



A Novel In Vitro Bioassay for Screening Matrix Metalloproteinase Activity in Human Cancer Cell Lines (2003)

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Abstract

Introduction:

Metastatic cancer cells secrete large amounts of matrix metalloproteinases (MMPs). MMPs have been identified as agents that degrade the ECM and basement membrane, which allows their spread to distal organs. Increased activity of MMPs is associated with a number of cancers. There is compelling evidence to suggest that MMP-2 and MMP-9 play important roles in tumor invasion and metastasis. The design of new drugs to inhibit MMP activity is, therefore, a priority. However, several types of cancer cells do not express MMP-2 and MMP-9 *in vitro*.

Objective:

In this presentation, we report our observation that co-culturing cancer cells with normal human dermal fibroblast (NHDF) results in an enhanced expression of MMP-2 and MMP-9. We suggest that this *in vitro* bioassay can be used to screen drugs for their ability to inhibit or enhance MMP activity.

Methods:

- The preferred MMP inducer was phorbol 12-myristate 13-acetate (PMA) and the preferred MMP inhibitors included cycloheximide, actinomycin D, epigallocatechin gallate (EGCG), trans-retinoic acid (RA), dexamethasone (DE), H-7, FN-439, tranexamic acid (TEA), sodium selenite and N-acetyl cysteine. These reagents were purchased from Sigma.
- Human skin cancer cells (melanoma A 2058), liver cancer cells (HepG2), human fibrosarcoma cells (HT-1080), breast cancer cells (MCF-7 and MDA-MB-231) and colon cancer cells (HCT 116) were obtained from ATCC, and normal human dermal fibroblast was obtained from Bio Whittaker, Inc.
- Cells were grown in a T-75 culture flask in DMEM supplemented with 10% fetal bovine serum and antibiotics (complete media). At near confluence, the cells were trypsinized, washed and re-suspended in culture flask and complete media. NHDF (5×10^3) was mixed with melanoma, HepG2, HT-1080 and breast cancer cells (5×10^3) separately in 1 ml of complete

media in 24 well tissue culture plates. Cells were also seeded separately (104), incubated at 37°C and equilibrated with 95% air and 5% CO₂.

- At near confluence, the media was aspirated, cells were washed twice with PBS, and challenged with either inhibitors or the inducer in serum free media.
- After 24 hours, condition media was collected and MMP expression was carried on pre-casted zymogram gels.

Results:

- When cultured alone, MMPs were minimally expressed. Very faint bands corresponding to MMP-2 and MMP-9 were seen for HepG2 cells and melanoma cells. MMP-9 was not expressed in human breast cancer cells. When human cancer cells were co-cultured with NHDF, MMP expression was increased. MMP-2 and MMP-9 were increased significantly in HepG2 cells, and MMP-9 was increased in MDA-MB-231 cells. Melanoma cells had the most increase in both MMP-2 and MMP-9.
- In this experiment, we investigated whether physical contact between cancer cells and NHDF is important for the enhancement of MMP expression. The cancer cells did not stimulate MMP expression when they were cultured along with the condition media from NHDF, which suggests that physical contact between cancer cells and NHDF is necessary for stimulation of MMPs.
- When NHDF was co-cultured with melanoma, HepG2 and HT-1080 cells, there was a dramatic increase in MMP expression. MMP bands are well distinct and discrete, and the intensity of the bands was enhanced several fold. Both MMP-2 and MMP-9 expression increased significantly in HepG2 and HT-1080 cancer cells; melanoma exhibited the greatest increase in MMP-2 and MMP-9. The expression of MMPs was further enhanced by PMA (stimulator) and decreased by EGCG (inhibitor).
- The maximum stimulation of MMPs occurred when melanoma cancer cells were co-cultured with NHDF in 1:1 ratio and also with PMA.
- EGCG inhibited the expression of MMP-2 and MMP-9 in a dose-dependent fashion. The expression of MMP-2 and MMP-9 was significantly inhibited at 50µM and virtually undetectable at 150 µM. Similar results were obtained with sodium selenite and NAC.
- Cycloheximide inhibited the expression of MMP-2 and MMP-9 in melanoma cells, as well as in NHDF co-culture with melanoma cells in a dose-dependent manner. However, actinomycin D at various doses did not change their expression. These data confirm that MMP expression in cancer cells is sensitive to inhibitors of protein translation but not DNA transcription.
- When co-cultured with NHDF, MMP expression was enhanced. PMA stimulated further increased expression of MMP. PMA-mediated MMP expression was inhibited by RA, EGCG and H-7, whereas DE, FN-439 and TEA had no effect. Thus, the expression of MMPs is sensitive to some inhibitors and insensitive to other inhibitors.

Conclusion:

These results demonstrate that this co-culture assay offers a superior screening system for potential cancer drugs or agents that have either enhancing or inhibitory effects on MMPs. When cancer cells are co-cultured with NHDF, there is dramatic increase in MMP expression. MMP bands are distinct and discrete, and the intensity of the bands is enhanced several fold. The intensity of the bands increases with enhancers and decreases with inhibitors. The inhibitors reduce the expression of MMP-2 and MMP-9 in a dose-dependent fashion. The technique is unique, elegant, simple, and quick. The co-culture bioassay may be applicable in

diseases that relate to the degradation of the extracellular matrix in various physiological and pathological conditions where MMP activity is altered.

Comment:

To reproduce and spread to other parts in the body, cancer cells degrade the extracellular matrix (ECM) by secreting various matrix metalloproteinases (MMPs), which have been correlated with the aggressiveness of tumor growth. There is compelling evidence to suggest that MMP-2 and MMP-9 play important roles in tumor invasion and metastasis. This study demonstrated that the technique of co-culture assay offers a superior screening system for potential cancer drugs or agents that have either enhancing or inhibitory effects on MMPs.