**EFFECTS OF VITAMIN D BINDING PROTEIN-DERIVED MACROPHAGE ACTIVATING FACTOR (GcMAF) ON HUMAN NEUROBLASTOMA CELLS AND PREDICTED MOLECULAR INTERACTION WITH THE VITAMIN D RECEPTOR**

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**Introduction 1**
- From the historical perspective, the concept of immunotherapy of cancer is associated with the early work of Dr. William Coley.
- In modern times, it has been re-proposed since 1990.

**Materials and Methods 2**
- **Cell Lines**: Human neuroblastoma cell line SH-SY5Y, normally maintained in culture at 37°C in a humidified atmosphere of 5% CO2 in air. This cell line was obtained from the American Type Culture Collection (ATCC) and was used in a quiescent state for all the experiments.
- **GcMAF**: Commercially available, highly active purified GcMAF was obtained from Immuno Biotech Ltd, Guernsey, Channel Isles.
- **Experimental Design**: The central role of macrophages in the immunotherapy of cancer has been further highlighted in the article pasted below.

**Discussion 3**
- The central role of macrophages in the immunotherapy of cancer has been further highlighted in the article pasted below.

**Results**
- GcMAF treatment of SH-SY5Y cells resulted in different effects depending on the proliferative activity of the cells.
- GcMAF inhibited cell proliferation in a dose-dependent manner and induced morphological changes indicative of differentiation (Fig. 1).
- In serum-starved, quiescent cells, GcMAF induced morphological changes indicating differentiation (Fig. 2 A, B).
- The effects of GcMAF were mediated by cAMP production (Fig. 3), possibly through cross-talk with the vitamin D receptor (VDR).

**Discussion 4**
- The results presented here demonstrate that GcMAF induces actively proliferating human neuroblastoma cells, whereas it induces the differentiation of serum-starved (quiescent) human neuroblastoma cells.
- The concentration of GcMAF necessary to inhibit proliferation of actively proliferating cells was 10 fold higher than that required to induce differentiation of quiescent cells.

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**Fig. 1.** SH-SY5Y cells proliferation was stimulated by 10% and 1% FBS (Fig. 1A). GcMAF treatment of SH-SY5Y cells resulted in different effects depending on the proliferative activity of the cells. (A) A comparison with the proliferation of untreated cells: comparison between untreated cells, cells treated with compound at 50 ng/ml and cells treated with 10% and 1% FBS. (B) Comparison between untreated cells and GcMAF-treated cells, the proportion of cells in S phase of the cell cycle was determined by flow cytometry. (C) Comparison between untreated cells, cells treated with compound at 50 ng/ml and cells treated with 10% and 1% FBS. (D) Comparison between untreated cells, cells treated with compound at 50 ng/ml and cells treated with 10% and 1% FBS.

**Fig. 2.** GcMAF-induced cell differentiation in serum-starved SH-SY5Y cells. The interaction between GcMAF and VDR could also occur inside the cell. A molecular interaction between the two proteins can therefore be proposed (Fig. 4).

**Fig. 3.** According to this model, vitamin D and oleic acid should facilitate the interaction between GcMAF and VDR.

**Fig. 4.** Oleic acid, taken as an example of an unminated fatty acid bound to GcMAF, could stabilize the complex at the level of the plasma membrane.

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