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 these 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 include:
- 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 common reagents were from Sigma Aldrich.
- *Cell lines*. Human breast cancer cells (MCF-7) were maintained at 37°C in a humidified atmosphere of 5% CO2 in Eagle's minimun essential medium in Earle's Balanced salt solution, supplemented with 1 mM sodium pyruvate, 10% foetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin.
- In experiments of **co-cultures**, macrophages (Raw 264.7, HPA Culture Collection) were activated by culturing them in the same medium of MCF-7 cells and in the presence of **100 ng/ml GcMAF for 72 h** prior to addition to the MCF-7 cell culture.

Materials and Methods (2)

- Before addition to the MCF-7 cell culture, the macrophages were gently centrifuged and re-suspended 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 examination.
- The cells were fixed and stained 40 hours after co-culturing them or photographed by time-lapse photography for 7 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 experimental point and the most representative were chosen.
- Papanicolaou and Hematoxylin Eosin staining were also performed. Slides were mounted with permanent mounting medium and observed under light microscopy.

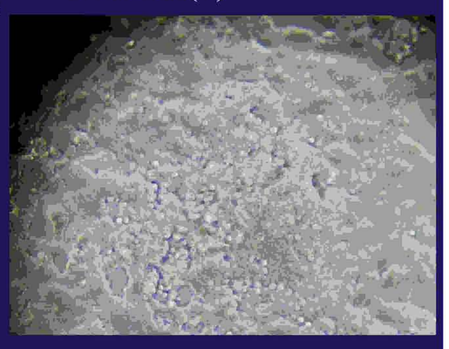
Materials and Methods (4)

- Time-lapse photography was taken over a 7 day period using an Olympus CK2 microscope and a GXCAM-3 with NCH Debut capture software.

RESULTS

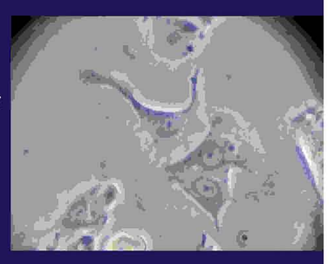
Results (1)

- Phase contrast microphotography of clusters of human breast cancer cells (300 X). Cells grow one on top of the other forming clusters that reflected the characteristic loss of contact inhibition.



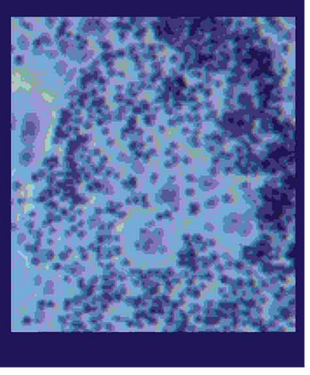
Results (2)

- At higher magnification (1200 X) the cells show linear, not fragmented, margins and with a clearly recognizable organization of chromatin inside the nucleus, indicative of a strong synthetic activity compatible with the high rate of proliferation of these cells.



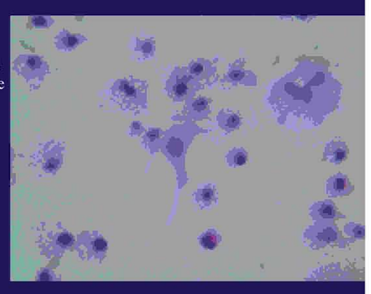
Results (3)

- When human breast cancer cells where co-cultured with macrophages that had been previously activated by GcMAF (100 ng/ml) for 72 h, GcMAF-activated macrophages appeared as small round cells that surrounded human breast cancer cells.
- Papanicolau staining, (100 X) shows a group of human breast cancer cells in the centre of the image surrounded by hundreds of small round macrophages.



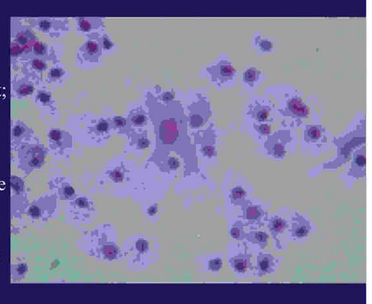
Results (4)

- Haematoxylin Eosin staining.
- The nucleus of the macrophages is well stained whereas the chromatin in the nucleus of the cancer cell appears fragmented and disorganized.
- The nucleoli, however, are still recognizable.
- The cytoplasm of macrophages appears vacuolized thus suggesting active phagocytosis.

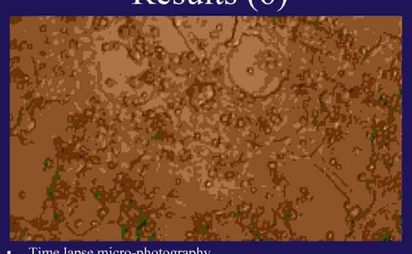


Results (5)

- Haematoxylin Eosin staining.
- The cell in the centre shows a peculiar aspect; the chromatin in the nucleus appears fragmented and the cytoplasm appears to be indented as if the macrophages were actively deconstructing the cytoplasmic assembly of the cancer cell.



Results (6)



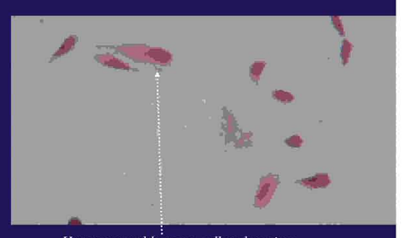
- Time lapse micro-photography.
- Human breast cancer cells and GcMAF activated macrophages at day 1; the cancer cells cover the field of observation. Individual cancer cells can be recognized.
- GcMAF-activated macrophages appear as small round cells that are attached to the cancer cells.
- Almost no macrophages can be observed in the naked areas of the plate, thus confirming the observation that GcMAF-activated macrophages seek for contact with the cancer cells.

Results (7)

- Time lapse micro-photography.
- After 7 days of co-incubation, no individual cancer cell can be recognized. After macrophage-induced apoptosis, their apoptotic bodies are all grouped together in the centre of the field of observation, and most of the field is empty of cancer cells. Most GcMAF-activated macrophages surround and infiltrate the mass of cancer cell debris in the centre.

Results (8)

- We also observed that GcMAF directly induced differentiation of human neuroblastoma cells (SH-SY5Y), thus reinforcing the hypothesis that GcMAF has multiple anti-cancer effects that can be exploited in the immunotherapy of a variety of tumors.

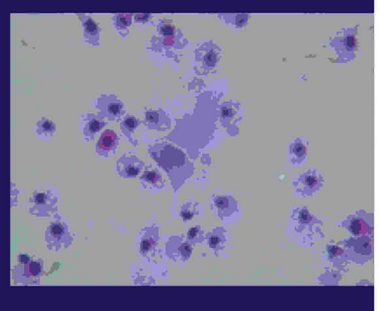


Human neuroblastoma cell undergoing differentiation after GcMAF treatment

DISCUSSION

Discussion (1)

- Our results demonstrate that GcMAF stimulates macrophages that in turn attack human breast cancer cells, induce their apoptosis and eventually phagocytise them.
- These results provide the scientific rationale for the clinical use of GcMAF in breast cancer treatment.



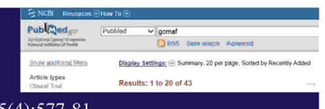
Discussion (2)

- In fact, it is well assessed that GcMAF is effective against a variety of experimental and spontaneous tumors as well as against various aspects of neoplastic transformation *in vitro*.
- Consistent with these observations, in a series of clinical cases recently observed, two trends emerge evident:
- All Patients presented with Nagalase levels well above the threshold of normal values indicating decreased endogenous GcMAF production and the appropriateness of GcMAF administration.
- All Patients showed significant decrease of Nagalase levels following GcMAF injections. In all cases, Nagalase levels were lower at the second determination and the decrease continued in the following determinations, thus confirming the effectiveness of GcMAF administration in restoring the immune system.

Discussion (3)

- As of today, GcMAF has been used, with encouraging results, in all types of cancers and at all stages by over 100 physicians who used it as compassionate treatment.
- However, it can be hypothesized that certain histological types as well as certain definite stages of cancer might have a differential response to GcMAF.
- Also the Patient's genotype as far as VDR gene polymorphisms are concerned, might influence the individual response to GcMAF.
- Finally, it should be remembered that the prognosis for all types of cancers is dependent upon the nutritional and inflammatory status of the patient that can be monitored by the Prognostic Inflammatory and Nutritional Index (PINI).
- The PINI score therefore could become part of the laboratory assessment during GcMAF treatment and together with Nagalase testing and VDR gene polymorphism determination it could help monitoring the response of each individual patient and adjusting the dose and the frequency of administration in order to obtain the best results.

References



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