Differential Biology of Tumor Cell Migration and Invasion Through Bio-Matrices Measured with 96-Well Live-Cell Kinetic Imaging Assays

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Summary and Impact

Cell migration and invasion is a pivotal event in a range of physiological and pathological processes including inflammation, wound healing & tumour development. We have evolved the scratch wound method into an image-based, facile, robust, fully kinetic 96-well model of both cell migration and invasion. The approach is amenable to many cell types and screening of small molecules, biologics and gene-interference reagents (e.g. siRNA, miRNA).

- Kinetic pharmacology reveals temporal differences in the profile of different pharmacological agents.
- Differential pharmacology was seen when a biomatrix was included in the model. Notably, bio-matrix-dependent effects were also observed.
- This model displays morphological, temporal and pharmacological hallmarks of in vitro tumour cell migration and invasion.

96-well Scratch Wound Assay - An Integrated Solution

Validation of Cell Morphology and Wound Quality

- Mean migration time-course (HT1080 cells). Data is expressed as the individual well value (grey symbol) and mean (black symbol). Inset: distribution histogram showing initial wound widths.
- Highly consistent wounds

Cell Signaling Inhibitors Yield Different Temporal Profiles

- HT1080 mean time-course data expressed as % RWD vs. time. Compounds added to cells immediately after wound creation (0h). Data from four 96-well plates.
- Note immediate attenuation of migration by wortmannin, consistent with a role of PIK in defining cell polarity and the leading edge.
- Note attenuation of later phases of migration by CCT081859 (IκBα inhibitor).

Differential Biology of Tumor Cell Migration and Invasion

- HT1080 cells migrate very rapidly when no ECM is present in the wound.
- Blebbistatin yields only small effects on migration and only at the highest concentration tested.
- GM6001 (up to 10 µM) does not effect the migration of HT1080.

More Complex Models: Invasion in Co-Culture

- The time-course of invasion on collagen-1 is even more rapid than that observed for Matrigel.
- Blebbistatin inhibits HT1080 invasion through Matrigel in a concentration-dependent manner.
- GM6001 inhibits HT1080 invasion through collagen-1 in a similar manner to that observed for Matrigel.

Use of fluorescent labels enables the investigation of migration and invasion phenotypes in mixed cultures.

The non-invasive MCF-7 cells fail to penetrate Matrigel, whereas the highly invasive HT1080 cells fully invade.

Notes:

- Cell morphology remains consistent
- Very rapid migration; note the even wound. The time-course of invasion is considerably slower than that observed for Matrigel
- HT1080 cell invasion has a slower time-course. Note spike like fillopodia and invasive tracks through which neighbouring cells follow.
- HT1080 cells migrate rapidly into the wound when no ECM is present.
- Only invasive cell types, such as HT1080 and MDA-MB-231 cells, enter the wounded area when ECM (Matrigel) is present in the wound. The time-course of invasion is considerably slower than migration and is gel density dependent.
- Non-invasive cell types, such as MCF-7 cells, fail to invade the wounded area when ECM is present.