UNRAVELING NEW STRATEGIES FOR EARLY DETECTION OF INVASIVE CANCER CELLS: A SPECIAL FOCUS ON THE NUCLEAR AND CYTOSKELETAL ARCHITECTURE

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M. Sofia Fernandes[1], J. Figueiredo[1], D. Vieira[2], S. Melo[1], A.M. Moreira[1], J. M. Sanches[2] and R. Seruca[1]

[1]Instituto de Investigação e Inovação em Saúde (i3S)/Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Epithelial Interactions in Cancer (EPIC), Porto, Portugal. [2]Instituto Superior Técnico (IST)/Institute for Systems and Robotics (ISR), Lisboa, Portugal.

INTRODUCTION

Cancer invasion is a hallmark of cancer progression and the leading cause of cancer mortality worldwide. However, the identification of invasive cancer cells at an early stage remains challenging. Therefore, there is an urgent need to develop novel biomarkers and innovative strategies to successfully tackle cancer invasion. It is well established that the structure and organization of the nucleus and the cytoskeleton are dynamically orchestrated during many cellular processes, including cancer invasion. Thus, in this study, we investigated fine-tuned nuclear and cytoskeletal patterns associated with invasion, taking into consideration the cell-extracellular matrix (ECM) interaction.

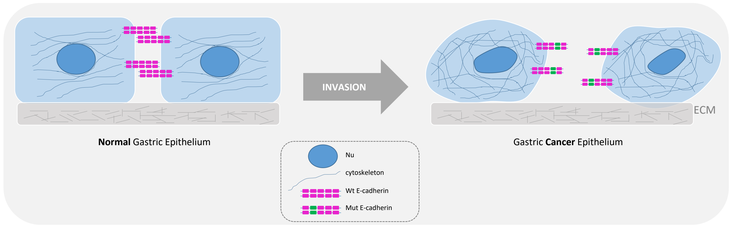
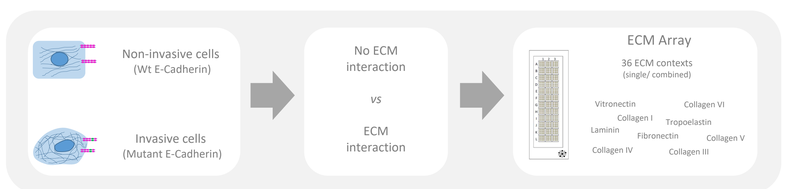


Figure 1 – Simplified representation of cell-cell and cell-ECM interactions in normal and gastric cancer epithelium. Dysregulation of E-cadherin leads to aberrant cell-cell and cell-ECM interplay and is associated with increased cell invasion in gastric carcinoma. ECM, Extracellular Matrix; Nu, nucleus; Wt, wild type; Mut, mutant.

MATERIALS AND METHODS

In this study, we took advantage of an ECM Array platform (MicroMatrix 36, MicroStem) and a panel of invasive and non-invasive gastric cancer cells. For validation purposes, we assessed cells transfected with wild type or mutant E-cadherin, which lead to distinct adhesion and invasion phenotypes. A comprehensive analysis of nuclei and cytoskeleton architectural features was performed. Nuclei area, intensity, perimeter, eccentricity, circularity, solidity and entropy were evaluated. Notably, a new computational pipeline was developed to characterize cytoskeletal structures, namely orientation, compactness, radiality, bundling, parallelism, morphology, among others.



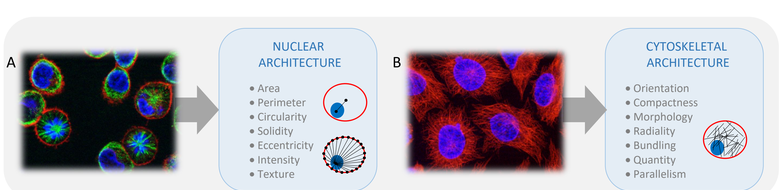


Figure 2 – Pipeline for the analysis of the nuclear and cytokeletal arquitecture of invasive and non-invasive cancer cells. (A) Nuclei (blue), Membrane marker (red) and α-tubulin (green). (B) Nuclei (blue) and α-tubulin (red).

RESULTS & DISCUSSION

1. The invasive abilitiy of cancer cells is associated with unique nuclear modifications

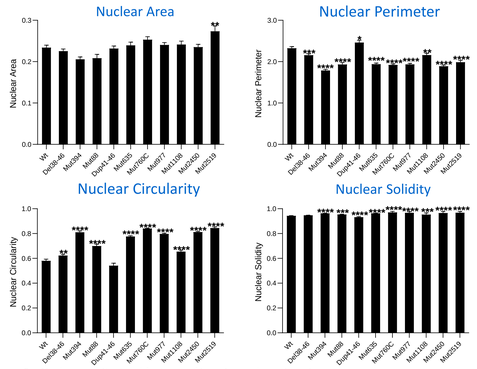


Figure 3 – Nuclear architectural features of invasive and non-invasive cancer cells. Briefly, CHO cells grown in standard conditions (no ECM coating) were transfected with Wt E-cadherin or a set of 10 E-cadherin mutants. Nuclei were labelled with DAPI and images evaluated using fluorescent microscopy. Data represent means ± SEM. *, p<0.05; **, p<0.01; ***,p<0.001; ****, p< 0.0001.

2. Invasive cells adhere preferentially to specific ECM components in contrast to non-invasive cells

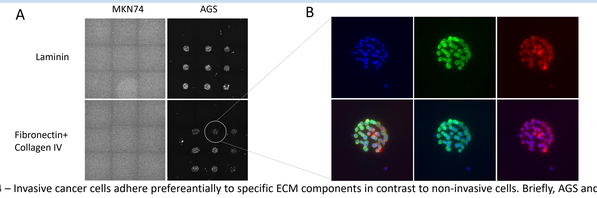


Figure 4 – Invasive cancer cells adhere prefereantially to specific ECM components in contrast to non-invasive cells. Briefly, AGS and MKN74 gastric cancer cells were grown in ECM Array Platforms (MicroMatrix 36, MicroStem) under the same conditions. Nuclei were stained with DAPI (blue) and cells were stained for α-tubulin (green) and for a plasma membrane marker (red). Nuclear and cellular morphological parameters were evaluated (data not shown). Representative images are shown in (A) and (B) (objective: 20x and 40x, respectively).

3. Nuclei of Wt E-cadherin cells are more compact than nuclei of mutant E-cadherin cells growing in Laminin and Vitronectin

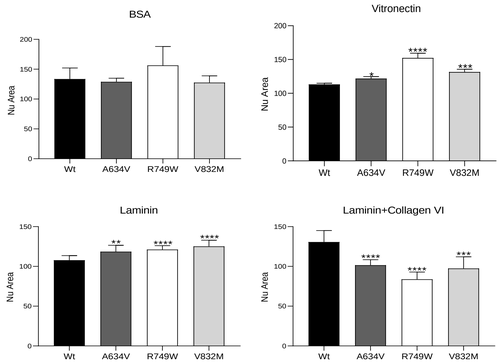


Figure 5 – Nuclear area of cells transfected with Wt or mutant E-cadherin. Briefly, AGS cells transfected with Wt or mutant E-cadherin were grown in an ECM Array Platform (MicroMatrix 36, MicroStem). Nuclei were stained with DAPI and cells were then evaluated for nuclear morphological parameters. The mutants A634V, R749W and V832M affect, respectively, the extracellular, the juxtamembrane and the intracellular domains of the protein. Data represent means ± SEM. *, p< 0.05; **, p< 0.01; ***, p< 0.001;****, p< 0.0001.

4. Nuclei of E-cadherin mutant cells have distinct textural features from those of Wt E-cadherin growing in Laminin

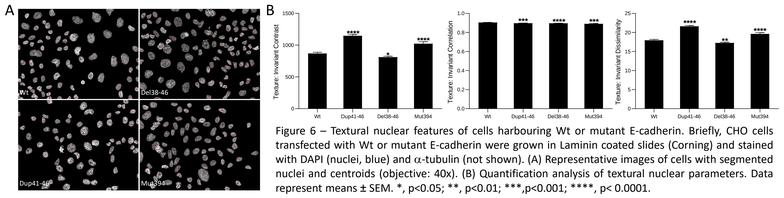


Figure 6 – Textural nuclear features of cells harbouring Wt or mutant E-cadherin. Briefly, CHO cells transfected with Wt or mutant E-cadherin were grown in Laminin coated slides (Corning) and stained with DAPI (nuclei, blue) and α-tubulin (not shown). (A) Representative images of cells with segmented nuclei and centroids (objective: 40x). (B) Quantification analysis of textural nuclear parameters. Data represent means ± SEM. *, p<0.05; **, p<0.01; ***,p<0.001; ****, p< 0.0001.

5. Microtubules of cells with disrupted E-cadherin are shorter, have uniform length patterns and are more compactly distributed

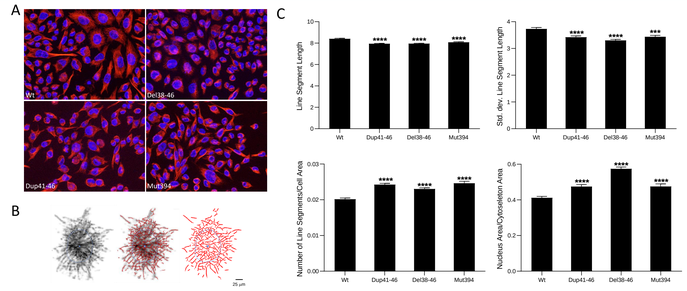


Figure 7 – Cytoskeletal features of cells harbouring Wt or mutant E-cadherin. Briefly, CHO cells transfected with Wt or mutant E-cadherin were grown in Laminin coated slides (Corning). (A) Representative images of cells stained with DAPI (nuclei, blue) and α-tubulin (red) (objective: 40x). (B) Example of cytoskeleton processing: deconvoluted image (left), skeleton structure following preprocessing (middle) and line segment detection (right). Nucleus and centroid are represented in blue. (C) Quantification analysis of cytoskeletal parameters. Data represent means ± SEM. ***,p<0.001; ****, p< 0.0001.

(1) The invasive abilities of cancer cells are associated with unique NUCLEAR and CYTOSKELETAL ARCHITECTURE.

(2) Invasive cancer cells adhere preferentially to specific ECM components.

(3) There is an association between nuclear phenotypes and increased attachment abilities, suggesting that the ECM may modulate nuclear morphometrics thus affecting the invasive behaviour of cancer cells.

(3) Cell lines harbouring E-cadherin mutations corroborate the existence of a nuclear signature associated with invasive potential.

(4) The proposed computational framework demonstrates that cells with disrupted E-cadherin have specific cytoskeleton architectural patterns.

CONCLUSION

Overall, this study demonstrates that the use of nuclear and cytoskeleton architectural features could provide an efficient strategy to identify cells with invasive potential. Further investigation and integration of this approach with other cellular properties could have an impact in diagnosis, prognosis and therapeutic strategies.

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