The use of high-content analysis to develop a high-throughput wound healing assay for cancer research

Dr. Remko de Pril

Galapagos, P.O. Box 127 2300, AC Leiden, Netherlands, remko.depril@glpg.com

The propensity of cancer cells to close an open wound in a cell monolayer is thought to predict their migratory ability in metastasis. Using the SilenceSelect™ adenoviral shRNA knock-down library we have established a high-throughput wound healing assay to identify novel genes involved in cell migration. We used a 96-pin scratch tool to apply a mechanical scratch-wound in a cellular monolayer and assessed wound healing using high-content imaging on an IN Cell Analyzer 1000 to measure the remaining scratch wound. We validated this model by demonstrating that several knock-down constructs targeting known players in motility, including CXCR4, PIK3CA, and ROCK2, inhibit wound healing. We then screened our adenoviral knock-down library, which focuses on drugable targets, and identified a high number of novel genes associated with tumor motility.

Introduction

Malignant cells have the ability to migrate throughout the body eventually resulting in distant tumors. Cancer cells maintain their migratory capacity *in vitro* and this is mediated by the same signaling pathways that affect the tumor *in vivo*. The basic mechanisms that underlie cellular movement, including polarization, actin remodeling, and growth cone formation, are preserved in both three-dimensional and two-dimensional migration. In general, the propensity of cells to close an open wound in a cellular monolayer is thought to predict their migratory ability.

To identify new targets that play a role in cancer cell migration, we used a human PC-3 prostate adenocarcinoma cell line, an adenoviral shRNA knock-down library (SilenceSelect) containing over 12 000 human shRNA vectors, and IN Cell Analyzer 1000 to develop a high-throughput wound healing assay to screen for drugable genes that affect the motile behavior of tumor cells. To validate this approach, we developed a 3D invasion assay as a secondary screen using cells transduced with targets that inhibit motility identified from the wound healing assay.

Method

For the wound healing assay, PC-3 cells were seeded in 96-well microplates and transduced with the SilenceSelect adenoviral knock-down shRNA library. Transduced cells were cultured for six days to allow for optimal knock-down of mRNA and to form a confluent monolayer. Next, a 96-pin scratch tool was used to apply a consistent mechanical scratch wound of 0.4 mm width (Fig 1A) in the cellular monolayer. After scratch formation, the plates were washed and refreshed on a liquid handling robot and the cells were allowed to migrate into the open space over a period of several hours. Subsequently, the cells were fixed and then imaged using IN Cell Analyzer 1000.

To validate the inhibitory effect of the knock down constructs in a secondary biological assay, we transferred human A549 lung carcinoma cells transduced with the targets from the primary screen of the wound healing assay to Matrigel™ coated Boyden chambers and performed a 3D invasion assay. FCS and EGF were used as chemoattractants and, after invasion, cells were stained with phalloidin and imaged. Cells were then segmented using phalloidin images to measure the number of cells that invaded.

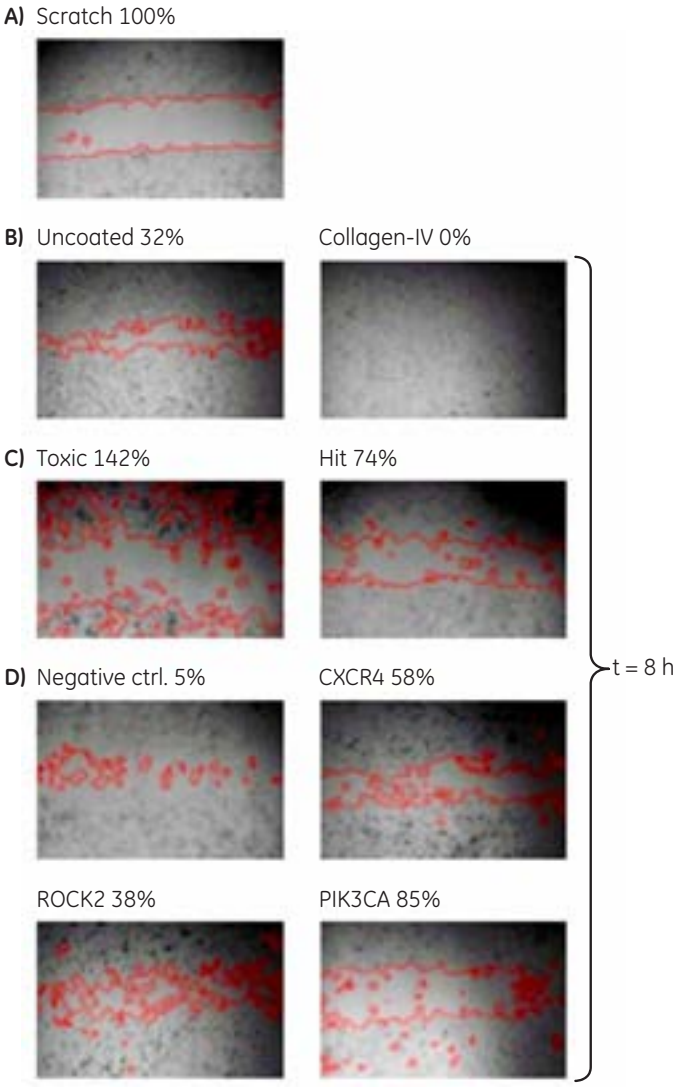

Fig 1. Inhibition of wound healing. Representative images of negative and positive functional controls 8 h after scratch formation (**B** to **D**). The percentage of open space remaining relative to the initial scratch at time 0 (**A**) is shown. Red overlay shows the open space segmentation. Images previously published as part of an article by Dr. R. de Pril *et al.* in *BTi*, **21(2)** (2009) and reproduced with permission (1).

Results

For the wound healing assay, we used transmitted light imaging for segmentation and quantitation of the scratch wound that remained open using an in-house analysis routine written with IN Cell Developer software. The algorithm enabled genes whose knock-down inhibit cell migration to be identified and open spaces between cells in the monolayer to be measured without additional staining or handling of the plates. This high-throughput approach allowed for identification of knock-down constructs that affect proliferation, adherence, or viability of the cells (Fig 1C) and these constructs could be excluded without the need for an additional toxicity assessment.

The tumor microenvironment is an important mediator of cancer cell behavior and therefore, to optimize the wound healing assay, we also used the analysis routine to evaluate the effect of the extracellular matrix coating on migration. The results show that a collagen-IV coating increased the motility of prostate cancer cells compared to uncoated plates (Fig 1B).

To benchmark the assay and to control for plate-to-plate and batch-to-batch variation, we included a series of positive and negative controls on each screen plate. Disease-relevant genes were selected as putative positive controls and tested for their effect on the phenotypic response in the respective screen. Two independent knock-down constructs targeting CXCR4 demonstrated a clear reduction in motility of the prostate cancer cells. Furthermore, we demonstrated that knock-down constructs for ROCK2 and PIK3CA also inhibited motility and that constructs directed against unrelated genes had no effect on migration (Fig 1D).

Conclusion

Aggressive spread of cancer cells is attributed to mesenchymal cells that have overcome the inhibitory effect of cell adhesion within the tumor. We thus identified a set of genes specifically involved in the migratory pathways involved in metastasis. In this high-content screen, we identified a large number of novel genes associated with tumor motility. These targets were further validated in the Boyden chamber assay and we will further assess the role of these targets in cell motility. As the adenoviral knock-down library focuses on drugable targets, this screen has yielded hits that can be employed quickly to generate small molecule compounds or antibody therapeutics. These results demonstrate the strength of combining relevant biological assays with adenoviral functional genomics and high-content screening.

Acknowledgements

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Reference

1. Dr. Pril, R. *et al.* A high-content screen for inhibitors of cell migration in cancer metastasis using adenoviral knock-down. *BTi*, **21 (2)**, 16–18 (2009).

The benefits of HCA for significantly increasing assay precision and productivity are described in more detail in a Case study article; visit www.gelifesciences.com and search for document 28-9906-37 under the Literature tab.

For more information on GE Healthcare Life Sciences' IN Cell Analyzer System, visit www.gelifesciences.com/incell