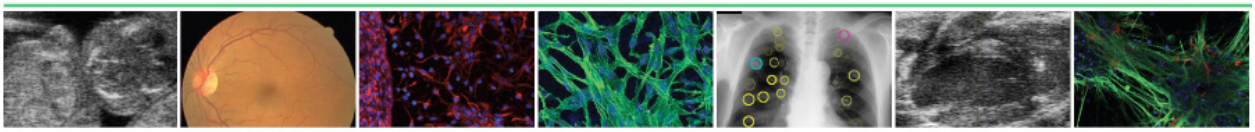
BIOIMAGING 2012
INTERNATIONAL SYMPOSIUM IN APPLIED BIOIMAGING
BRIDGING DEVELOPMENT AND APPLICATION
BIBLIOTECA ALMEIDA GARRETT, PORTO, SEPTEMBER 20-21, 2012



Submitting Abstract: *Oral*

Characterization of E-Cadherin Distribution from Fluorescence Images

J. Miguel Sanches[1], Joana Figueiredo[2], Isabel Rodrigues[1], Raquel Seruca[2]

[1]Institute for Systems and Robotics,
Department of Bioengineering-Instituto Superior Técnico / Technical University of Lisbon

[2]IPATIMUP - Institute of Molecular Pathology and Immunology of the University of Porto, Portugal

(corresponding author[1]: J. Miguel Sanches (jmrs@ist.utl.pt))

Introduction

In epithelia, cell-cell adhesion is mainly mediated by E-cadherin. Cadherins are known to undergo dynamic changes within the cell and their localization is dependent on protein trafficking. In a homeostatic situation transient expression of E-cadherin can be observed at the cytoplasm, but the highest level of expression is seen at the plasma membrane where the protein is stably clustered.

In cancer, alterations of E-cadherin are very common and significantly associated to loss of cell-cell adhesion. In familial forms of gastric cancer E-cadherin mutations may occur at germline level and mutant carriers are at high risk of developing early-onset (<40 years) invasive gastric cancer without any evidence of symptoms. E-cadherin mutants are unstable at the plasma membrane and are prematurely degraded, leading to E-cadherin loss/reduced or aberrant expression and abnormal cell morphology and behavior. We have established a functional assay to predict the clinical relevance of E-cadherin mutants but we still need to improve on the classification of these mutants found in a cancer context. To achieve this goal we developed a quantitative method to determine the profile of E-cadherin expression at cellular level.

AIM

The main goal of this work is to extract morphological and textural features from the microscope images in order to characterize the distribution of wild-type and mutant E-cadherin, predict its role in the adhesion process and advance in the underlying molecular mechanisms in order to determine their clinical impact.

Materials and Methods

Cells with normal E-cadherin can be discriminated from cells with mutated molecules trough the characterization of its intra- and inter-cellular distribution. This distribution can be observed from microscopy images (40x) of fluorescence where the E-cadherin molecules were immunostained with fluorescent dyes (Alexa 488).

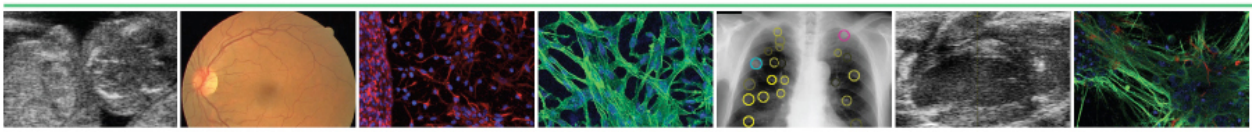
In normal cells these molecules are absent at the nucleus, uniformly distributed in the cytoplasm with a characteristic pattern and highly concentrated at the membrane where they play a key role in the cellular adhesion process. On the contrary, the mutated molecules follow a different distribution, characterized by lack/reduced E-cadherin signals at the membrane and/or large concentrations of the molecule at the perinuclear region at the cytoplasm.

The following measures associated with E-cadherin distribution assume particular relevance for oncologists and biologists dedicated to cancer; i) width of signal intensity in cell to cell contact, ii) number of cell to cell contact points between cells, iii) loss of signal intensity at cell membrane and vi) aberrant cytoplasmic signal intensity.

Two main image processing challenges are addressed here to extract useful biological information from E-cadherin immunofluorescence images.

---

[1] This work was supported by the FCT project [PEst-OE/EEI/LA0009/2011]



First, the amplification gain needs to be enlarged due to the low amount of detected fluorescence dots in the images. Therefore, the images present low signal-to-noise ratio (SNR) and are corrupted by a type of multiplicative noise with Poisson distribution. Second, to discriminate normal from mutated E-cadherin, the relevant distribution features were computed in a radial basis by taking the nucleus as references, as shown in Figure 1. These features are strongly dependent and distorted by morphological differences between cells, namely, by deviations from the ideal circular geometry.

Here, an automatic procedure is used to segment the nuclei of the cells and to extract radial intensity profiles (Figure 1) that reflect the distribution of the molecule at the cytoplasm and intercellular space (membrane expression) (Figure 2).

Denoising and geometric compensation procedures are applied to these profiles to attenuate the noise and compensate for geometrical differences along the angle of the profile.

Results and Discussion

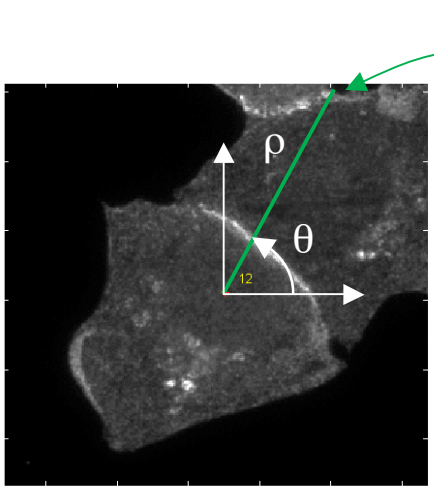


Figure 1

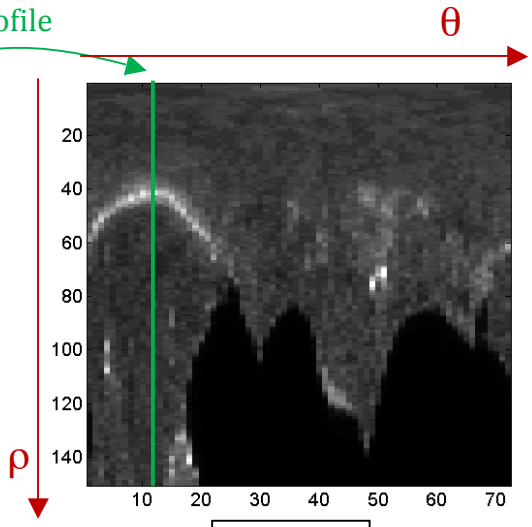


Figure 2

Herein, we present a novel image processing method to characterize the intra and intercellular distribution of E-cadherin. We are able to generate profiles (Figure 2) that can distinguish wild-type and mutant forms of E-cadherin and advance in the mechanisms of protein regulation. This method will allow the assessment of the functional impact of E-cadherin mutants in adhesion and help on the genetic counseling and surveillance of individuals that are at high risk of invasive cancer.

References

Suriano G, Oliveira C, Ferreira P, Machado JC, et al. Identification of CDH1 germline missense mutations associated with functional inactivation of the E-cadherin protein in young gastric cancer probands. Hum Mol Genet. 2003 Mar 1;12(5):575-82.

Figueiredo J, Söderberg O, Simões-Correia J, Grannas K, Suriano G, Seruca R. The importance of E-cadherin binding partners to evaluate the pathogenicity of E-cadherin missense mutations associated to HDGC. Eur J Hum Genet. 2012 Aug 1. [Epub ahead of print]

Rodrigues I. and Sanches J. Convex Total Variation Denoising of Poisson Fluorescence Confocal Images with anisotropic filtering. IEEE TRANSACTIONS ON IMAGE PROCESSING. VOL. 20, NO. 1, JANUARY 2011.