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RELATIONSHIP BETWEEN ELECTROROTATION SPECTRUM AND MEMBRANE ASSOCIATED REGION1 BINDING PROTEIN (SMAR1) AND FOCAL ADHESION KINASE (FAK) GENE EXPRESSION.

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BACKGROUND

The potential correlations between the genes *SMAR1* and *PTK2* and cellular membrane alterations have garnered significant research interest due to their implications in cancer biology. In this study, we extended our previous evaluation of the membrane genes *SMAR1*, *CK8*, and *PTK2* by including a third cell line, HCT-116, alongside CCD-841 (healthy colon) and CaCo-2 (colon adenocarcinoma). We aimed to investigate the differential expression of these genes across the three cell lines and explore their potential correlations with variations in electrorotation. Our findings provide a comprehensive analysis of gene expression profiles and their potential impact on the biophysical properties of cellular membranes in healthy and cancerous colon cells. This study offers new insights into the role of membrane-associated genes in colorectal cancer and underscores the importance of understanding gene expression dynamics in the context of cellular membrane behavior.

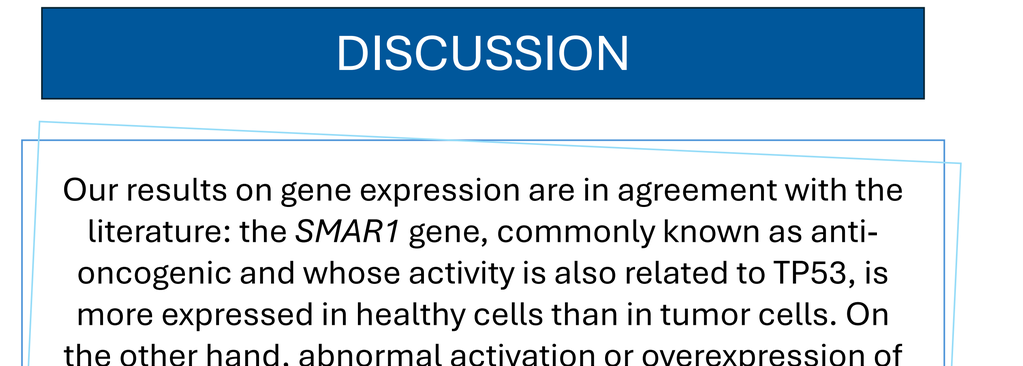


Figure 2. Variation of the rotational speed of the three cell lines as the frequency varies.

DISCUSSION

Our results on gene expression are in agreement with the literature: the *SMAR1* gene, commonly known as anti-oncogenic and whose activity is also related to TP53, is more expressed in healthy cells than in tumor cells. On the other hand, abnormal activation or overexpression of PTKs is frequently associated with various cancers. In fact, HCT-116 and CaCo-2 cells express *PTK* more than CCD-841 cells. In addition to its anti-oncogenic role, it has been demonstrated that *SMAR1* is involved in the reorganization of the cell membrane. Specifically, the greater its expression, the greater the smoothness of the membrane. This is also highlighted by the electrorotation experiment: CCD-841 cells, having a smoother structure, are able to reach greater speeds compared to the other two tumor lines which, expressing less *SMAR1*, offer more resistance to rotation due to greater membrane roughness.

MATERIALS

The CCD-841, CaCo-2 and HCT-116 cell lines were grown in their respective media [1]. The expression of *SMAR1* and *FAK* genes was evaluated via RTqPCR, with gene expression normalized to the β-actin. For electrorotation experiments, cells were washed and resuspended in an appropriate buffer [1] up to a final concentration of 150 cells/µL. Cells were then subjected to ER in an engineered plug-and-play chamber using a prototypal DEP chip (Figure 1), administering a frequency range of 10^4-10^7 Hz, and evaluating their velocity (ω) in radians per second.

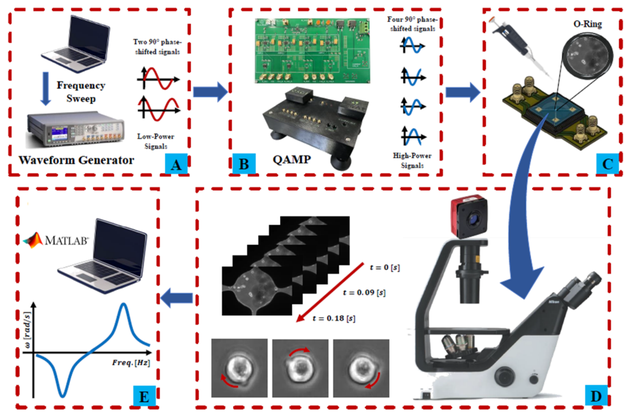


Figure 1A. Working principle schematic: (A) Low-power signal generations, (B) high-power quadrature stage, (C) ER-Device, (D) sensing and acquisition, and (E) offline analysis and result evaluation.

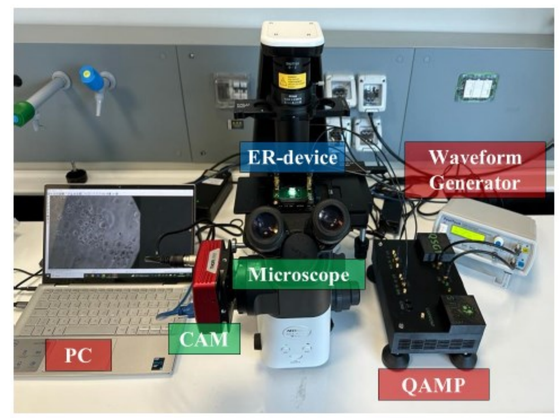
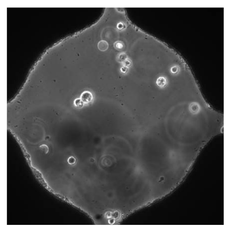
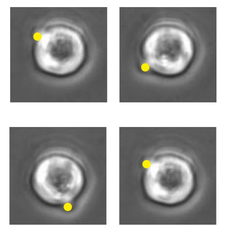


Figure 1B. experimental setup

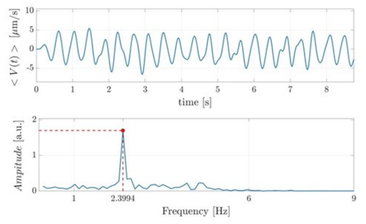
RESULTS



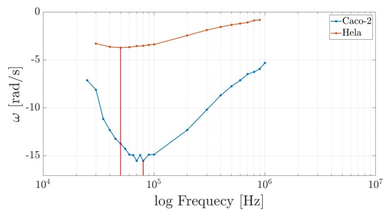
CaCo-2 cells in the plug-and-play chamber seen under a microscope at 20% magnification.



Tracking algorithm to follow the rotation of the single cell at different times and at all analyzed frequencies.



Extrapolation of velocity as a function of time and amplitude as a function of frequency.



Comparison of velocity in rad/s as a function of applied frequency for two different cell lines.

Our results show how there is a direct correlation between *SMAR1* expression and different behavior in electrorotation. Basically, it seems like the greater the expression of *SMAR1*, the greater the change in speed of the cell line as the frequency varies (Figure 2). RT-qPCR showed that healthy CCD-841 cells present the highest expression of SMAR1, CaCo-2 an intermediate situation while the lowest expression of *SMAR1* is presented by HCT-116. At the same time, the CCD-841 have speed values expressed in rad/s ranging from -3 to -24, the CaCo-2 from -0.6 to -14.3 and the HCT-116 from -2.3 to - 11.7. PTK expression, on the other hand, follows a diametrically opposite trend, with the highest expression value reported by HCT-116 and the lowest by CCD-841, with CaCo-2 always placed in the middle.

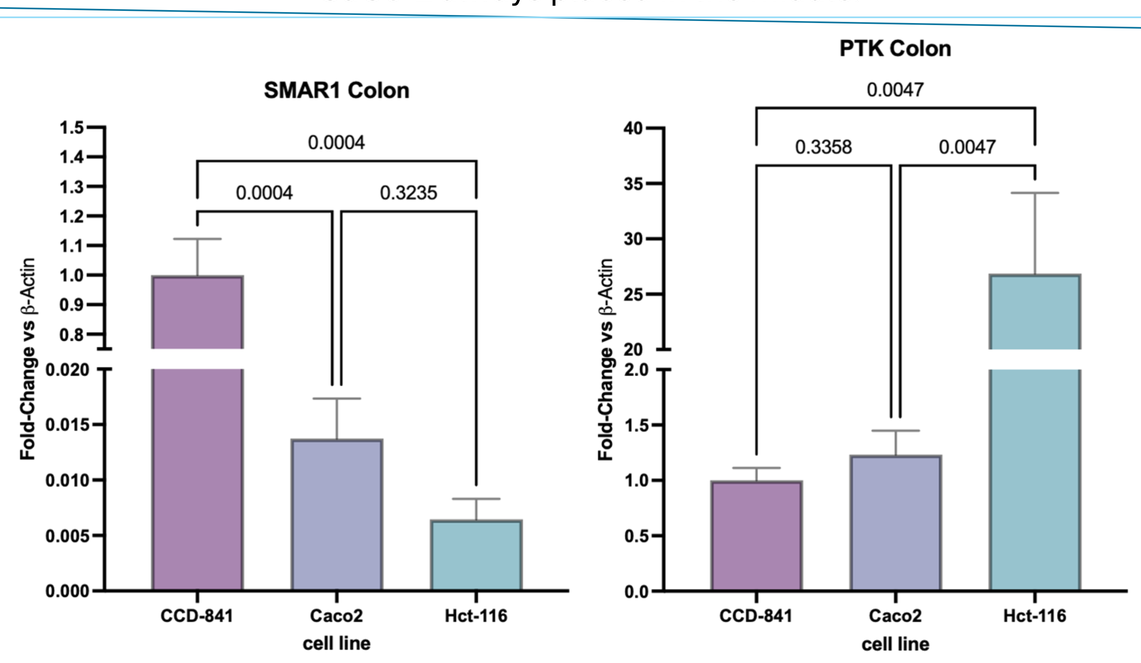


Figure 2. Expressions of the SMAR1 and PTK genes for the different cell lines normalized for beta actin.

CONCLUSIONS and PERSPECTIVES

Our study elucidates the significant correlations between the expression of SMAR1 and PTK2 genes and the biophysical properties of cellular membranes in both healthy and cancerous colon cells. These findings provide a comprehensive understanding of how membrane-associated gene expression influences cellular behavior, particularly in the context of colorectal cancer. The observed variations in electrorotation velocities among the cell lines offer a novel perspective on the mechanical properties of cell membranes, potentially paving the way for new diagnostic and therapeutic approaches. Future research should focus on further elucidating the molecular mechanisms underlying these correlations and exploring the broader implications of membrane gene expression in cancer biology. By integrating these insights with clinical data, we can enhance our understanding of cancer progression and develop more effective strategies for diagnosis and treatment.

[1] Bonacci, Paolo G et al. "Impact of buffer composition on biochemical, morphological and mechanical parameters: A tare before dielectrophoretic cell separation and isolation." *Translational oncology* vol. 28 (2023): 101599. doi:10.1016/j.tranon.2022.101599

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