

IJAE

Italian Journal of Anatomy and Embryology

Official Organ of the Italian Society
of Anatomy and Histology

68° CONGRESSO
della Società Italiana di Anatomia e Istologia

68TH MEETING
of the Italian Society of Anatomy and Histology

Ancona 18-20 september 2014



Vol. 119
N. 1 (Supplement)

2014

ISSN 1122-6714



IJAE

Italian Journal of Anatomy and Embryology

Official Organ of the Italian Society of Anatomy and Histology

Founded by Giulio Chiarugi in 1901

Editor-in-Chief

Paolo Romagnoli

Assistant Editors

Maria Simonetta Pellegrini Faussonne

Gabriella B. Vannelli

Past-Editors

I. Fazzari – E. Allara – G.C. Balboni – E. Brizzi – G. Gheri

Editorial Board

Giuseppe Anastasi (Messina)

Pepa Atanassova (Plovdiv)

Antonio Filippini (Roma)

Menachem Hanani (Jerusalem)

Giovanni Orlandini (Firenze)

Nadir M. Maraldi (Bologna)

Hanne B. Mikkelsen (Copenhagen)

Laurentiu M. Popescu (Bucharest)

Alessandro Riva (Cagliari)

Ajai K. Srivastav (Gorakhpur)

Contact

Prof. Paolo Romagnoli

Department of Anatomy, Histology and Forensic Medicine

Section "Enrico Allara", Viale Pieraccini 6, 50139 Firenze (Italy)

Phone: +39 055 4271389 - Fax: +39 055 4271385

E-mail: paolo.romagnoli@unifi.it - ijae@unifi.it

Journal e-mail: ijae@unifi.it

Web site: <http://www.fupress.com/ijae>

© 2014 Firenze University Press

Firenze University Press

Borgo degli Albizi 28

I-50122 Firenze, Italy

E-mail: journals@fupress.com

Available online at

<http://www.fupress.com/ijae>

For Subscriptions

Licosa Libreria Commissionaria Sansoni Spa

Via Duca di Calabria 1/1

I-50125 Firenze, Italy

Phone +39 055 6483201

Fax +39 055 641257

E-mail: laura.mori@licosa.com

Human and murine microglial cell lines: two experimental models to deeply understand the mechanism of microglia involvement in human brain diseases

Jacopo J.V. Branca¹, Gabriele Morucci¹, Massimo Gulisano¹, Marco Ruggiero², Ferdinando Paternostro¹, Alessandra Pacini¹, Lorenzo Di Cesare Mannelli³, Stefania Pacini¹

¹ Department of Experimental and Clinical Medicine, University of Firenze, 50134 Firenze, Italy

² Department of Clinical and Biomedical Experimental Sciences, University of Firenze, 50134 Firenze, Italy

³ Department of Neurosciences, Psychology, Drug Research and Child Health, Section of Pharmacology and Toxicology, University of Firenze, 50134 Firenze, Italy

Microglia act as a first line of defence against pathogen invasion, by recognizing, sequestering and processing antigens. Once microglia become activated, they produce and release many substances that activate nearby astrocytes, microglia and neurons (Martin et al., 2005). In this study we compare two microglial cell lines: the human cell line c13-nj and the murine microglia cell line BV-2. BV-2 are commonly available on the market but their main limitation consists in the fact that they are not human thus limiting their use for the study of human brain diseases. On the contrary, the human c13-nj cell line, which would be the most appropriate to investigate the role of microglia in human brain diseases, is not commercially available. In this study we compare the cell viability, the cAMP formation, the VEGF expression as well as the expression of a specific marker of microglial activation (B7-2) after treatment with a toxicant (oxaliplatin) and with a protective agent (GcMAF) on BV-2 and on c13-nj (kindly donated). Our results show that the human microglial cell line is more resistant to toxicants such as oxaliplatin; however, the signal transduction pathways activated when the two cell lines are treated both with oxaliplatin and with GcMAF, are the same. This lead us to hypothesize that the murine microglial cell line (BV-2) can be considered as a superimposable model in studies concerning human brain representing an excellent experimental model, not expensive, easy to culture and to retrieve.

References

- [1] Martin et al. (2005) Neurotensin and the Neurotensin Receptor-3 in Microglial Cells. *J Neurosci Res* 81: 322-326.

Keywords

Human microglia, VEGF, B7-2, oxaliplatin, GcMAF.

Gc-protein derived macrophage activating factor (GcMAF) counteracts the neuronal damage induced by oxaliplatin

Gabriele Morucci¹, Jacopo J. V. Branca¹, Massimo Gulisano¹, Marco Ruggiero², Ferdinando Paternostro¹, Alessandra Pacini¹, Lorenzo Di Cesare Mannelli³, Stefania Pacini¹

¹ Department of Experimental and Clinical Medicine, University of Firenze, 50134 Firenze, Italy

² Department of Clinical and Biomedical Experimental Sciences, University of Firenze, 50134 Firenze, Italy

³ Department of Neurosciences, Psychology, Drug Research and Child Health, Section of Pharmacology and Toxicology, University of Firenze, 50134 Firenze, Italy

Oxaliplatin-based regimens are effective in metastasised advanced cancers. However, a major limitation to their use is represented by neurotoxicity leading to peripheral neuropathy (Wolf et al., 2008). In this study we evaluate the effects of an immunotherapeutic agent (Gc protein-derived macrophage activating factor, GcMAF) in preventing oxaliplatin-induced neuronal damage and in restoring microglial activation. The effects of oxaliplatin was studied in human neurons (SH-SY5Y) and microglial cells (c13-nj). Cell density, morphology and viability as well as production of cAMP and expression of vascular endothelial growth factor (VEGF), markers of neuron regeneration and markers of microglia activation were determined. GcMAF reverted the damage inflicted by oxaliplatin on human neurons and preserved their viability; it also increased cAMP production, VEGF and neuromodulin expression. GcMAF did not revert the effects of oxaliplatin on microglial cell viability. However, it induced microglial activation resulting in an increased expression of a specific marker without any increase in cell number. Our results demonstrate that GcMAF may significantly contribute to neutralize the neurotoxicity induced by oxaliplatin, at the same time concurring to an integrated anti-cancer effect.

References

- [1] Wolf et al. (2008) Chemotherapy-Induced Peripheral Neuropathy: Prevention and Treatment Strategies. *Eur J Cancer*; 44: 1507-1515.

Keywords

Oxaliplatin, human neurons, human microglia, vitamin D, cancer, immunotherapy, GcMAF.

Gc-protein-derived Macrophage Activating Factor (GcMAF) induces ERBB2 shift in human breast cancer

Marco Ruggiero^{1,3}, Massimo Gulisano², David Noakes³, Jacopo J. V. Branca², Gabriele Morucci², Stefania Pacini²

¹Department of Experimental and Clinical Biomedical Sciences, University of Firenze, 50134 Firenze, Italy

²Department of Experimental and Clinical Medicine, University of Firenze, 50134 Firenze, Italy

³Immuno Biotech Ltd; GY1 6NB Guernsey, Channel Islands, UK

HER2/Neu/ERBB2 is a receptor tyrosine kinase overexpressed in a high percentage of human breast cancers. Gc-protein-derived Macrophage Activating Factor (GcMAF) is a powerful stimulant of the immune system endowed with intrinsic anticancer properties (Pacini et al., 2012). We recently demonstrated that molecular complexes of oleic acid (OA) and GcMAF (OA-GcMAF) show significant therapeutic activity in a variety of tumours (Ward et al., 2014). Here we demonstrate that OA-GcMAF eradicates ERBB2 expression in human breast cancer. A biopsy taken before OA-GcMAF treatment showed strong positivity to ERBB2. The patient was then treated with OA-GcMAF administered through subcutaneous injections and with food naturally rich in OA-GcMAF for 3 weeks prior to mastectomy. The subsequent surgery specimen was negative for ERBB2. These results lead to hypothesize: 1. OA-GcMAF completely reversed the neoplastic phenotype. 2. OA-GcMAF induced the apoptosis of all ERBB2-positive cancer cells.

References

- [1] Pacini et al. (2012) Effects of Vitamin D-binding protein-derived Macrophage-Activating Factor on Human Breast Cancer Cells. *Anticancer Res* 32: 45-52.
- [2] Ward et al. (2014) Clinical Experience of Cancer Immunotherapy integrated with Oleic Acid Complexed with De-glycosylated Vitamin D Binding Protein. *Am J Immunol* 10: 23-32.

Keywords

Breast cancer, GcMAF, immunotherapy, oncogene, human EGF receptor.

Intra-tumoural nitric oxide release by macrophages activated by Gc-protein-derived Macrophage Activating Factor (GcMAF)

Marco Ruggiero^{1,3}, Massimo Gulisano², Jacopo J.V. Branca², Gabriele Morucci², David Noakes³, Stefania Pacini²

¹ Department of Experimental and Clinical Biomedical Sciences, University of Firenze, 50134 Firenze, Italy

² Department of Experimental and Clinical Medicine, University of Firenze, 50134 Firenze, Italy

³ Immuno Biotech Ltd; GY1 6NB Guernsey, Channel Islands, UK

Over past decades, nitric oxide (NO) has emerged as a molecule of interest in cancer treatment because of its tumouricidal properties (Choudhari et al., 2013). Gc-protein-derived Macrophage Activating Factor (GcMAF) induces the synthesis and release of NO by activated macrophages. It was previously demonstrated that molecular complexes of oleic acid (OA) and GcMAF (OA-GcMAF) stimulate macrophage activation in cancer patients (Ward et al., 2014). Here we demonstrate that intra-tumoural injection of OA-GcMAF leads NO synthesis and release inside the tumour. Under ultrasound guidance, OA-GcMAF was injected into patients harbouring different types of solid tumours; a metastasis from a melanoma, and a metastasis from breast cancer. Intra-tumoural NO synthesis and release was monitored in real-time by power-doppler ultrasonography. One to two minutes after injection, we observed a significant increase in blood flow and in blood vessels diameter, a clear indication of vasodilation due to NO synthesis and release. These observations substantiate the dramatic clinical results previously observed by Ward et al. (2014), and open the way to further investigation in the role of GcMAF as a powerful anticancer agent.

References

- [1] Choudari et al. (2013) Nitric oxide and cancer: a review. *World J Surg Oncol* 11: 118-129.
- [2] Ward et al. (2014) Clinical Experience of Cancer Immunotherapy integrated with Oleic Acid Complexed with De-glycosylated Vitamin D Binding Protein. *Am J Immunol* 10: 23-32.

Keywords

Nitric Oxide, GcMAF, immunotherapy, breast cancer, ultrasonography.