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Identification of Actin-Based Stress Fibers with a Morphometric Shape Factor

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The laboratory employs an assay for cell shape phenotypes, which is based on an analysis of contours obtained from cells that are grown on solid substrate interferometers. The shapes of several contours are analyzed by computing the values for a total of 102 variables. A few of these variables, e.g. shape factor, are dimensionless at the outset, and the remainder are rendered dimensionless by normalizing them either to dimensions of the cell or the ellipse of concentration. The variables' values comprise a unique description of each cell. Using various classification methods, we studied cell lines that tested negative for tumorigenicity in whole animals at the outset, but gradually became tumorigenic [1]. The results suggest that lines becoming cancerous share some common changes, even if they originated from different tissues. One of the drawbacks of this assay, however, is that the variables are not intuitively related to morphological features of cells. This was addressed by making different combinations of variables to extract factors, which are based on common changes in variance of the variables' values. Of 20 factors extracted from a database of morphometric information, several correspond to specific features. When shape changes are broken down by factors, the biggest single, quantitative difference between normal and cancer cells is in factor #4 (microspikes/filopodia) [2].

The goal of the current work was to determine whether a morphological feature corresponding to factor #7 could be found. The important variables used in computing #7 were ALTI (mean altitude of projections), LNNC (mean length of negative curvature regions), MEDN (mean length of projection medians), WDTH (mean width of projections at base), and MINP (area of polygon formed by joining local minima). Some variables that were heavily weighted in computing #4 values had little weight in computing #7. WDTH was entered in both cases but with opposite sign (positive with #7 and negative with #4). The identification of #7 with a morphological feature was suggested by data obtained from a preneoplastic cell line, which had been derived and followed through the time course to tumorigenicity as described above. Cells transiently took on a phenotype like that of tumorigenic cells following treatment with a tumor promoter [3]. They showed enhancement of ruffling activity over a period of 0.5 to 5 hours, followed by ruffling suppression [4]. Visualizing actin in the cells, we found that the actin filament arrangement coinciding with the phase of ruffling suppression was suggestive of stress fiber formation (FIG. 1). Data on the frequency of cells exhibiting stress fibers over the time course confirmed this result (FIG. 2). Stress fibers form in response to activation of the RhoA GTPase downstream of EDG receptors. Since these receptors can be stimulated by adding lysophosphatidic acid (LPA) to the culture medium, further work was done to analyze cells treated with promoter alone or with

promoter and LPA. When these samples were processed for shape analysis, the results indicated that changes occurred in variables contributing to factor #4 (FIG. 3).

References

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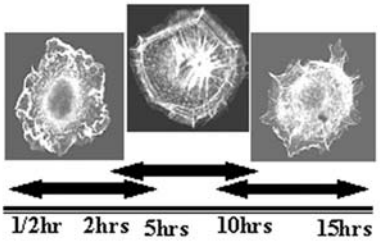

FIG. 1. Typical morphologies seen following administration of the phorbol ester tumor promoter, phorbol 12-myristate 13-acetate, at time zero. Views were taken in a Zeiss 410 laser confocal scanning microscope for times of 30 minutes to 15 hours. Ruffling is suppressed by 5 hours, and stress fibers begin to appear in a sizable fraction of the cells.

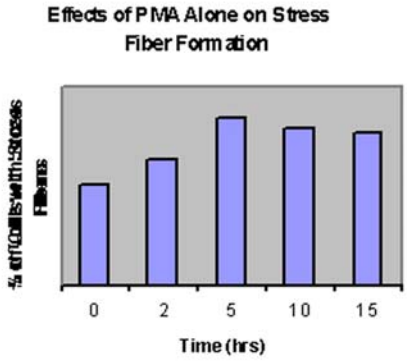

FIG. 2. Number of cells displaying stress fibers following administration of the tumor promoter at time zero. Cells were stained with a fluorochrome-tagged phalloidin and observed using a Zeiss Axiophot with FITC filter. Stress fibers increased during the initial time course, peaked at 5 hours, and then declined.

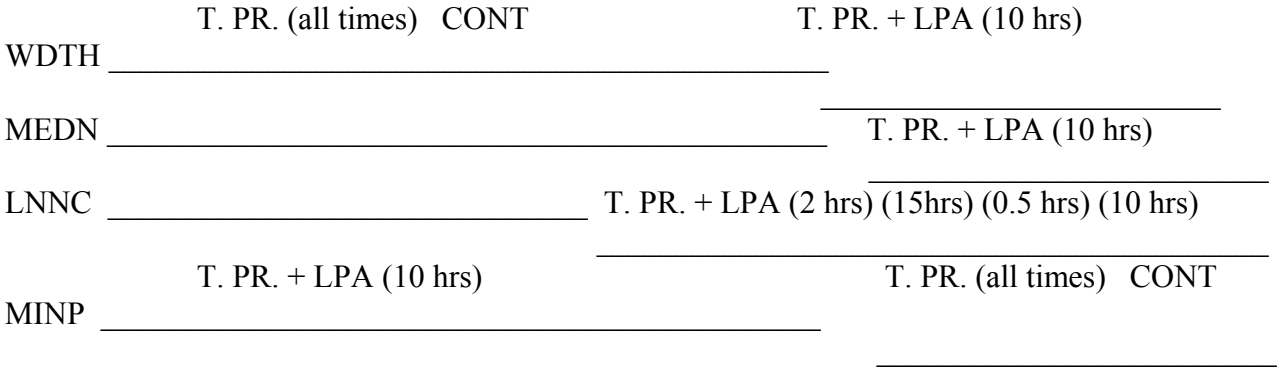

FIG. 3. Statistically significant differences among samples, for cells treated with the tumor promoter alone (T. PR.) or together with LPA (T. PR. + LPA) compared to untreated or control (CONT) cells. Cells were processed for shape analysis, and the derived values of the variables WDTH, MEDN, LNNC, and MINP were compared. The higher value of each variable is indicated at the right end of the scale. Note that values of the variable, MINP, are negatively weighted in the computation of factor #7, so the value for the control sample should exceed those of experimental.

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