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 analysis reveals temporal differences in the profile of different pharmacological agents.
- Differential pharmacology was seen when a bio-matrix 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

- Highly consistent wounds
- Migration time-courses (HT1080 cells). Data is expressed as the individual well value (gray symbol) and mean (black symbol). Inset: distribution histogram showing initial wound widths.
- Compatible with many cell types
- HT1080 cell invasion has a slower time-course
- Note sparse filopodia and invasive tracks through which neighbouring cells follow.

Migration and Invasion Time - Courses

96-well plate view

Migration vs invasion (BD-Matrigel™)

- Migratory cell types migrate rapidly into the wound when no ECM (Matrigel) is present in the wound.
- 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.

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 (0 h). Data from four 96-well plates.
- Note immediate attenuation of migration by wortmannin, consistent with a role of PI3K in defining cell polarity and the leading edge.
- Note attenuation of later phases of invasion by CCT081859 (siAkt inhibitor).

Differential Biology of 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.
- The time-course of invasion into Matrigel is markedly slower than migration.
- Blebbistatin, but not GM6001, inhibits HT1080 invasion through Matrigel in a concentration-dependent manner.
- The time-course of invasion into collagen-1 is more rapid than that observed for Matrigel.
- Blebbistatin inhibits HT1080 invasion through collagen-1 with a similar potency to that observed for Matrigel.
- GM6001 inhibits HT1080 invasion through collagen-1 in a concentration-dependent manner.

More Complex Models: Invasion in Co-Culture

- 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.