Hepatocarcinoma-associated pathways modulated by soybean peptide lunasin

Samuel Fernández-Tomé1, Pedro Indiana-Romacho2, Samuel Paterson2, Alfredo Galvez3, Blanca Hernández-Ledesma2

1 Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IIS-IP), Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Madrid, Spain.
2 Department of Bioactivity and Food Analysis, Institute of Food Science Research (CIAL, CSIC-UAM, CEI UAM+CSIC), Nicolás Cabrera 9, 28049 Madrid, Spain.
3 Reliv International, Inc. 38 Chesterfield Industrial Blvd. Chesterfield, MO 63005, USA.

Corresponding Author: Blanca Hernández-Ledesma, Ph.D., Department of Bioactivity and Food Analysis, Institute of Food Science Research (CIAL, CSIC-UAM, CEI UAM+CSIC), Nicolás Cabrera 9, 28049 Madrid, Spain.

Keywords: bioactive soybean peptide, hepatocarcinoma, apoptosis, cell cycle, cell adhesion

Background: Liver tumours are one of the most frequently found tumours in cancer patients. Among them, hepatocellular carcinoma (HCC) is the most prevalent form of primary liver cancer, being expected to become the 3rd leading cause of cancer mortality by 2030 worldwide. The initiation and progression of HCC are triggered by multiple factors like long term alcohol consumption, metabolic disorders, fatty liver disease, hepatitis B and C infection, age, and oxidative stress. Moreover, the intake of a high-fat Westernized-style diet has been recognized to play an important role (1). On the contrary, accumulating research has revealed that certain dietary natural products and their bioactive components may prevent the onset and advance of liver cancer through different mechanisms including inhibition of cancer cell growth, and reduction of oxidative stress and chronic inflammation (2).

Lunasin is a 43-amino acid peptide naturally present in soybean with a variety of biological functions, such as antioxidant, anti-inflammatory, chemopreventive, and immune- and neuro-modulatory effects (3). Although lunasin has been demonstrated to exert beneficial effects against several types of cancer such as colorectal, breast, and prostate cancer, no data are still available about its effects on liver cancer.

Objective: To evaluate the modulatory activity of soybean peptide lunasin on carcinogenesis-associated events in hepatocarcinoma HepG2 cells.

Methods: A soybean extract rich in albumin was used as starting material to obtain a lunasin-enriched sample (LES) which protein profile was analyzed by SDS-PAGE, and its lunasin content was determined by Western-Blot and ELISA, using a lunasin polyclonal primary antibody. Hepatocarcinoma HepG2 cells were treated with LES (concentrations of lunasin ranged from 10 to 150 μM) for 6-48 h, and the effects
on cell viability were measured by the 3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The action of peptide on cell cycle distribution and early and late-apoptosis was evaluated by flow cytometry. Moreover, the capacity of synthetic lunasin to attach cancer cells through its RGD domain was analyzed by crystal violet staining using poly-D-lysine as positive control.

**Results:** A total of 13 proteins with molecular weight between 8 kDa (Bowman-Birk protease inhibitor (BBI)) and 127.3 kDa (lipooxygenase-1) were identified in LES. The protein concentration was 2 mg/mL that corresponded to 36 µg bioactive lunasin/mg LES. Although LES inhibited cancer cells viability in a dose-dependent manner at all assayed incubation times, the highest effects were observed at 48 h. After this time, lunasin reduced cell viability up to 62.0% at 10 µM while 100 and 150 µM reduced viability up to 85.7 and 94.6%, respectively. The IC50 values (lunasin concentration needed to inhibit 50% of cell viability) calculated after 15 and 24 h-treatment with LES were 128.8 and 95.5 µM, respectively. Lunasin exerted its effects through induction of apoptosis, increasing the percentage of apoptotic cells from 2.0% in non-treated cells to 17.0% in cells treated with 100 µM lunasin for 15 h. Moreover, the treatment of HepG2 cells with lunasin resulted in a dose-dependent arrest of cell cycle at S-phase. Thus, the percentage of cells at this phase increased from 21.0% in non-treated cells to 36.0% in cells treated with 50 µM lunasin for 15 h. The percentage of cells at subG1-phase also increased from 2.0% (control cells) to 26.0 and 46.0% in cells treated with 100 and 150 µM lunasin, respectively. The adherence capacity assay showed that lunasin was able to attach HepG2 cells in a dose-dependent manner at concentrations higher than 50 µM.

**Conclusion:** Our study demonstrated potent chemoprotective effects of LES in hepatocarcinoma HepG2 cells. These inhibitory effects were mediated by arrest of cell cycle, induction of apoptosis and necrosis, and cell morphological changes. Moreover, lunasin showed a notable capacity to attach cancer cells that might be associated to the high expression level of integrins acting as receptors of lunasin through the RGD domain of the peptide and allowing its internalization into cells.

**Acknowledgements:** This work has received financial support from projects AGL2015-66886-R (Spanish Ministry of Science and Innovation, MICIU) and PID2019-103919RB-100 (CSIC).

**References:**

FFC's 29th International Conference:

**Corresponding Author:**
Blanca Hernández-Ledesma, Ph.D., Department of Bioactivity and Food Analysis, Institute of Food Science Research (CIAL, CSIC-UAM, CEI UAM+CSIC), Nicolás Cabrera 9, 28049 Madrid, Spain, e-mail: b.hernandez@csic.es, phone number: (0034) 910017970

**Main Presenting Author:**
Samuel Fernández-Tomé, Ph.D., Junior Researcher, Hospital Universitario de La Princesa, Instituto de Investigación Sanitaria Princesa (IIS-IP), Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), Madrid, Spain, e-mail: fernandeztome.samuel@gmail.com, phone number: (0034) 915202200 (17529)

**Co-authors:**
Pedro Indiano-Romacho, MS, e-mail: pedro.indiano@csic.es
Samuel Paterson, e-mail: samuelpatersonmoreno@gmail.com
Alfredo Galvez, PhD, e-mail: agalvez@relivinc.com

**Presentation Type:** poster

**Session:**
8. Bioactive Compounds and Chronic Diseases
   f. The Role of other Bioactive compounds in Chronic Disease