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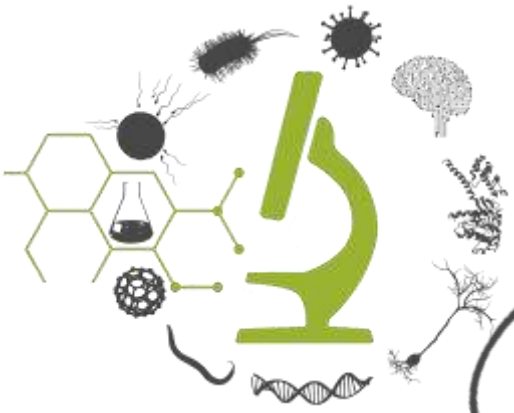

4th – 6th November 2020,

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BOOK OF ABSTRACTS

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P02-8 Narrow-leafed lupin (Lupinus angustifolius L.) seed β-conglutin proteins induce G0/G1 arrest and apoptosis in human colorectal cancer cells

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"Introduction: Lupins seed proteins seem to be promising as innovative source of functional food with positive health aspects: prevention of cardiovascular diseases and reduction of glucose and cholesterol blood levels.

Lupinus angustifolius (narrow-leafed lupin, NLL) seeds are a valuable source of proteins with multiple nutraceutical properties. Among these proteins, research attention has been focused on the β-conglutin family.

Recently, the potential anti-diabetic, antioxidant and anti-inflammatory activities of β-conglutins 1, 3 and 6 have been studied (decrease of pro-inflammatory cytokines, inhibition of chemotaxis and cell adhesion capacity among others).

Methodology: We purified NLL recombinant β-conglutins (rβ1, rβ3, and rβ6) using affinity-chromatography and evaluated their effects on colorectal cancer (CRC) cell lines HCT-116 (p53 wild-type) and HCT-116 p53 null (p53 inactive) by cell culture and flow cytometry assays.

Results: The results showed that β-conglutins are capable of inhibiting the growth of CRC cells. In HCT-116 cell line, the IC50 values are 0.8, 5.8 and 30.1 µg ml for β1, β3 and β6 respectively. Moreover, for HCT-116 p53 null line, the IC50 values are 3.0, 3.4 and 51.8 µg/ml for β1, β3 and β6 respectively. Thereby, in both cell lines, the effect is higher in the case of β1, followed by β3 and β6. Additionally, cell proliferation decreases, inducing cell cycle arrest independently of p53. Specifically, β1 and β3 induce accumulation in G0/G1 phase while β6 induces, in addition, accumulation in G2/M. In all cases, very few cells were found in S phase. Finally, this treatment considerably increases the apoptosis independently of p53.

Discussion: β1, β3 and β6 conglutin proteins from NLL seeds affect the viability in CRC cells at very low concentrations, inducing apoptosis and decreasing cell proliferation by cell cycle arrest: these proteins may be natural chemotherapeutic agents with potential uses for treatment of human CRC.

P02-9 Biocompatible Glucose Oxidase-Powered JanusPt-MSN Ultra-Fast Nanomotors for Controlled Doxorubicin Delivery to Cancer Cells

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The use of mesoporous silica nanoparticles with “molecular gates” to specifically deliver therapeutic payloads to cancer cells, among other target destinies, has been successfully proved in the past due to their well-known benefits1. Nevertheless, their displacement to the target is only provoked by the passive EPR effect, resulting in poor tumor penetration and deficient cytotoxic results. For this reason, have recently emerged the development of self-propelled nanodevices to improve the tumor reach an infiltration, named as nanomotors. However, most of the nanomotors reported present a dichotomy between velocity and biocompatibility, to achieve high speeds they need to use toxic exogenous fuels 2-4. Therefore, in this work we report the design and evaluation of a novel multifunctional nanomotor with potential “smart” anticancerogenic properties. Consist in a Janus nanodevice, presenting two differentiate faces: a platinum nanopartide (Pt NP) and a mesoporous silica nanoparticle (MSN). The MSN part acts as a nanocontainer, being loaded with an anticancer drug (Doxorubicin). Externally is functionalized by amide bonds with the high active enzyme Glucose Oxidase (GOx), acting at the same time as “molecular gate” and first step propelling system, transforming glucose into hydrogen peroxide (H_2O_2). While the Pt NP gives the final pushing to the nanomotor, catalyzing the reduction of H_2O_2 into water and oxygen (gas). The advantages of our Janus Pt-MSN-Doxo-GOx nanomotor reside in the bioavailability of the fuel, glucose, in the tumor microenvironment and in its anisotropy, that guide their spatial trajectories in a directional manner.

References

1. Iturrioz-Rodríguez, et al. Controlled drug delivery systems for cancer based on mesoporous silica nanoparticles. Int. J. Nanomedicine 2019, 14, 3389-3401.

Narrow-leafed lupin (Lupinus angustifolius L.) seed β-conglutin proteins induce G0/G1 arrest and apoptosis in human colorectal cancer cells

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INTRODUCTION:

- Lupins seed proteins seem to produce promising positive health aspects, preventing cardiovascular disease, and reduction of glucose and cholesterol blood levels.
- Among these proteins, particular research attention has been focused to those from the vicilin or beta-conglutin family, which are the most abundant proteins in NLL seeds.
- Recently, we have demonstrated the potential antidiabetic, antioxidant and anti-inflammatory activities of particular beta-conglutin proteins β1, β3 and β6: decrease of the production of pro-inflammatory cytokines; inhibition of chemotaxis and cell adhesion capacity. These properties could be attributed to the ability of these conglutins to interact with insulin.

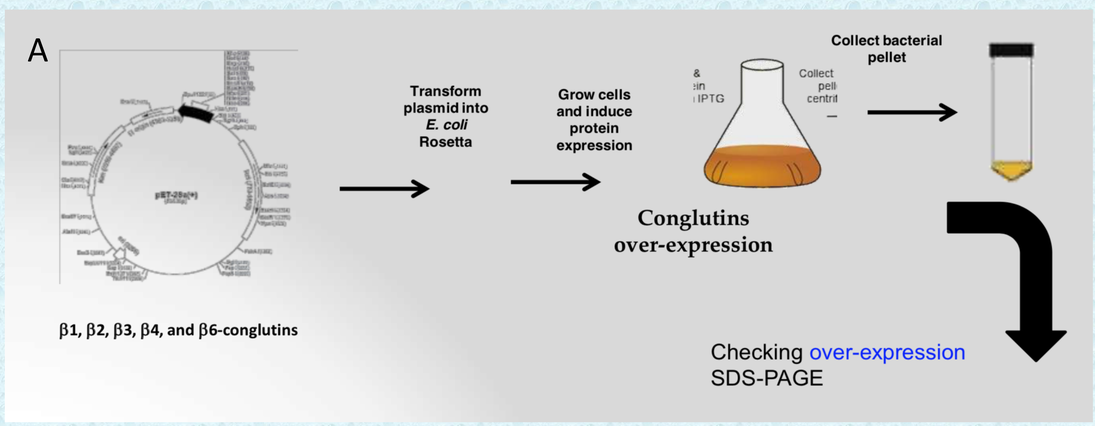

Figure 1. Overexpression, purification and selection of recombinant β-conglutins using affinity chromatography and western-blotting. **A:** Overexpression of β-conglutins using *E.Coli* Rosetta.
B: Purification with Affinity-Chromatography and dialysis. **C**: Checking of β-conglutins with Western Blotting.

CONCLUSIONS:

In summary, β-conglutin proteins from blue lupine seeds, affect the **viability in colorectal cancer cells**, inducing **apoptosis** and **decreasing cell proliferation** by cell cycle arrest, either in G0/G1 phase or in G2/M phase. Therefore, our results suggest that NLL β-conglutin proteins may be **potential chemotherapeutic agents** with potential uses for treatment of human colon cancer.

REFERENCES: C. Delgado-Andrade, R. Olías, J.C. Jimenez-Lopez, A. Clemente. *Arbor* 2016, **192**, a313- ; R.C. Foley, J.C. Jimenez-Lopez, L.G. Kamphuis, J.K. Hane, S. Melser, Singh K.B. *BMC Plant Biology* 2015, **15**, 106. ; E. Lima-Cabello, V. Alche, R.C. Foley, S. Andrikopoulos, G. Morahan, K.B. Singh, J.D. Alche, J.C. Jimenez-Lopez. *Molecular Nutrition & Food Research* 2017, **61**. ; E. Lima-Cabello, S. Morales-Santana, J. León, V. Alche, A. Clemente, J.D. Alche, J.C. Jimenez-Lopez. *Food & Function*, 2018, DOI: 10.1039/C8FO01164H; E. Lima-Cabello, S. Morales-Santana, R.C. Foley, S. Melser, V. Alche, K.H.M. Siddique, K.B. Singh, J.D. Alche, J.C. Jimenez-Lopez. *Journal of Functional Foods* 2018, **40**, 510 – 519.

ACKNOWLEDGMENTS: Supported by European Research Program MARIE CURIE (FP7-PEOPLE-2011-IOF), Project ref.: PIOF-GA-2011-301550; by the Spanish Government (MINECO), project ref.: RYC-2014-16536 (Research Program Ramon y Cajal), and project ref.: BFU2016- 77243-P; and by CSIC – Intramural project, Ref: 201540E065

MATERIALS AND METHODS:

In the present study, we purified NLL recombinant betaconglutin proteins (rβ1, rβ3, and rβ6) using affinity–chromatography (Figure 1) and evaluated their effects on colon cancer cell lines HCT-116 (p53 wild-type), and HCT-116 p53 null (p53 inactive) using flow cytometry and tetrazolium (MTT) assay for cellular viability and activity (Figures 2 and 3).

RESULTS:

- β1, β3 and β6 conglutins are capable of inhibiting the growth of colorectal cancer cells.
- When the HCT-116 p53 null line is treated, the IC50 values are 3.0, 3.4 and 51.8μg/ml for β1, β3 and β6 conglutin, respectively (figure 2).
- In both cell lines β-conglutin proteins effect is higher in the case of β1, followed by β3 and β6.
- β1 and β3 conglutins induce accumulation in G0/G1 phase, while β6 induces, in addition, the accumulation in G2/M phase (figure 3A).
- On the other hand, the treatment with the three conglutins considerably increases the apoptosis, independently of p53 (figure 3B).

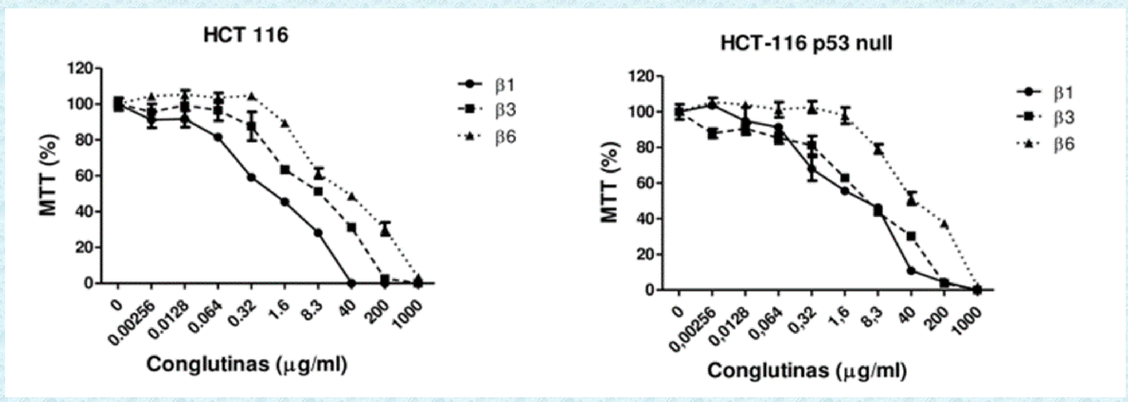

Figure 2. Results of the tetrazolium (MTT) assay for cellular viability for HCT-116 and HCT-116 p53 null cell lines. For both cell lines, the 24h treatment with the three β-conglutin proteins produces a decrease in the cellular viability and this effect is increased with higher concentrations of the conglutins.

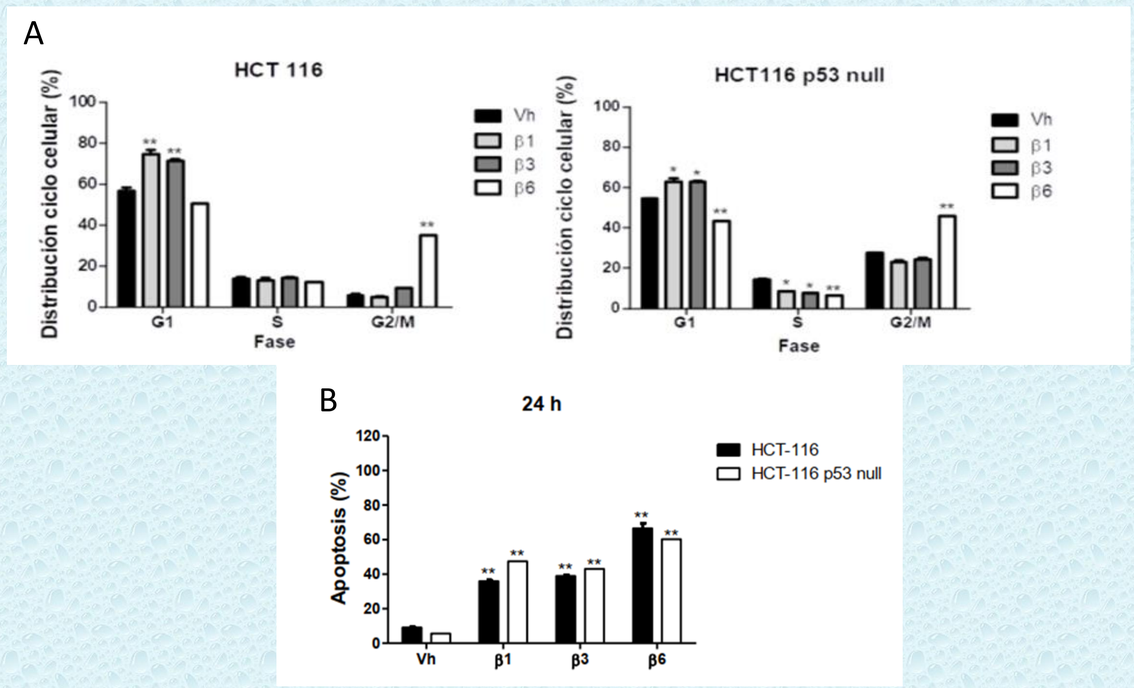

Figure 3. Results of flow cytometry assays for HCT-116 and HCT-116 p53 null cell lines with a control (Vh) without treatment and three 24h treatments with β-conglutins 1, 3 and 6. **A:** Decrease of cell proliferation by cell cycle arrest in G0/G1 or G2/M phase. **B**: β-conglutins induce apoptosis in colorectal cancer cells.