GROUP COMPONENT PROTEIN-DERIVED MACROPHAGE ACTIVATING FACTOR (GcMAF) STIMULATES MACROPHAGES THAT INDUCE HUMAN BREAST CANCER CELL APOPTOSIS

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INTRODUCTION

- ✓ The first publication on GcMAF in a peer-reviewed journal is dated 1994 (*J Immunol.* 1994 May 15;152(10):5100-7).

A Defect in the Inflammation-Primed Macrophage-Activation Cascade in Osteopetrotic Rats[1]

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- ✓ In this article, a group of researchers from the Temple University School of Medicine in Philadelphia, USA, described the effects of a protein, defined as GcMAF, on rat macrophages.
- ✓ In 2008 it was demonstrated that GcMAF treatment was effective against metastatic breast cancer with reported eradication of tumors and no recurrence for more than 4 years (*Int J Cancer.* 2008 Jan 15;122(2):461-7).
- ✓ We recently demonstrated (*Anticancer Res.* 2012 Jan;32(1): 45-52) that the anticancer efficacy of GcMAF can be ascribed to different effects:

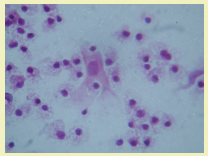
1. Direct inhibition of human breast cancer cell proliferation and reversion of the transformed phenotype.
2. Inhibition of human breast cancer cell-induced angiogenesis.
3. Stimulation of tumoricidal macrophages.

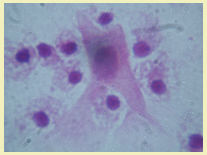
- ✓ GcMAF activates macrophages that infiltrate experimental tumours in animal models (*Oncol Lett.* 2011 Jul;2(4): 685-691. *J Surg Res.* 2012 Jan;172(1):116-22.).
- ✓ This evidence, however, refers to experimental tumors other than human breast cancer.
- ✓ Therefore, in order to fill this gap of knowledge, we performed experiments to provide clear-cut evidence that GcMAF, as part of the vitamin D axis, activates normal macrophages that in turn exert a tumoricidal action against human breast cancer cells.

MATERIALS and METHODS

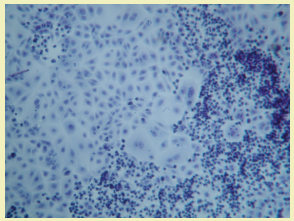
- ✓ Highly purified, activity-tested GcMAF was obtained from Immuno Biotech Ltd, Guernsey, Channel Isles. Common reagents were from Sigma Aldrich (Milan, Italy).
- ✓ *Cell lines.* Human breast cancer cells (cell line MCF-7) were obtained from the Istituto Zooprofilattico Sperimentale della Lombardia e dell' Emilia-Romagna, Brescia, Italy. In experiments of co-cultures, macrophages (cell line 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. Gc-protein was used as control. The macrophages were added at a ratio of 1:1 to the MCF-7 cell culture.
- ✓ *Study of cell morphology.* Cell morphology was studied by phase-contrast microscopy using an Optika inverted microscope (Model XDS-2; Optika Microscopes, Bergamo, Italy).

RESULTS

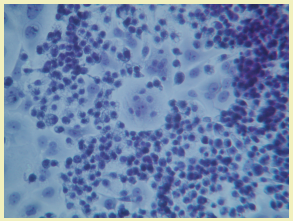




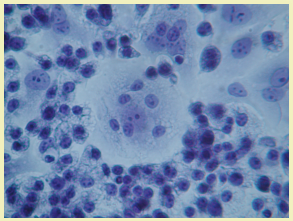
A human breast cancer cell in the centre of the image is surrounded by small round GcMAF-activated macrophages. The nucleus of the macrophages is well stained. The cytoplasm of macrophages appears vacuolized thus suggesting active phagocytosis.



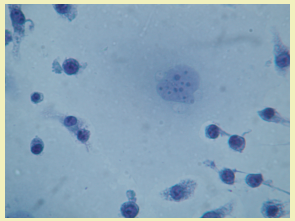
Human breast cancer cells surrounded by hundreds of GcMAF-activated macrophages.



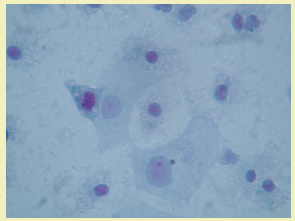
Another field of observation where human breast cancer cells surrounded by hundreds of GcMAF-activated macrophages can be observed.



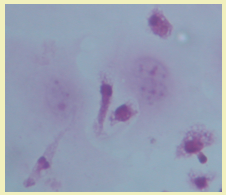
The nucleoli of the human breast cancer cell can be recognized; this phenomenon can be interpreted as an index of remaining synthetic activity as expected in cells undergoing active apoptosis.



GcMAF-activated macrophages deconstruct the cytoplasm of the large human breast cancer cell in the centre of the image.



GcMAF-activated macrophages deconstruct the cytoplasm of the human breast cancer cells in the centre of the image.



GcMAF-activated macrophages deconstruct the cytoplasm of the human breast cancer cells in the centre of the image. The fragmented chromatin in the nuclei of the cancer cells can be observed.

DISCUSSION

- ➢ Our results demonstrate that GcMAF stimulates macrophages that in turn attack human breast cancer cells, deconstruct their assembly and eventually phagocytise them.
- ➢ These results are consistent with the observation that macrophages infiltrated experimental tumours implanted in severely immunodeficient mice after GcMAF injections (*J Surg Res.* 2012 Jan;172(1):116-22).
- ➢ However, at variance with the observation reported above, in our experiments we could rule out indirect effects due to the adaptive response of the whole organism to the presence of an advanced tumour and to the GcMAF-induced inhibition of angiogenesis with consequent tumour hypoxia and necrosis (*J Surg Res.* 2012 Jan;172(1):116-22).
- ➢ Taken together these results are consistent with clinical reports describing the therapeutic efficacy of GcMAF in human breast cancer patients.

Int J Cancer: 122, 461–467 (2008)
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Immunotherapy of metastatic breast cancer patients with vitamin D-binding protein-derived macrophage activating factor (GcMAF)

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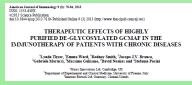

THERAPEUTIC EFFECTS OF HIGHLY PURIFIED DE-GLYCOSYLATED GCMAF IN THE IMMUNOTHERAPY OF PATIENTS WITH CHRONIC DISEASES

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ORIGINAL RESEARCH
GC protein-derived macrophage-activating factor decreases α-*N*-acetylgalactosaminidase levels in advanced cancer patients

The observation reported here confirm and extend the results presented in (*Int J Cancer.* 2008 Jan 15;122(2):461-7; *Cancer Immunol Immunother.* 2008 Jul;57(7):1007-16; *Transl Oncol.* 2008 Jul;1(2):65-72; *J Med Virol.* 2009 Jan;81(1):16-26; *Autism Insights* 2012:4 31–38; *Anticancer Res.* 2013 Jul;33(7):2917-9), and open the way to further studies aimed at assessing the role and indications of GcMAF in the immunotherapy of cancer and other chronic diseases.