variables ${ }^{5}$. In mathematical terms, PCA maximizes the variance of a linear combination of the original predictor variables. Principal components are orthogonal to each other and are defined in such a way that the first principal component is the direction that describes the highest degree of variance, the $2^{\text {nd }}$ principal component describes the second highest degree of remaining variance, and so on. If we are considering a matrix of spectra, where each spectrum is a vector in a n-dimensional space ( n is the number of masses in this case), PCA is basically a matrix rotation into a new set of axes, based on a combination of the original data set, that best describes the spread of the data. The new axes are called loadings and they are a measure of the importance of the original variables to the new direction. When a particular peak has high magnitude of a loading then it means that this peak was significant in defining the new direction of maximum difference. The original mass spectrum can be projected on to the new directions of principal components by means of scores - the distances along these directions of that spectrum for the particular principal components. In the presented work, the PCA was applied to analyze spectra recorded by ToF SIMS. The mass spectra were analyzed within the range $0-500 \mathrm{Da}$. In the presented approach, for all the collected spectra the same method of data analysis, without predefining of any particular masses, was applied. Firstly, each recorded spectrum was normalized to the sum of intensities for all peaks. Then, autoscaling was applied as a pre-processing method. In autoscaling, after mean-centering, each variable is divided by the corresponding variable standard deviation. Such a way of pre-processing results in scaling of each variable in a way providing an equal impact on a final result of multivariate analysis. After pre-processing, the PCA was carried out using the PLS Toolbox 7.5.2 (Eigenvector Research, Manson, WA) for MATLAB 8.1.0.604 R2013a (MathWorks, Inc., Natick, MA) software. Original data were analysed with the same weights. The cross-validation algorithm was applied to select the number of components in PCA. From PCA analyses, both
scores and loading plots were obtained. First, the scores plots were reviewed to find the best separated PCs. To visualize ellipsoids in the 3D scores plot double SD range along the axes of PC1, PC2, PC3 were calculated and ellipsoids were constructed by means of MATLAB 8.1.0.604 R2013a software.

Figure S2. Exemplary mass spectra recorded for all studied cell lines.


Figure S2. Exemplary mass spectra of investigated cell lines obtained with $\mathrm{Bi}_{3}+$ primary ion gun in the range 0-500Da: a) HEMa-LP - human melanocytes; b) WM793 - cell line derived from a vertical growth phase (VGP) in the primary melanoma site; c) WM115-cell line derived from a vertical growth phase (VGP) in the primary melanoma site; d) WM239 - cell line derived from a cutaneous skin metastasis; e) WM266-4 cell line derived from a cutaneous skin metastasis; f) 1205Lu cell line originated from a lung metastasis; g) A375P cell line originated from a lung metastasis; h) RPMI-1640 - culture medium for melanoma cells; i) MEDIUM 254 - culture medium for melanocytes.

Figure S3. Scores and loading plots for PCA obtained for all cells
Below, in Figures S3-S6 the 2D distributions of PCA scores plots (a-c) and loadings (d) are presented, corresponding to the Figures 2 and 3.


Figure S3. a-c) 2D scores plot of PCA calculated for the cellular spectra together with the spectra of silicon treated with culture media. d) loadings plot for the presented principal components.

Figure S4. Scores and loading plots for PCA during a comparison between cells originating from a) VGP melanoma and skin metastasis, and b) VGP melanoma and lung metastasis.


Figure S4. a) PCA outcome for a comparison of melanoma cells from VGP primary melanoma and metastatic sites in skin (The corresponding scores and loading plots for PC1, PC2 and PC3). b) PCA outcome for a comparison of melanoma cells from VGP primary melanoma and metastatic sites in lung (the corresponding scores and loadings plots for PC1, PC2 and PC3).

Figure S5. Loading plots for PCA outcome obtained for cells originating from two pairs of cells derived from the same patient.


Figure S5. (a) Loadings plot for PC1, PC2 and PC3 components resulted from PCA of ToF SIMS mass spectra recorded for WM115 (primary melanoma site, VGP cells) and WM266-4 (secondary tumor site, skin metastasis). (b) Loadings plot for PC1, PC2 and PC3 components resulted from PCA outcome of ToF SIMS mass spectra recorded for WM793 (primary tumor site, VGP cells) and 1205Lu (secondary tumor site, lung metastasis).

Table S2. Molecular masses that contribute to largest separation between cells from skin and lung metastasis.

| Molecular mass <br> $(u)$ | Proposed assigment |
| :---: | :---: |
| 104.11 | sphingomyelin or phosphatidylcholine |
| 124.99 | sphingomyelin or phosphatidylcholine |
| 184.10 | sphingomyelin or phosphatidylcholine |
| 224.11 | phosphatidylcholine |
| 206.09 | sphingomyelin or phosphatidylcholine |
| 246.11 | sphingomyelin or phosphatidylcholine |

Assignment was performed based on:

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Measurement, and Phenomena, 34, 051804 (2016); doi: 10.1116/1.4961461
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3. Tipping, M. E. \& Bishop, C. M. Probabilistic Principal Component Analysis. J. R. Stat. Soc. Ser. B (Statistical Methodol. 61, 611-622 (1999).
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