"THE EFFECT OF GROWTH DIFFERENTIATION FACTOR 11 (GDF11) ON THE RESPONSE OF TUMOR-ASSOCIATED MACROPHAGES IN HEPATOCELLULAR CARCINOMA DERIVED CELLS"

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Introduction and Objectives: Hepatocellular carcinoma (HCC) is the most common and aggressive form of liver cancer. Until 2020, worldwide, it has been ranked 5th and 3rd in terms of incidence and mortality, respectively. HCC has various etiologies, but tumors formed by high cholesterol intake induce an aggressive phenotype and high tumor-associated macrophages (TAM) infiltration. TAM acquires an alternative polarity (M2) with immunosuppressive and pro-tumoral activities. HCC patients with high TAM recruitment have a poor prognosis with a low free-survival rate. The design of therapies represents a challenge. The growth differentiation factor 11 (GDF11) has been proposed as a promising molecule for treating HCC, favors a reduction in proliferation, invasion, and lipid metabolism, and therefore reduces the aggressive phenotype. In our research group, we have found that GDF11 also has various effects on cells belonging to the tumor microenvironment, particularly in TAM.

Aim: To evaluate TAM polarity and their intercommunication with HCC-derived cells during treatment with GDF11.

Materials and Methods: THP-1-macrophages cell line was used; lipopolysaccharides were used for the M1 polarity acquisition and IL-4/13 or conditioned medium (CM) derived from Huh-7 cells for M2 polarity acquisition, flow cytometry (FCM) was used. GDF11 (50 ng/mL) was applied every 24 h up to 3 days. We used anti-Smad2 and 3 antibodies in Western blot (WB). Cell viability and proliferation studies were performed using crystal violet. The quantification of cholesterol was performed using o-Phthaldialdehyde and free radicals' detection was performed using dihydroethidium. Data were presented as mean ± standard error of the mean (SEM). The analysis of variance (ANOVA) test was used to compare the mean values between groups. Each result has at least 3 independent experiments. Statistical significance was indicated with an asterisk (*P<0.05).

Results and Discussion: GDF11 treatments induced Smad proteins activation by specific phosphorylation, indicating that THP-1 macrophages respond to treatment in times from 5 to 60 min, but maintaining their response up to 72 h. This could suggest that the difference in the subsequent response may be involved in the binding or activation of additional adapter proteins of this signaling pathway. Furthermore, it was observed that GDF11 does not affect the cell viability, proliferation, and morphology studied in their different polarities. Macrophage re-education or re-polarization assays were carried out by adding GDF11 for an additional 72h. The data obtained show that TAM populations lost CD206 marker, going from 90% to 40% of the population, suggesting a loss of this pro-tumor polarity and a re-education towards anti-tumor macrophages responses. However, experiments show a dual role of GDF11 in macrophages due to the increase of specific markers in non-activated cells, indicating that it has a different effect depending on the activation state. Since GDF11 compromises cholesterol metabolism, which is observed in a decrease in total cholesterol levels in non-activated macrophages, which is observed in other studies using HCC cell lines. Also, it was possible to corroborate that GDF11 is behaving like a statin used as a positive control in macrophages and Huh-7 cells. Finally, GDF11 increases ROS levels, specifically superoxide anion, characteristic of phagocytic M1 macrophages. To evaluate response on HCC cells, wound-healing assays indicated that macrophage secretion is a key factor for cell displacement rather than viability and proliferation.

Conclusion: In the present study, we found that GDF11 has immunomodulatory effects in TAM, decreasing pro-tumoral markers and lipid content that could be important to design HCC therapies.

The authors declare that there is no conflict of interest.

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EFFECT OF SUPPLEMENTATION WITH TYPICAL MEXICAN FOODS (OPTUNA FICUS INDICA, THEOBROMA CACAO AND EDIBLE CRICKETS) IN HIGH-FAT/HIGH-SUGAR DIET-FED MICE

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Introduction and objectives: The obesogenic environment, including high fat/ high sugar diet, are risk factors for developing multiple diseases associated with obesity, such as metabolic-associated fatty liver disease (MAFLD) and metabolic syndrome, among others. An increase in physical activity and change of diet is the first therapeutic line to prevent or treat obesity; however, adherence to this treatment is negligible. Therefore, this communication's objective was to evaluate the effect of supplementation with a mixture of foods of Mexican origin: *Optuna ficus indica*, *Theobroma cacao* and edible crickets (MexTHER) on a diet high in saturated-fats and fructose-sucrose in obesogenic mice.

Material and methods: Twenty male C57BL/6J mice were divided into three groups between 7 and 8 weeks of age. Control group: Normal diet (ND) for 16 weeks; HF Group: Diet with 45% of Kcal from fat and water added with 55% fructose and 45% sucrose *ad libitum* for 16 weeks; and Therapeutic group: HF diet up to week eight and the last 8 weeks switched to a high fat/sugar diet supplemented with 10% nopal, 10% cocoa and 10% cricket (MexTHER). The animals were sacrificed at 16 weeks and histological, biochemistry and cognitive analysis were performed.

Results: Mice fed with MexTHER diet showed a significant reduction in weight at sacrifice. After two weeks MexTHER group equaled their weight to the ND group (ND = 35.92 ± 4.76, HF = 47 ± 7.11, MexTHER = 34.20 ± 6.71 p <0.001). Liver weight, visceral fat, and epididymal fat were also significantly reduced. The MexTHER group significantly decreased levels of triglycerides, cholesterol, LDL, insulin, glucose, GIP, leptin, PAI-1 and resistin. The livers of MexTHER and ND mice did not show histological alterations. The size of adipocytes showed significantly smaller diameters in the MexTHER group against HF group. Mice supplemented with MexTHER improved