THE IMMUNE STIMULANT PROPERTIES OF VITAMIN D BINDING PROTEIN - DERIVED MACROPHAGE ACTIVATING FACTOR CAN MINIMISE MORBIDITY IN GYNAECOLOGICAL CANCERS

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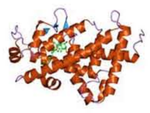
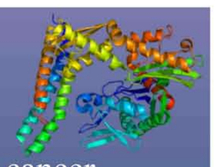
Introduction 1



- Immunodeficiency and consequent increase of morbidity is a common occurrence in advanced cancer patients (Comp Biochem Physiol A Mol Integr Physiol. 2002;132:1-8).
- One of the events responsible for immunodeficiency and increased morbidity in cancer patients is excess production of alpha-N-acetylgalactosaminidase (nagalase) an enzyme that de-glycosylates (inactivates) vitamin D binding protein, which is the precursor of vitamin D binding protein-derived macrophage activating factor (GcMAF).

Introduction 2



- The increase in nagalase activity in cancer patients is due to the fact that cancer cells release nagalase (Cancer Lett. 2009; 283:222-9).
- Thus, nagalase activity reflects tumour burden, aggressiveness and progression of the disease (Cancer Lett. 2000;158:61-4) up to the point that determination of nagalase activity is a non-invasive way of evaluation of cancer severity (Cancer Lett. 2009;283:222-9).

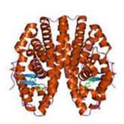
Introduction 3

- GcMAF proved effective against metastasized breast cancer (Int J Cancer. 2008.122:461-7), metastasized colon cancer (Cancer Immunol Immunother. 2008. 57:1007-16), and prostate cancer (Transl Oncol. 2008. 1:65-72).
- In these studies, the anti-cancer effects of GcMAF were evaluated by measuring serum nagalase activity, a marker of tumour burden and progression as well as of immunodeficiency.

Introduction 4

- Here we demonstrate that GcMAF stimulates human monocytes/macropahges.
- Stimulation of human monocytes/macropahges restores a competent immune system.
- We elucidate the molecular mechanism of action responsible for GcMAF signal transduction.
- We propose an integrated strategy to obtain the best effects from immuno-therapy with GcMAF in order to minimise morbidity in cancer patients

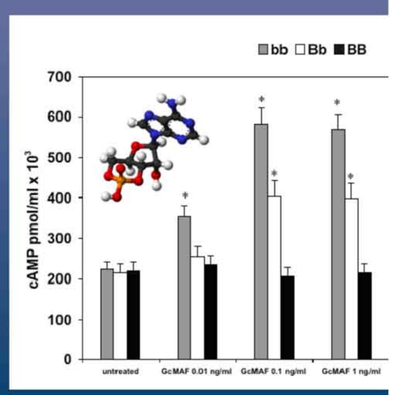
Materials and Methods



- Purified, activity-tested GcMAF was from Immuno Biotech. Ltd.
- Monocytes were collected from healthy volunteers. Separation procedure to obtain PBMC was performed by Polymorphoprep (Axis-Shield).
- Assessment of cell viability was determined by Calbiochem Rapid Cell Proliferation Kit.
- cAMP levels were measured by a competitive EIA assay (Cyclic AMP EIA kit, Cayman Chemical).
- Vitamin D receptor (VDR) restriction site polymorphism was evaluated on genomic DNA extracted from peripheral blood cells using a QIAamp DNA Blood Mini Kit
- Absence or presence of the *Bsm*I restriction site was denominated "B" and "b" respectively.

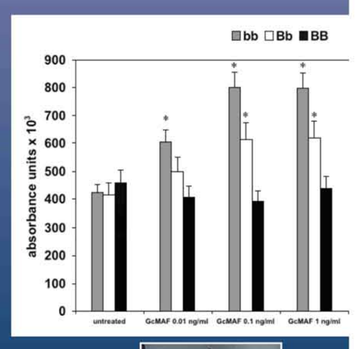
Results 1

- Healthy donors were selected for their *Bsm*I genotype and 3 different groups of subjects were identified according to *Bsm*I polymorphisms: subjects with "bb"genotype, subjects with "BB" genotype, and subjects with "Bb" genotype.
- GcMAF significantly stimulated cAMP formation in a dose-dependent manner. Intracellular cAMP formation was highest in the "bb" genotype. In the "Bb" genotype, the response was intermediate.



Results 2

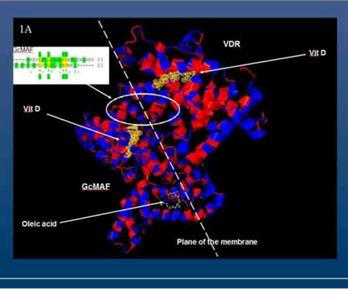
- When we challenged monocytes from subjects harbouring different *Bsm*I polymorphisms, we observed that the "b" allele of the VDR gene and the "bb" genotype were associated with highest stimulation of proliferation by GcMAF.
- Subjects with "Bb" genotype showed an intermediate response to GcMAF.
- These data suggest that there is an association between the presence of "b" alleles and the degree of the response to GcMAF.



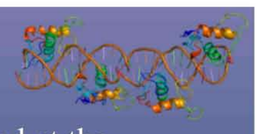
Results 3

- These results demonstrate that GcMAF counteracts immunodeficiency by stimulating macrophage signalling and proliferation and these events are mediated through VDR.

Proposed mode of interaction between GcMAF and VDR at the plasma membrane. Vitamin D and oleic acid stabilize the complex by binding to the hydrophobic domains of the two vitamin D binding proteins.

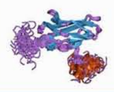


Discussion 1



- Taken together, the results obtained at the molecular, cellular and experimental level, support the observation that GcMAF administration to patients with advanced cancer resulted in dramatic results that include tumour growth control and decrease of morbidity.
- (Int J Cancer. 2008; 122:461-7; Cancer Immunol Immunother. 2008; 57:1007-16; Transl Oncol. 2008; 1:65-72).

Discussion 2



- On the basis of the results presented here, it can be hypothesized that the patient's genotype, as far as VDR gene polymorphisms are concerned, influences the individual response to GcMAF.
- Therefore, the use of GcMAF to restore a competent immune system and minimise morbidity in gynaecological cancers has to be calibrated on the individual patient's genotype adopting an integrated approach.

Discussion 3

- In fact, in order to minimise morbidity, 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) (Am. J. Immunol. 2012; 8: 65-70).

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ROLE OF ANGIOTENSIN-CONVERTING ENZYME AND VITAMIN D RECEPTOR GENE POLYMORPHISMS IN CANCER ANOREXIA-CACHEXIA SYNDROME

Discussion 4

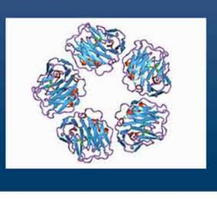
The PINI score

PINI is calculated by dividing the product of serum alpha-1-glycoprotein and CRP levels by that of albumin and pre-albumin.

Prognostic Inflammatory and Nutritional Index:

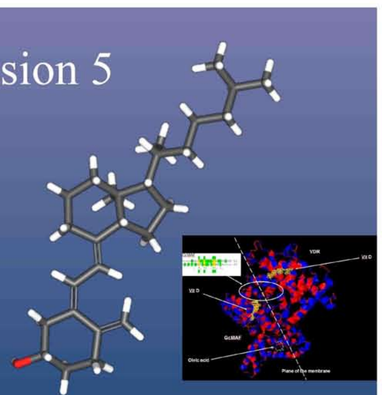
$$\frac{\text{alpha}-1\text{ acid}-\text{glycoprotein (mg/dl)} \times \text{CRP (mg/dl)}}{\text{albumin (g/dl)} \times \text{prealbumin (mg/dl)}}$$

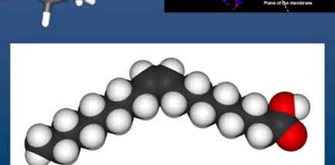
PINI
- > 30 predicts a **very high** risk of morbidities during hospitalization
- [21-30] predicts a **high** risk of morbidities during hospitalization
- [11-20] predicts an **intermediate** risk
- [1-10] predicts a **low** risk
- < 1 **normal**



Discussion 5

- The observation that vitamin D and oleic acid participate in the GcMAF/ VDR interaction leads to propose an integrated individual approach designed to exploit the characteristics of GcMAF and reduce morbidity in cancer patients.





Discussion 6
The integrated approach to minimise morbidity through GcMAF

1. Determination of VDR genotype to assess the most appropriate individual dose for GcMAF.
2. Determination of PINI score.
3. Design of individual nutritional plan to provide adequate amount of vitamin D and oleic acid.
4. Design of individual nutritional plan to lower PINI score.

Discussion 7

- The observation reported here open the way to design GcMAF/nutrition-based individual integrated approaches that will lead to:

1. Minimise morbidity in cancer patients.
2. Decrease the probability of cancer anorexia cachexia syndrome (CACS).
3. Significantly increase survival.


View of the *Asklepion* of Kos, the best preserved instance of an Asklepieion.