APPLICATION NOTE

Functional Characterization of Anti-Metastatic Agents Using Multi-Parametric Readouts

INTRODUCTION

Metastasis is a complex process by which cancer cells spread from a primary tumor to other parts of the body, forming secondary tumors in distant organs and tissues. This process is responsible for the majority of cancer-related deaths and represents a formidable challenge in cancer research and treatment. The ability of cancer cells to metastasize requires a range of biological adaptations, including enhanced cell motility, invasion, resistance to cell death, and the ability to interact with the microenvironment of distant organs. Understanding the mechanisms that underly metastasis is crucial for developing effective therapeutic strategies to prevent or treat the spread of cancer.

Existing approaches to evaluate metastasis encompass *in vivo* models and select low-throughput *in vitro* assays. The development of a scalable phenotypic assay for metastasis is essential for identifying therapeutics that can target this process, ultimately enhancing patient prognosis.

In this application note, we use a known inhibitor of metastasis in a triple negative breast cancer model, to assess *in vitro* phenotypes that may serve as indicators of anti-metastatic properties. We observe responses across multiple functional and morphological parameters,[1] demonstrating the utility of electrical imaging as a tool to study metastasis.

RESULTS

Modulation of MDA-MB-231 Cells with Dexamethasone Treatment

MDA-MB-231 cells are a model of triple negative breast cancer, known for their aggressive metastatic behavior *in vivo* and extensively used in *in vitro* studies.[4] Dexamethasone is a synthetic glucocorticoid, a type of corticosteroid hormone known for its anti-inflammatory and immunosuppressive properties. It has been shown to be anti-metastatic, specifically in mouse models of triple negative breast cancer[2] and inhibits cell migration in cells prone to metastasis.[3,4]

MDA-MB-231 cells were seeded on CytoTronics' microplates, allowed to grow for 24 hours, and then treated with Dexamethasone (1 µM). Morphological and functional changes across various parameters were monitored.

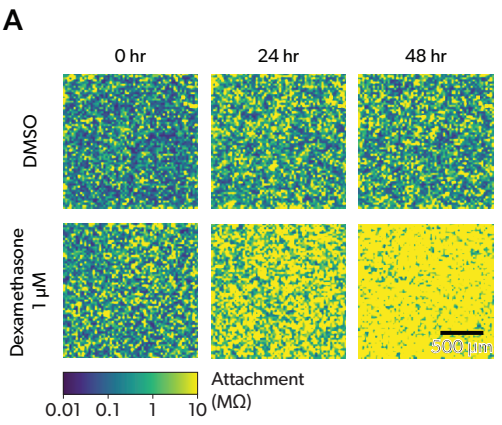

Figure 1. (A) Attachment images for MDA-MB-231 cells treated with DMSO or 1 µM Dexamethasone. (B) Time-normalized attachment upon compound treatment with DMSO (gray) or 1 µM Dexamethasone (blue).

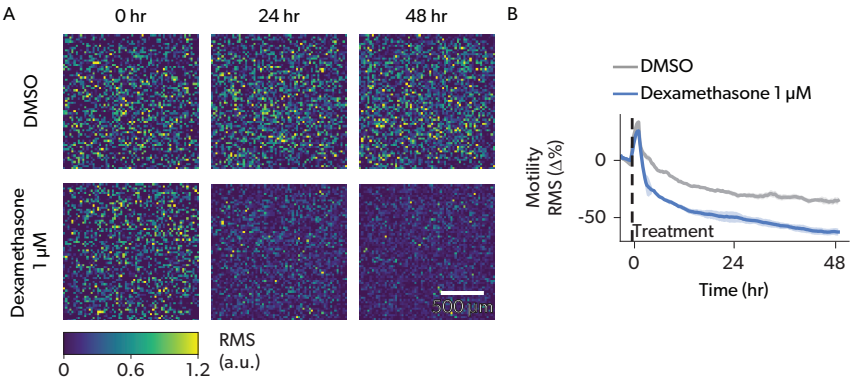

Figure 2. (A) Motility images for MDA-MB-231 cells treated with DMSO or 1 µM Dexamethasone. (B) Time-normalized motility upon compound treatment with DMSO (gray) or 1 µM Dexamethasone (blue).

We observed changes in certain key parameters throughout the course of the experiment, with cell attachment and motility being of particular interest. Figure 1 shows the effect of Dexamethasone on cell attachment. Cell attachment increases after Dexamethasone addition, as seen in the attachment images (Figure 1A), and plots of median well attachment over time (Figure 1B). Loss of cell attachment to the extracellular matrix (ECM) is one of the first steps in the metastasis process, whereas an increase in attachment could indicate a lower metastatic potential of cells.

Figure 2 shows the effect of Dexamethasone on motility. Cell motility significantly decreases after Dexamethasone addition, as seen in the motility images (i.e. frame to frame difference) (Figure 2A), and plots of median well motility over time (Figure 2B). Metastatic cells generally show an increase in invasion, migration, and general motility of which motility is shown here to decrease upon treatment with Dexamethasone.

CONCLUSION

Studying intricate biological phenotypes, such as cancer metastasis, presents challenges *in vitro*, where a singular readout may prove insufficient. Our research showcases the effectiveness of multiparametric readouts, facilitating the creation of *in vitro* assays tailored for metastasis studies. This assay could be further extended to model physiological conditions with the addition of ECM or monitoring phenotypes in heterogenous cell populations. This approach not only enhances the comprehensiveness of data but also offers scalability, enabling high-throughput applications in primary discovery with remarkable ease.

METHODS

Cell Lines and Culture

MDA-MB-231 (HTB-26) were obtained from ATCC, cultured in DMEM supplemented with 10% FBS and 100 U/mL Penicillin-Streptomycin, and maintained in a humidified incubator at 37°C and 5% CO_2.

Treatment and Measurement

Impedance measurements were taken inside a humidified incubator at 37°C and 5% CO_2 every 15 minutes throughout the experiment. MDA-MB-231 cells were seeded at 20k cells per well and grown for 24 hours. Dexamethasone (Selleck Chem S1322) was added at 1 µM with a 0.5% (v/v) DMSO control. Compound in media was temperature and CO_2 equilibrated prior to addition.

Data Analysis

The well median of each measurement (attachment and motility) was plotted over time, with the standard error calculated across three technical replicates. Relative measurements were calculated by normalizing to values one hour before compound addition.

REFERENCES

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