A Novel Assay for Cell Invasion using Cytostar-T Scintillating Microplates

INTRODUCTION
Cell invasion is typically measured using micro-porous filters coated with extra cellular matrix (ECM), which forms a barrier between an upper chamber containing cells and media, and a lower chamber containing media plus chemo-attractant. Counting the cells which invade through the filter to the lower chamber can be difficult and time consuming. We have developed a scintillation proximity assay using 96-well Cytostar-T, scintillating microplates, to measure the invasion of [14C] and [35S] labeled cells through ECM gel. In the test wells, a lower layer of ECM gel is added to form a barrier preventing the labeled cells from reaching the scintillating containing baseplate. The labeled cells are then added in an upper layer of ECM gel, and the microplate is incubated overnight. Only cells invading the lower layer of ECM gel and gaining proximity to the scintillant generate a signal in the assay. A variety of cell lines with invasive and non-invasive phenotypes have been examined, including the effects of inhibitor compounds.

METHOD
Control wells contained either 30 µl 100% ECM gel or 30µl medium. ECM gel was diluted with complete medium on ice, then added to the plate and incubated at 37°C for 2 hours to gel. To the wells containing a lower layer of gel, cells (50,000/well) were added on top as a suspension in 4mg/ml ECM diluted in complete culture medium. To the control wells without gel, the cells were added in culture medium. The microplate was incubated overnight and counted on the MicroBeta.

RESULTS

Figure 2: Photomicrographs of PC-3 cells (invasive prostate adenocarcinoma) after o/n incubation in (A) medium only (F-12K/7% FBS), in (B) 4mg/ml ECM gel over a lower layer of undiluted gel, or in (C) 4mg/ml/gel over a similar dilution of gel in the lower layer. The microscope was focused on the base of the well.

In order to quantify the number of cells reaching the baseplate, the cells were added to the microplate in an upper layer of 4mg/ml ECM gel containing 25Ci per well [14C]-lucine, methionine or [35S]methionine.

Figure 3A: Cytostar-T counts for cell invasion assay using various cell lines. CHO (non-invasive) and PC-3 (invasive) were added to 4mg/ml ECM gel diluted with F-12K/7% FBS. SVEC4-10 (invasive vascular endothelial) and BPH-1 (non invasive benign prostate hyperplasia) were added in ECM diluted with DMEM/10% FBS.

HT-1080 (invasive fibrosarcoma) were added in ECM gel diluted with MEM/10%FBS. The microplate was incubated overnight before counting in a MicroBeta. Results are presented as means (n=3).

CONCLUSIONS
- A simple assay for cell invasion has been developed using Cytostar-T microplates, which uses as a barrier a layer of ECM gel diluted with the culture medium of the test cell line.
- The response of several invasive and non-invasive cell lines has been investigated and the results match the reported invasive potential of these cells. Inhibition of this response was observed using Cytochalasin B and Paclitaxel.
- The assay incorporates in-well amino acid labelling of the cells, using either [14C] or [35S], and the isotope at 25Ci/well.

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