International Immunopharmacology 62 (2018) 86–95

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Genistein reduces proliferation of EP3-expressing melanoma cells through inhibition of PGE2-induced IL-8 expression

Isabella Venza[a,1], Maria Visalli[b,1], Rosaria Oteri[b], Concetta Beninati[c], Diana Teti[d], Mario Venza[a,*]

[a] Department of Biomedical, Odontostomatology Sciences and of Morphological and Functional Images, University of Messina, Messina, Italy
[b] Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy
[c] Department of Human Pathology of Adult and Developmental Age "Gaetano Barresi", University of Messina, Messina, Italy
[d] Charybdis Vaccines Srl, University of Messina, Messina, Italy

ARTICLE INFO

Keywords:
Genistein
Melanoma
IL-8
PGE2
EP receptors

ABSTRACT

Genistein, a natural isoflavone found in soybean products, is considered as a powerful anti-cancer agent, although the involved mechanisms are not fully understood. There is a growing body of evidence that, among the genes inhibited by genistein and responsible for cell cycle progression, invasion, metastasis, and angiogenesis, IL-8 occupies a relevant place. On the other hand, it is equally well documented that IL-8 is upregulated by prostaglandin E2 (PGE_2) in different pathological conditions, particularly in neoplastic disease. Here we investigated whether genistein could affect cell growth in a panel of oral, uveal and cutaneous melanoma cell lines by interfering with basal or PGE2-induced IL-8 production. To this end, experiments were performed to evaluate the effect of PGE2 treatment on IL-8 levels, the expression and the role of PGE2 receptors and whether genistein could be able to interfere with these events. Finally, it was evaluated whether the inhibition of oral, uveal and cutaneous melanoma cell proliferation in the presence of genistein could be related to a reduction of IL-8 levels. We show that PGE2 enhances IL-8 synthesis via the EP3 receptor and that genistein is able to down-regulate the latter, as well as to decrease IL-8 mRNA and protein expression, thereby inhibiting oral, uveal and cutaneous melanoma cell proliferation. Taken together, our data provide new insights into the anti-cancer properties of genistein by showing that this flavonoid may affect the development and growth of melanoma at oral, uveal and cutaneous sites. Moreover, these results provide evidence that genistein may exert its therapeutic activity through its ability to prevent PGE2-mediated IL-8 induction.

1. Introduction

Melanoma is the most serious type of skin tumor due to its high propensity to invasion, metastasis and angiogenesis [1, 2]. This condition is strictly related to chronic exposure to solar ultraviolet radiation [3] that promotes chronic inflammation and high levels of proinflammatory cytokines and prostaglandins (PGs) [4, 5]. In particular, PGE2, the most ubiquitously produced PG, was found to act as a promoter of melanoma tumorigenesis [6], and its elevated production has been associated with an unfavourable prognosis in melanoma patients [7]. Interestingly, increased expression levels of cyclooxygenase-2 (COX-2), a rate-limiting enzyme that catalyses the conversion of arachidonic acid to PGs, were correlated with the development and progression of human melanoma [8], while inhibition of microsomal prostaglandin E synthase-1 (mPGES1), the dominant enzyme that converts the COX derived intermediate product, PGH_2, to PGE_2, was linked to the suppression of melanoma cell survival [7].

Indeed, PGE2 exerts a variety of pro-oncogenic effects either directly or indirectly through the enhancement of the expression of cytokines, such as interleukin-8 (IL-8) [9, 10], that in turn promotes invasion, angiogenesis and metastasis in many cancers, including melanoma [11]. In this regard, we previously showed that PGE2 epigenetically activates the IL-8 gene by inducing histone H3 hyperacetylation and DNA demethylation at a specific site of the IL-8 promoter [12].

Moreover, PGE2 is produced either by melanoma or by tumor-surrounding cells [13], and its effects are associated with the expression and the activation of PGE2 receptors (EPR) [14]. It is worth to note that the G protein-coupled receptors EP1, EP2, EP3 and EP4 have been implicated in angiogenesis, decreased host immunity, enhanced

* Corresponding author at: Department of Biomedical, Odontostomatology Sciences and of Morphological and Functional Images, Azienda Policlinico Universitario G. Martino, via Consolare Valeria, 1, 98125 Messina, Italy.
E-mail address: mario.venza@unime.it (M. Venza).
[1] These authors contributed equally to this work and therefore should be considered equal first authors.

https://doi.org/10.1016/j.intimp.2018.06.009
Received 17 November 2017; Received in revised form 17 May 2018; Accepted 4 June 2018

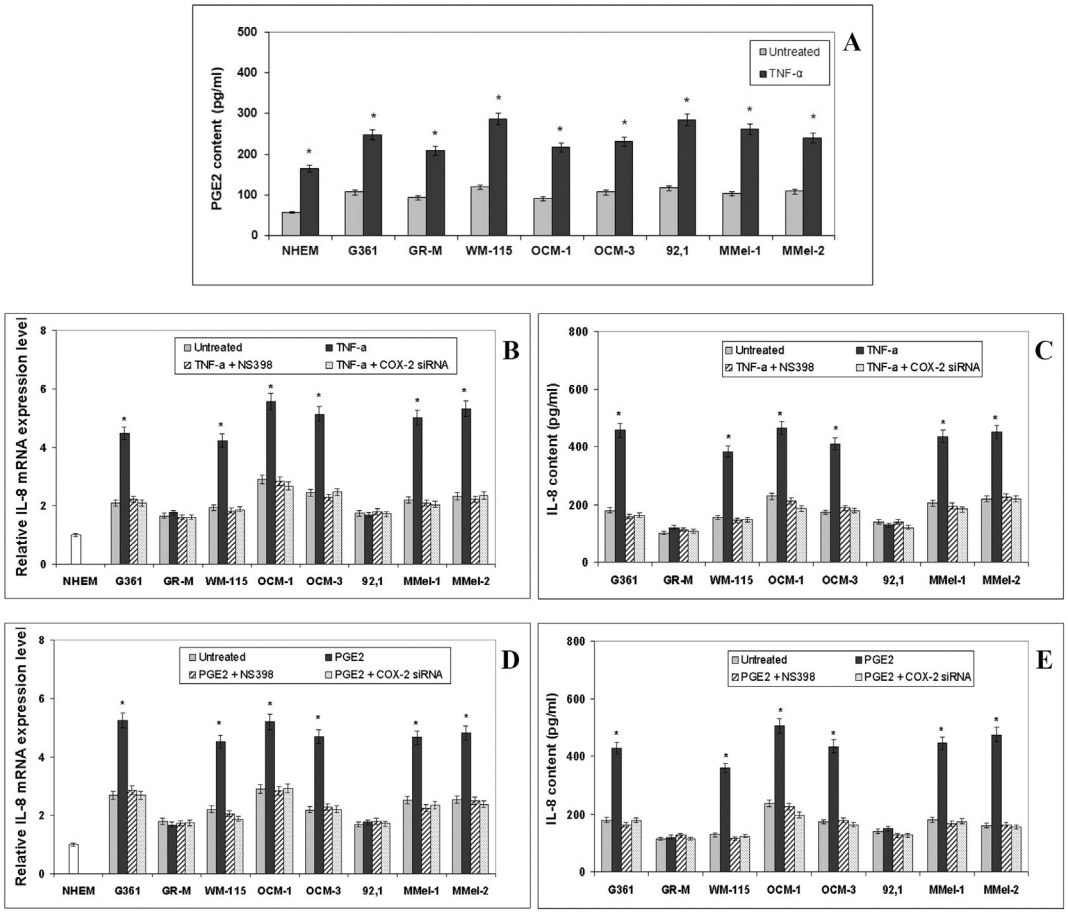

Fig. 1. IL-8 expression is upregulated by endogenous and exogenous PGE2. (A) Exogenous TNF-α enhances PGE2 secretion in normal melanocytes and melanoma cell lines. (B and C) Exogenous TNF-α stimulated IL-8 mRNA expression and protein secretion in G361, WM-115, OCM-1, OCM-3, MMel-1, and MMel-2 melanoma cells. TNF-α treatment was not able to stimulate IL-8 mRNA expression and protein production in melanoma cells pre-treated with COX-2 inhibitor (NS398) or specific siRNA. (D and E) Exogenous PGE2 (10 μM) promoted IL-8 mRNA expression and protein secretion in G361, WM-115, OCM-1, OCM-3, MMel-1, and MMel-2 melanoma cells. PGE2 treatment was not able to stimulate IL-8 mRNA expression and protein production in melanoma cells pre-treated with COX-2 inhibitor (NS398) or specific siRNA. Data are depicted as means ± SD of three independent experiments. Significant *$p < 0.01$, as calculated by ANOVA test.

invasion, and metastasis in cancer [15, 16].

In the last decades much attention was given to the potential role of various biological agents as new adjuvant cancer therapies for their newly recognized ability to modify the expression of many genes involved in tumorigenesis, so that some of them are successfully employed in antitumor therapy, particularly in the treatment of melanoma [17–20]. Interestingly, the reduction of EPR expression in melanoma, together with the inhibition of COX-2 and PGE2, is believed to be essential for the suppression of melanoma cell invasion and migration induced by green tea catechins [15, 21, 22].

Genistein, the predominant isoflavone found in soy products, has been lately employed in cancer chemoprevention and treatment with encouraging results, which might be linked to its reversal effects on radio- and chemo-resistance [23–25]. Moreover, genistein has antioxidant properties, prevents the expression of stress and inflammation related genes, i.e. IL-8, and induces apoptosis, cell cycle arrest, and inhibition of angiogenesis in various cancer cells [26–34]; however, the functional relationship among such distinct activities of genistein remains unknown.

On these bases it seemed interesting to: i) assess whether PGE2 has any effect on IL-8 expression in oral, uveal, and cutaneous melanoma; ii) identify the PGE2 receptor/s that might be involved in this effect; iii) investigate if genistein could interfere with such effects; iv) test whether the known inhibitory activity of genistein on melanoma cell growth is attributable to changes in IL-8 expression. Here we presented evidence that PGE2 enhances IL-8 synthesis in melanoma cells via the EP3 receptor and that genistein is able to downregulate this receptor and, as a consequence, to decrease IL-8 expression and cell proliferation. These results, besides adding to our knowledge of the inhibiting activity of genistein on IL-8 expression and melanoma growth, open up hopeful perspectives for the newly emerging “integrated therapy” that combines biologically based complementary and alternative medicines with conventional treatments.

2. Materials and methods

2.1. Cell cultures and treatment

Normal Human Epidermal Melanocytes (NHEM) cells (Lonza, Walkersville, USA) were grown in Melanocyte Medium plus Bullet Kit (Lonza). G361 cutaneous melanoma cells (ECACC, Salisbury, UK), established from a malignant melanoma of a 31 year old male Caucasian, were grown in McCoy's 5a medium modified with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 1% penicillin/streptomycin. WM-115 cutaneous melanoma cells (ATCC; Manassas, VA), derived from primary skin tumor of a 55-year-old female, were cultured in Eagle's Minimum Essential Medium supplemented with 10% FBS and 1% penicillin/streptomycin. OCM-1 cells (provided by J. Mellon, Department of Ophthalmology, UT Southwestern Medical Center, Dallas, TX), derived from a spindle cell ocular melanoma, OCM-3 cells (provided by Martine J. Jager, Leiden University Medical Center, Leiden, The Netherlands), obtained from an epithelioid cell ocular melanoma, 92.1