Gilles Carpentier: Angiogenesis Analyzer for ImageJ

Abstract

Scope: In the field of cancer therapy, it is now well admitted that the use of anti-angiogenic molecules is a promising strategy to cure cancer. In this context, several in vitro as well as in vivo experimental models have been developed to select angiostatic molecules and further study their properties. Several of these tests have been developed and the most used of them is the in-vitro differentiation of primary endothelial cells culture in biocompatible gel (Endothelial Tube Formation Assay (ETFA)) [1]. The cells used in these assays, most often coming from the umbilical cord (Human Umbilical Vein Endothelial Cells, (HUVEC)) or from bovina aorta (adult bovine aortic endothelial cell (ABAE)), differentiate by presenting long extensions and cell alignments. These structures that can branch, mimic a pseudo capillary in-vitro formation. At later stage, this differentiation leads to a formation of a meshed network from different sizes. Although widely used, the interpretation of this assay still presents some problems, especially to obtain a quantitative evaluation of the vessels-like and their organisation. We propose the “Angiogenesis Analyzer” [2] as a simple and precise tool to quantify the ETFA experiment images.

Materials and Methods: HUVEC cells were cultured in matrigelTM during 24 hours. Phase contrast images were taken with an inverted microscope coupled to a CCD camera at different time of the cell culture. The obtained images were analyzed using the “Angiogenesis Analyzer” tool, programmed in ImageJ’s macro language.

Analysis and Specifications: The first steps of the analysis consisted in a segmentation of the cell areas followed by a skeletonization. The skeleton analysis was then first performed through the detection of pixels corresponding to extremities (1 neighbor pixel), nodes (3 neighbor pixels) and groups of nodes forming real junctions (one or more nodes). Secondly, from these connection elements, the pseudo vascular tree analysis was continued by sorting the different peaces resulting from the cut off of the tree into branches (peaces connected to one extremity and one node or junction) and segments (peaces connecting to 2 nodes or junctions). To improve the precision of the analysis, the distinction was made between junctions implicated into branches, and junctions delimiting segments forming meshes. Finally, the tool which can manage batch of images, returned an Excel like table containing the different extracted parameters among whom branching index and meshing index. The program contains the generally required online functionalities of software : documentation, demo images for training and update facilities.

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Keywords

angiogenesis, cancer therapy, endothelial cells, meshed network, skeleton

Short CV

Research Assistant in Biochemistry starting in 1987, I first worked on proteomic and crustacean endocrinology at the Laboratoire de Biochimie et Physiologie du Développement, Ecole Normale Supérieure de Paris URA CNRS 686 (France). After an experience in cryobiology of sea organisms at the “Centre Océanologique du Pacifique de l’IFREMER (French Polynesia), I joined the Université Paris 12 in 1991. Here, I first worked on biochemistry of muscle differentiation, and I was led to be responsible of a pole of image facilities in the laboratory. I have been developing image processing and analysis tools using the ImageJ macro language since 2005. Engineer since 2006 at the Faculté des Sciences et Technologie, Université Paris Est Créteil, I'm now managing the confocal microscopy imaging facility of the Laboratoire CRRET, EAC 7149.

Administrative data

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