QUANTITATIVE MODEL-BASED IMAGE ANALYSIS OF SUB-VISUAL CHANGES IN NuMA DISTRIBUTION LINKS NUCLEAR ORGANIZATION WITH CELL PHENOTYPE

David W Knowles, Sophie A Lelievre, William S Chou, Aaron Lee, Wanling Wen, Carlos Ortiz de Solorzano, Mina J Bissell, Stephen J Lockett, Lawrence Berkeley National Laboratory, MS84-171 1 Cyclotron Road, Berkeley, CA 94720

The extracellular matrix (ECM) plays a critical role in directing cell behaviour and morphogenesis by regulating gene expression and nuclear organization. Using non-malignant human mammary epithelial cells (HMECs), it was previously showed that ECM-induced morphogenesis is accompanied by the redistribution of nuclear mitotic apparatus protein (NuMA) from a diffuse pattern, in proliferating cells, to a multi-focal (punctate) pattern as HMECs growth arrested and completed morphogenesis. When these cells were cultured as monolayers on plastic, NuMA distribution was diffuse in proliferating HMEC, while it appeared slightly aggregated upon induction of growth-arrest. Interestingly there was no visual difference in NuMA distribution between proliferating non-malignant and malignant HMECs. Here we present a novel model-based image analysis algorithm which quantifies the punctateness of NuMA and allows clear distinction not only between growth arrested and proliferating non-malignant cells but also between proliferating non-malignant and malignant cells, cultured as monolayers. Cell cultures were imaged in 3D using confocal microscopy, for fluorescently labeled NuMA, Ki-67 and DNA. Nuclear segmentation, based on the DNA staining, allowed image analysis of NuMA staining within individual nuclei. Ki-67 staining was used to identify cells in the cell cycle. The image analysis algorithm was based on a multi-scale Gaussian blurring method and measured intensity variations within each nucleus. Averaging results over cells in each population resolved significant, yet, sub-visual differences in NuMA punctateness. Non-malignant growth arrested cells were most punctate, non-malignant proliferating cells produced intermediate values and malignant cells were the least punctate. This ability to discern cell phenotype based on quantifying the spatial distribution of a nuclear protein has broad application in furthering fundamental understanding of biological processes.