Anti-angiogenic effects of Mangiferin in metastatic melanoma. A fair tale of two worlds.

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We described here how international collaboration can validate the preclinical research of a new use for a given molecule produced in Cuba while strengthening the scientific capacity of the country.[b]

Mangiferin is a bioactive xanthonoid $C_{19}H_{18}O_{11}$, 1,3,6,7-tetrahydroxyxanthone-C2-β-D-glucoside present in the mango tree (*Magnifera indica L.*) widely grown in tropical and some sub-tropical countries. Although many medicinal properties of the plant have been known for a long time among the rural populations where mango trees grow, it is only in the last 20 years that the research into the molecular mechanisms of its active component Mangiferin (Mg) has been revealed [1]. Interestingly, those molecular mechanism of actions are also involved in diseases of the developed world, such as cancer and degenerative diseases [2]. In this respect, we report to the TCS journal some novel insights of this particular research elucidating the anticancer mechanism of action of Mg against metastatic melanoma to show that the cooperation between the North and South can generate frontline science while strengthening the scientific capacity of the developing partner.

The Cuban Centre of Pharmaceutical Chemistry and other institutions in the scientific pole of Havana has been investigating for more than 20 years this natural bioactive glucosylxanthone as a potential molecule (either monoceutical or natureceutical) with new well-defined pharmacological properties. Previous research in Cuba demonstrated that Mg has antioxidant, anti-proliferative, anti-tumor, and anti-angiogenic properties in different ***in vitro*** and ***in vivo*** models [2]. On the other hand, Cuban researchers have been advancing the collaboration with Flemish researchers in Belgium through one of the VLIR-UOS projects [c]. One of the aims in VLIR-UOS is to offer partnership that can continue advancing in the developing country [d]. Indeed, the research published in the high-impact journal Melanoma Research [3] was the result of the long-term partnership between the University of Havana, and the University of Antwerp [4], in a network with other institutions of both countries: Cuba and Belgium. A Cuban Belgium researcher was also part of the team working at the University of Antwerp. The Belgium-Cuba research partnership owed its success to the leadership of Professor Gay Haegeman from the University of Gent who sadly passed away last year, and to Professor Wim Vanden Berghe from the University of Antwerp currently leading the project. Through this project, the Cuban partner institution received the expensive equipment used during training in Belgium.

Malignant melanoma continues being one of the most aggressive and deadly skin cancers. Despite the availability of novel therapies, there is still poor improvement of patient survival. Previous studies suggested that Mg might reduce aggressiveness of this tumor by interfering with vascular angiogenesis, a pivotal feature of the metastatic melanoma.

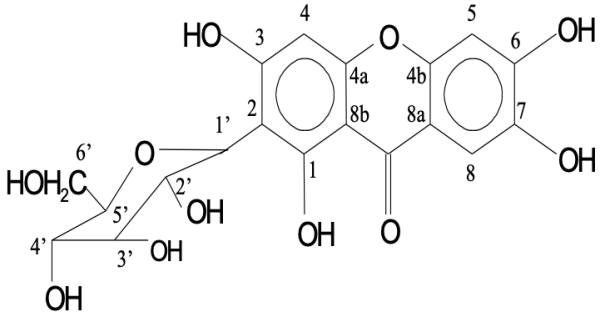

Figure 1: $C_{19}H_{18}O_{11}$, 1,3,6,7-tetrahydroxyxanthone-C2-β-D-glucoside

For this study, the glycosylated xanthone mangiferin was purified in Cuba as a mayor bioactive component (Figure 1) of extracts obtained from leaves and the stem bark of specific varieties of the mango tree *Mangifera indica L.*

Accordingly, a set of ***in vitro*** assays was carried out including cell scratch migration assay, human placenta blood vessel explant assay, capillary tube formation by endothelial cells, and the chorioallantoic mem-

brane (CAM) angiogenesis assay in order to find and validate markers of vascular angiogenesis.

In this paper [3] we demonstrate that Mg has the ability to inhibit the formation of new vessels from pre-existing vessels (angiogenesis) ***in vitro*** and ***in vivo***. These results support the hypothesis that its antiangiogenic activity is essential to explain its antitumor effect. Aggressive and metastatic tumors create their own vascular bed providing oxygen and nutrients to fast proliferating cancer cells. Finding signal transductions activated in this microenvironment is part of current strategies for the development of new antitumor products.

Mangiferin inhibits the gene expression of key signal molecules associated with processes of migration, proliferation, survival and vasculogenesis in metastatic B16 melanoma cells (B16F10 cell line). Using the Ingenuity Pathway Analysis (IPA, Ingenuity System) for functional enrichment and detection of significant pathways, our experiments illustrate that Mg selectively inhibits the up-regulation of the expression of genes such as IL6, TNF, IFNG, VEGFR2, PLAU, FGF1, MMP19, CCL2 and PGF, typical inflammatory and angiogenic mediators. Moreover, by showing dose-dependent Mg specific inhibition of phosphorylation of NF-κB, a key mediator up-stream of the inflammatory response, we further demonstrate significant immunosuppressive effects of Mg treatment in metastatic melanoma cells.

Finally, our study demonstrated that Mg is also capable to inhibit TNFα induction of angiogenesis ***in vivo*** in a dose-response manner. The antiangiogenic activity was carried out in melanoma syngeneic studies ***in vivo*** (Figure 2), in models of tumor angiogenesis induced by TNFα and by the highly metastatic melanoma cells (B16F10).

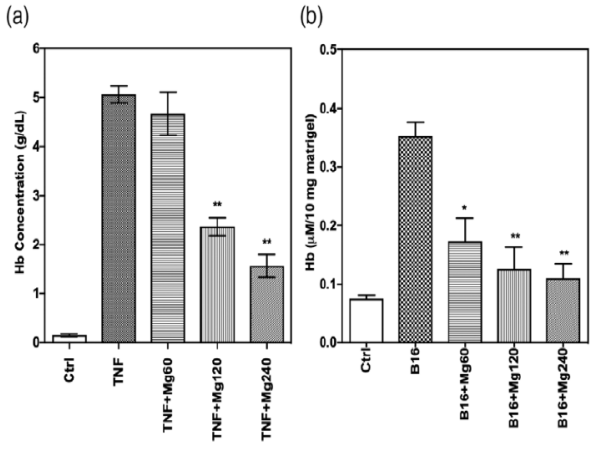

Figure 2: Mangiferin (Mg) (60-240 μM) inhibits neovascularization ***in vivo*** on (a) TNF alpha (10 ng/mL) and (b) B16F10 10^5 cells-induced angiogenesis in mouse abdominal subcutaneous connective tissue.

In conclusion, in this study we demonstrate that Mg holds promise for a novel pharmacophore candidate for cancer treatment. Although Mg showed a potent anti-angiogenic and anti-metastatic effects, highly tolerated, widely available and attractive due to its cost-effective production, these results require the development of pharmaceutical and clinical studies that validate the preclinical findings that are presented in this investigation.

The authors would like to dedicate this work to the memories of Janet Rodriguez Morales (Cuba) and Professors Sandra Apers and Guy Haegeman (Belgium).

Notes

a. Email: rdh231259@gmail.com

b. Original version of this article is Ref. [3]

c. ZEIN2011PR383 and ZEIN2016PR418. Capacity building-Cuban bioactive compounds. `https://www.vliruos.be/en/projects/project/22?pid=3947`

d. VLIR-UOS supports partnerships between universities `https://www.vliruos.be/en/about_vlir_uos/2#annual-reports`

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