QUANTIFICATION OF F-ACTIN STRUCTURES IN ASTROCYTOMA CELLS IN RESPONSE TO CANDIDATE PHARMACEUTICALS

Chi Cui[1,3], Tommy Turbyville[1], Prabhakar Gudla[1], Kaustav Nandy[1], John Beutler[2], Joseph Jaja[3], and Stephen Lockett[1]
[1]Image Analysis Laboratory, National Cancer Institute, SAIC-Frederick, Frederick, MD, USA, [2]Molecular Targets Development Program, National Cancer Institute, Frederick, MD, USA, [3]Electical and Computer Engineering, University of Maryland, College Park, MD, USA
E-mail: cch2000@umd.edu, slockett@ncifcrf.gov

KEY WORDS: Living cells, F-actin, confocal microscopy, 2D image quantification

The distribution, directionality and motility of the actin fibers control cell shape [1], affect cell function and are different in cancer versus normal cells [2]. Quantification of actin structural changes is important for further understanding differences between cell types and for elucidation the effects and dynamics of drug interactions. We developed software to quantify key features of F-actin from confocal microscope images of glioma cells. Cells were treated with three candidate pharmaceuticals that interact with actin pathways: OSW1, Schweinfurthin A (SA) and a synthetic cephalostatin 1 derivative (ceph). For controls, cells were treated with reagents well known to inhibit actin: cytochalasin B (cytoB) that directly interacts with actin, and Y-27632 (Y) a ROCK inhibitor that indirectly affects actin, as well as with the vehicle control, DMSO. The inhibitors and the candidate pharmaceuticals have been observed visually to cause discreet changes in F-actin organization. Fixed cell cultures were stained with DAPI to label nuclei and facilitate single cell segmentation, and Phalloidin to label F-actin. Since images were quite small in the z dimension, maximum intensity projections in the z direction sufficed for analysis. The following measurements were made to quantify F-actin: cell area, nuclear area, F-actin area, and the occurrence of four categories of F-actin structures: cortical actin along cell borders, bright intracellular punctate points, stress fibers that traverse the cell, and "leading edge" F-actin at cell extremities. Validation of the automatic segmentation results was manual. Preliminary results showed uniquely significant increases in cortical F-actin to stress fiber ratio for increasing doses of OSW1 and SA and a less marked increase for ceph. This increase was not observed for the actin inhibitors: cytoB and Y. Future studies will further validate the algorithms, and elucidate the molecular pathways and kinetics underlying the F-actin changes. This is the first study quantifying different structural formations of the same protein in intact cells. Since many anti-cancer drugs target the cytoskeleton, we believe that the quantitative image analysis method reported here will have broad applications to understanding the mechanisms of candidate pharmaceuticals. Funded by NCI Contract No. HHSN261200800001E.

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