ON THE EFFECTS OF VITAMIN D-BINDING PROTEIN- DERIVED MACROPHAGE ACTIVATING FACTOR (GcMAF) ON HUMAN BREAST CANCER AND NEUROBLASTOMA CELLS

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INTRODUCTION

Introduction (1)
- Breast cancer is the most common type of cancer in women and one of the most common causes of cancer death. This leads to the continuous search for additional therapies that could be used as a complement to or alternative to surgery, radiation therapy and chemotherapy.
- Among the novel approaches, those that target the immune system, often referred to as immunotherapy, appear promising.

Introduction (2)
- Vitamin D-binding protein-derived macrophage-activating factor (GcMAF) is a good candidate for immunotherapy of breast cancer because it has the ability to stimulate macrophages, and inhibit angiogenesis.
- It was previously demonstrated that administration of GcMAF to 16 women as treatment for metastatic breast cancer yielded significant results with eradication of cancer.

Introduction (3)
- These powerful anti-cancer effects of GcMAF can be ascribed to different biological properties of the molecule that includes:
  - Inhibition of tumor-induced angiogenesis;
  - Direct inhibition of cancer cell proliferation, migration and metastatic potential;
  - Stimulation of tumoricidal macrophages.

Introduction (4)
- However, no studies have so far been performed in order to assess whether GcMAF is indeed capable of activating macrophages that could in turn "attack" human breast cancer cells in vitro.
- Here we present clear-cut evidence that GcMAF activates normal macrophages that in turn exert a tumoricidal action against human breast cancer cells.

MATERIALS AND METHODS

Materials and Methods (1)
- Purified, activity-tested GcMAF was obtained from Immuno-Biotech Ltd, Guernsey, Channel Isles. All other commercial reagents were from Sigma-Aldrich.
- Cells were human breast cancer cells (MCF-7) were maintained at 37°C in a humidified atmosphere of 5% CO2 in Eagle's minimum essential medium in Eagle's balanced salt solution, supplemented with 10% fetal bovine serum, gentamicin (50 µg/ml), penicillin (100 U/ml), streptomycin (100 µg/ml), and fungizone (25 µg/ml).
- Cells were maintained in 24-well plates with 0.5x10^6 cells per well (4x10^6 cells in total) for 72 h in the presence of 200 ng/ml GcMAF or DMSO.

Materials and Methods (2)
- Before addition to the MCF-7 cell culture, the macrophages were gently centrifuged and resuspended in fresh medium in order to avoid transferring GcMAF to the co-culture. In this way we could rule out direct effects of GcMAF on MCF-7 cells.
- The macrophages were added at a ratio of 1:1 to the MCF-7 cell culture. The cells were then allowed to settle for 1 h before co-culture.
- The cells were fixed and stained 48 hours after co-culturing them or photographed by time-lapse photography for 5 days.

Materials and Methods (3)
- Cell morphology was studied by phase-contrast microscopy. Phase-contrast imaging was performed on living cells without any fixation or treatment. A series of digital images of living cells were recorded for each treatment point and the most representative were chosen.
- Papanicolaou and Hamatoxylin Eosin staining were also performed. Slides were mounted with permanent mounting medium and observed under light microscopy.

Materials and Methods (4)
- Time-lapse photography was taken over a 7 day period using an Olympus C22 microscope and a C9603-C with NCL D70 capture software.

RESULTS

Results (1)
- Phase contrast micrographs showing clusters of human breast cancer cells (MCF-7).
- Cells grow out of the clusters that inhibited the characteristic loss of cancer inhibition.

Results (2)
- Actin reorganization (128X with effabio)
- Few effabio stained filaments are visible and with a clearly morphologically defined distribution inside the myofibroblastic cells of the tumor.
- There is a strong morpho-stylistic activity relatively high density of proliferation of these cells.

Results (3)
- What human breast cancer cells (MCF-7) are in contact with macrophages (100 ng/ml GcMAF).
- GcMAF induced transformation and activation of normal macrophages to tumor necrosis factor.
- Proportion of cells (1:1) shows a strong between carcinoma cells and the cancer of the breast tumoricidal macrophages.

Results (4)
- Human breast cancer cells (MCF-7) with macrophages (100 ng/ml GcMAF)
- GcMAF induced transformation and activation of normal macrophages to tumor necrosis factor.
- Proportion of cells (1:1) shows a strong between carcinoma cells and the cancer of the breast tumoricidal macrophages.

Results (5)
- Hamatoxylin Eosin staining.
- The cell is at the centre of an area that shows differentiation of the clump to the eosin in the presence of fragmented and the cell has a characteristic loss of cancer inhibition.

Results (6)
- The macrophages were fixed and stained 48 hours after co-culturing them or photographed by time-lapse photography for 5 days.

Results (7)
- Time-lapse microscopy of GcMAF treated macrophages.
- GcMAF treated macrophages were observed to show a pronounced increase in the number and size of the tumoricidal macrophages, and also cause a cell kill of normal cells in the culture.

Results (8)
- We also observed that GcMAF significantly increased the proliferation of breast cancer cells (MCF-7), thus confirming the tumoricidal action of GcMAF has an inhibitory action on cancer cells that is not only effective in vitro but also effective in vivo.

DISCUSSION

Discussion (1)
- One result demonstrated that the GcMAF stimulation caused an attack by human breast cancer cells to immobilize, infect their epithelial and eventually phagocytize them.
- This confirms the scientific rationale for the effects of GcMAF in human breast cancer cells.

Discussion (2)
- In fact, it is well known that GcMAF is effective against a variety of experimental and spontaneous tumours as well as against various normal tissues with implications in transplantation in vitro.
- Consistent with these observations, a series of clinical cases have been observed, two-tcell-driven ex vivo.
- All patients treated with Vascular-affected level above the threshold of normal values indicating decreased expression of GcMAF production and the appropriate GcMAF introduction.
- All patients showed significant decrease of Vascular-affected level below the threshold of normal values indicating decreased expression of GcMAF production and the appropriate GcMAF introduction.
- All patients showed significant decrease of tumor density or size indicating decreased expression of GcMAF production and the appropriate GcMAF introduction.

Discussion (3)
- As noted, GcMAF has been used with encouraging results in all types of tumors for the treatment of advanced tumors, demonstrating significant improvement in response to the therapy.
- Inhibiting tumor cell proliferation and migration may be a critical step in the development of this therapy.
- The GcMAF was shown to inhibit tumor cell proliferation, migration and tumorigenicity, which may explain the effectiveness of GcMAF administration in inducing the immune system.

Discussion (4)
- The GcMAF may be an effective treatment for cancer, and may have potential for use in other diseases as well.
- The GcMAF was shown to inhibit tumor cell proliferation, migration and tumorigenicity, which may explain the effectiveness of GcMAF administration in inducing the immune system.

REFERENCES