BandW : The 2-DE image analysis software using MATLAB

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Since genomics represents only the first step in understanding celluar physiology, it needs to be complemented by systematic analysis of the proteins, termed proteomics. The primary means studying protemics has been with use of 2-demer sional electrophoresis (2DE) since 1975. 2DE initially separate each protein according to its electric charge content by using isoelectric focusing and further separation of similar charged proteins are accomplished by using soduim dodecyl sulfate polyacrylamide gel electrophoresis(SDS-PAGE). But Analyzing two-dimensional gel electrophoresis(2DE) is complicated and time-consuming. In order to facilitate the process, we compiled "BandW" which is an image analysis tool made using MATLAB(Matrix oriented computing engine). BandW analyzes the input image using M by N matrix which has a value between 0 to 255. Each value represent a pixel intensity. Image output is processed to TIF(Tagged Indexed Fileformat) format. BandW has simplified layout which utilizes image processing toolbox and graphic user interface engine embedded in MATLAB 5.3. During analysis, spot volume is calculated using contour algorithm. Volume calculation starts with combining outer boundary of same pixel intensity in the adjacent matrix until the circular loop is completed. The area within the loop actually represent a three dimentional volume. We tested BandW by analyzing 2DE in liver mitochondria of young (LCY) or liver mitochondria of old (LCO) rats. Our results, using BandW, were identical to values obtained from available commercial softwares. In addition, BandW is 3D capable as only one other highly priced software.

[PC1-50] [04/18/2002 (Thr) 14:00 - 17:00 / Hall E]

Activation of PI3K is not Sufficient but Required for H-ras-induced Invasive Phenotype of MCF10A Cells

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We have previously shown that H-ras, but N-ras, induces an invasiveness and cell motility in human breast epithelial cells (MCF10A), while both H-ras and N-ras induce transformed phenotype. It has been recently shown that phosphatidylinositol 3-kinase (PI3K) plays an important role on cell migration. In the present study, we wished to investigate the functional role of PI3K in H-ras-induced invasive phenotype in MCF10A cells. The activation of PI3K was examined by detecting phosphorylation of Akt, a downstream molecule of PI3K, by Western blot analysis. We show that phosphorylated Akt level was upregulated both in H-ras MCF10A cells and N-ras MCF10A cells comparing to the parental MCF10A cells while the amount of Akt was equal in the parental, H-ras and N-ras MCF10A cells. The data indicate that activation of PI3K is not sufficient for invasiveness and motility since PI3K is also activated in the non-invasive and non-motile N-ras MCF10A cells. We investigated the functional significance of PI3K activation in invasion and motility by using PI3K inhibitors, LY294002 and wortmannin. Treatment of LY294002 and wortmannin significantly reduced invasive phenotype and motility of H-ras MCF10A cells, suggesting the requirement of PI3K activation for H-ras-induced invasion and motility. We then examined the effect of the PI3K inhibitors on matrix metalloproteinase(MMP) expression. Treatment of LY294002 inhibited secretion of MMP-2 and MMP-9 in a dose-dependent manner while wortmannin did not affect MMP levels in H-ras MCF10A cells. The possible role of Rac1 in H-ras-induced invasive phenotype in MCF10A cells are currently under investigation.

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Enhancement of Proliferation and Migration of Glioma Cells by Glial Cell-derived Neurotrophic Factor (GDNF) for the Development of an Artificial Nerve Tubing

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