MOLECULAR INTERACTION OF VITAMIN D BINDING PROTEIN - DERIVED MACROPHAGE ACTIVATING FACTOR WITH VITAMIN D RECEPTOR; A NEW PERSPECTIVE IN ENDOMETRIAL CANCER TREATMENT

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Introduction 1

- Vitamin D is a promising therapeutic treatment against endometrial cancer (Mol Cancer Res. 2011. 9:1479-92).
- Interaction of vitamin D with its receptor (VDR) induces genomic and non-genomic responses that inhibit cancer cell proliferation, metastatic potential and angiogenesis.
- Here we demonstrate that another member of the vitamin D axis, that is vitamin D binding protein-derived macrophage activating factor (GcMAF), may directly interact with VDR and exert anti-cancer effects.

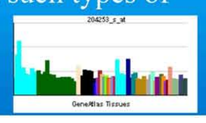
Introduction 2

- 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).
- The efficacy of GcMAF as an anti-cancer agent is due to the multiple biological properties of the molecule that are:

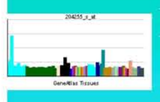
1. Activation of tumoricidal macrophages.
2. Inhibition of cancer cell proliferation.
3. Inhibition of cancer cell metastatic potential.
4. Inhibition of cancer cell-induced neo-angiogenesis.

Introduction 3

- Here we demonstrate that these multiple effects of GcMAF can be explained through its interaction with VDR.
- VDR is expressed in a variety of normal and transformed tissues including gynecologic neoplasms (Gynecol Oncol. 1992. 44:131-6).
- Therefore, interaction between GcMAF and VDR can be exploited to treat such types of cancer.



Materials and Methods



- Purified, activity-tested GcMAF was from Immuno Biotech. Ltd.
- Analyses were carried out on the nucleotide and amino-acid sequences of the genes coding for vitamin D binding protein/GcMAF (isoform 1 precursor; gi|324021743|ref|NP_001191235.1) and VDR (gi|38511972|gb|AAH60832.1) in *Homo sapiens*.
- Three parameters were taken into account: sequence identity, sequence similarity, hydrophobic profile.

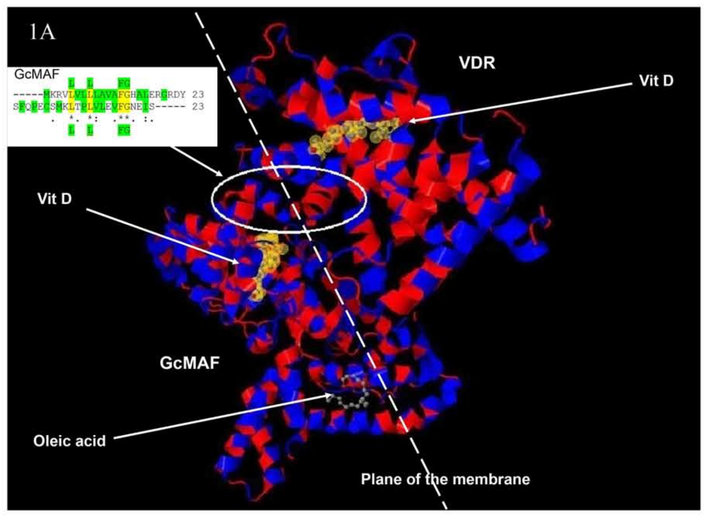
Results

- There are 23 hydrophobic amino acids near the amino terminus of **GcMAF** (-----MKRVLVLLLAVAFGHALERGRDY) and 23 amino acids near the carboxyl terminus of the **VDR** (SFQPECSMKLTPLVLEVFGNEIS-----).

```
              L   L     FG
-----MKRVLVLLLAVAFGHALERGRDY 23
SFQPECSMKLTPLVLEVFGNEIS----- 23
      .  *. *:  .**. :.
              L   L     FG
```

- If these two sequence are aligned (see below), it is possible to observe that:
- In both proteins there is a long stretch (13-14) of hydrophobic amino acids (highlighted in green).
- 4 hydrophobic amino acids are identical (L L FG; indicated in yellow and in green above and under the alignment. The sequence of GcMAF is above).
- 11 amino acids have similar functional valence (as indicated by the conventional symbols [*], [.] and [:].

```
              L   L     FG                GcMAF
-----MKRVLVLLLAVAFGHALERGRDY 23
SFQPECSMKLTPLVLEVFGNEIS----- 23
      .  *. *:  .**. :.
              L   L     FG                VDR
```



- According to the model depicted in the Figure, the last 23 hydrophobic amino acids of VDR (on the right of Fig. 1A), located at the inner part of the plasma membrane (represented as a dotted line), interact with the first 23 hydrophobic amino acids of the GcMAF (on the left of the Figure) located at the external part of the plasma membrane, with vitamin D (represented in yellow) sandwiched in between the two vitamin D-binding proteins.
- Oleic acid, taken as an example of an unsaturated fatty acid bound to GcMAF (Biochem Biophys Res Commun. 1988. 15:1019-24), stabilizes the complex at the level of the plasma membrane.

- In addition to the mode of interaction proposed in Fig. 1A, there is also another one that takes into consideration the fact that GcMAF binds unsaturated fatty acids.
- The fatty acid binding site is located between domains II and III of GcMAF, that is between the positions 304 and 387.

```
DSTTCFNAKGPLLKKEISSFIDKLQELC   88
--------LTPLVLEVFGNEIS------   23
        **: :..  *.            VDR
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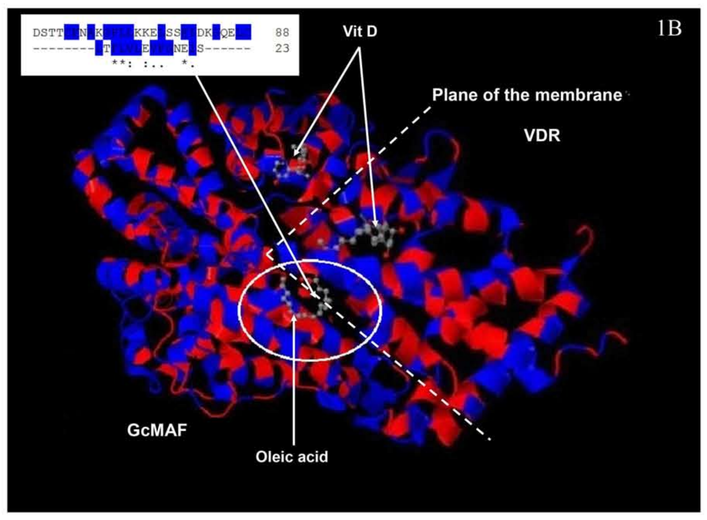
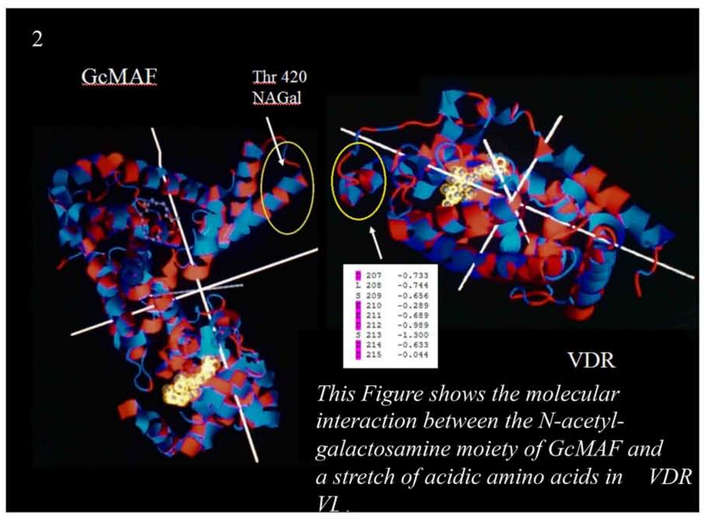


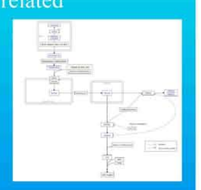
- When we aligned the 23 hydrophobic amino acids of the VDR quoted above (represented in the insert in Fig. 1B) and the corresponding hydrophobic amino acids of the unsaturated fatty acid binding site of GcMAF (in particular, those in position 356-386), we observed that there was a significant degree of functional homology.
- There are 8 amino acids with similar functional valence in a long stretch of hydrophobic amino acids (highlighted in blue).



This Figure shows the molecular interaction between the N-acetyl-galactosamine moiety of GcMAF and a stretch of acidic amino acids in VDR

- The complex GcMAF/VDR is then internalized (J. Nutr. 2008. 138: 1323–1328) and regulates a number of genes that in turn are responsible for the biological effects of GcMAF.

- Among these:



- BAG oncogene, implicated in apoptosis and age related neurodegenerative diseases as Alzheimer's.
- MED1, regulating apoptosis.
- MED12, associated with prostate cancer
- Retinoid X receptor alpha.
- RUNX1, implicated in breast cancer.
- SNW1, involved in oncogenesis.
- ZBTB16, associated with acute promyelocytic leukemia, Natural Killer and gamma-delta T cell function.

Discussion 1

- Endometrial cancer is considered vitamin D-sensitive because of activation of VDR.
- Activation of VDR leads to anti-mitogenic, pro-apoptotic and pro-differentiating signaling that contribute to anti-cancer effects (Anticancer Res. 2009. 29:3687-98).
- GcMAF, by activating VDR, elicits a variety of anti-cancer effects that synergize with those of vitamin D itself.

Discussion 2

- These observations at the molecular level are corroborated by observations at the clinical level.
- Here we report the preliminary observation of a series of clinical cases describing the results obtained administering GcMAF to patients with diverse types of cancers at advanced stages.
- In all cases, GcMAF treatment was initiated at late stages of tumour progression.

Discussion 3

- The response to GcMAF was robust and, even though statistical analysis is inappropriate in such an heterogeneous recollection of clinical stories, certain trends emerge evident.
- All patients (n= 19), but one, presented with nagalase levels well above the threshold of normal values.
- This indicates high tumour burden and relative immune deficiency (Cancer Lett. 2000.158:61-4).

Discussion 4

- All patients, but one, showed significant decrease of nagalase levels following GcMAF weekly injections.
- In all cases, but two, such as decrease was associated with improvement of the clinical condition.
- Conversely, in one patient, clinical improvement was not associated with a decrease of nagalase level.
- No adverse side effects were reported.

Discussion 5

- The observation reported here confirm and extend the results presented in 2008 (Int J Cancer. 2008. 122:461-7; Cancer Immunol Immunother. 2008. 57:1007-16; Transl Oncol. 2008.1:65-72).
- They open the way to further studies aimed at assessing the precise role and indications for GcMAF in the immunotherapy of endometrial cancer.