A Microscopy Approach To Quantitatively Study Cell Morphology

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Introduction

U2OS is a human osteosarcoma cell line used widely throughout research. Due to its relatively large size and ease of culture, it has been used here to investigate genes that may play a role in cell morphology.

Small interfering RNAs (siRNAs) can be used to deplete the activity of specific genes. In this study, the effects of various genes associated with the plasma membrane and the cytoskeleton were examined: DNM2, KIF20A, RAC1, and RHOA.

The effect of down-regulating these genes can be monitored through fluorescence staining and microscopy. The fluorescent dye phalloidin-AlexaFluor 568 is used to visualize actin filaments within the cell, which play a major role in maintaining cell morphology. Paxillin is a focal adhesion protein and using fluorescently-tagged antibodies its presence in focal adhesions around the cell edges can be detected, providing insight into how cell adhesion and cell morphology might be affected by down-regulation of the target genes selected.

Research Techniques

Transfection

Cells grown in culture were transfected with specific siRNAs targeting genes associated with cell morphology

Immunofluorescence Staining

The cells were then stained with fluorescent dyes and fluorescently-tagged antibodies

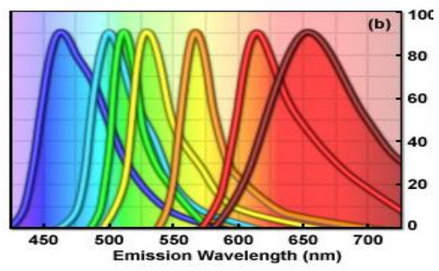
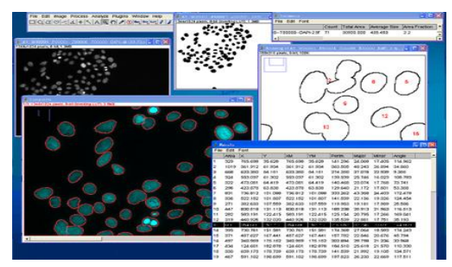


Image Acquisition

Images were acquired using a wide-field fluorescence microscope and then exported for analysis

Image Analysis

Imaging software, known as ImageJ, converted the cells' morphological characteristics into quantitative data



Results

1

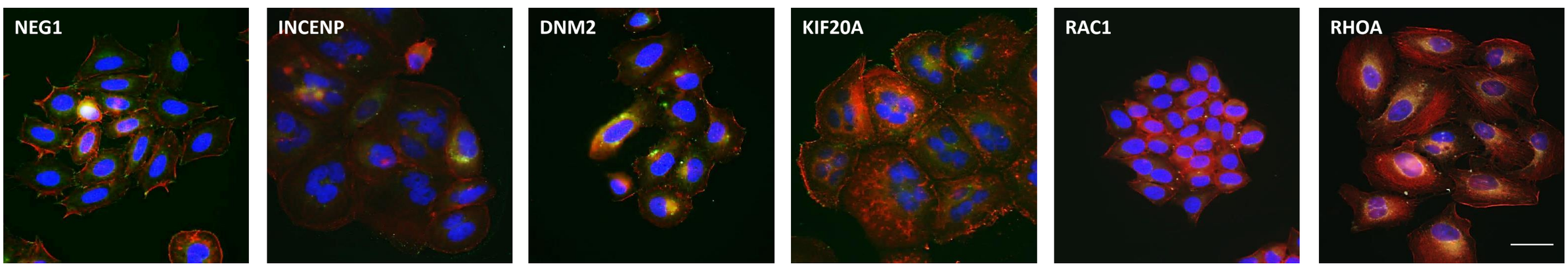


Figure 1: Selected composite images of each siRNA treatment (including controls – NEG1 and INCENP). Nuclei are stained with DAPI (blue), actin filaments are stained with phalloidin-AlexaFluor 568 (red) and focal adhesions are stained with AlexaFluor 488 tagged anti-paxillin antibodies (green). Scale bar is 50µm.

2

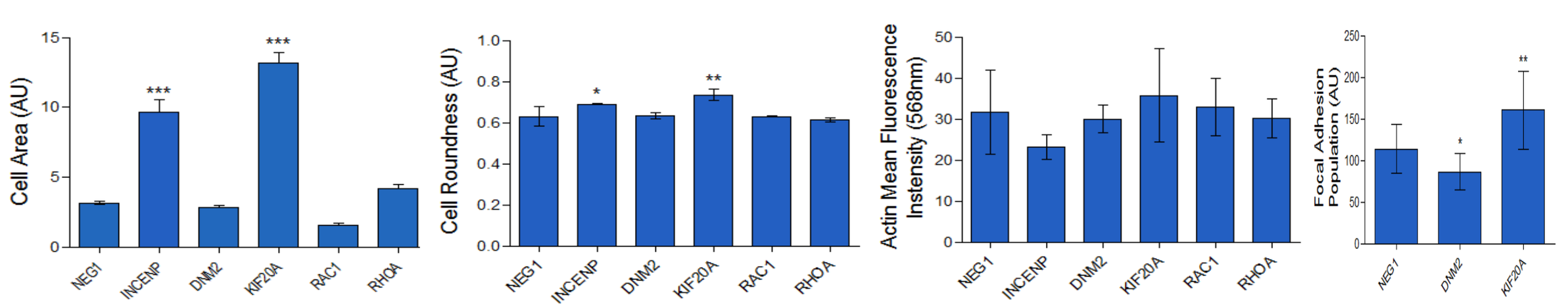


Figure 2: Changes in cell morphology and focal adhesion population were observed in response to certain siRNA treatments. NEG1 and INCENP serve as experimental controls. Depletion of KIF20A exhibits the most pronounced changes in morphological features and cell adhesion (*p<0.05, **p<0.01, ***p<0.001, n=3, ANOVA comparing all columns to NEG1). Data presented as Mean±SD.

Conclusions

INCENP acts as positive control targeting the inner centromere protein causing disruption to normal cell division. The appearance of multi-lobed nuclei indicate successful transfection of the siRNA.

Depletion of KIF20A induces significant morphological changes compared to NEG1 (negative control siRNA). KIF20A is a microtubule associated motor protein involved in cell division. When the activity of this gene is depleted the cell is unable to effectively divide, thus continues to replicate its material causing the cell to grow larger and rounder. The number of focal adhesions also increases in proportion to the amount of cell spreading.

RAC1 is a GTPase that has a variety of functions including the control of cell growth and cytoskeletal reorganization. Although the data do not reach statistical significance, there was an observed decrease in cell area compared to control when RAC1 activity was depleted.

No significant changes were observed in actin mean fluorescence intensity, which may indicate that although actin distribution is affected by the silencing of the genes selected for this study, its expression remains unchanged within the cells.