Nutraceuticals molecular targets

Nutraceuticals molecular targets (II)

A novel way of approaching health by using nutraceuticals: Combined -omics and virtual screening

This is a series of articles of the regular column for 2017 entitled "**Nutraceuticals Molecular Targets**". This column will be focused on the **analysis of the nutraceuticals market** within the last years under the new regulatory status on health claims and the big gap between science of phytochemicals, legislation and consumers.

As a continuation of our series on "Nutraceuticals Molecular Targets" let me introduce a modern approach that is being utilized in the scientific field for several years in the pharmaceutical field and is starting to be transferred and implemented to determine the beneficial effects of dietary components in human health. This is another proof that the scientific field, market and legislation work at different speeds and that science is much further ahead than market.

Traditionally, research on bioactive compounds focused to prove their health effects involve, as a primary step, testing food components and mixtures on cellular models that mimic metabolic stress conditions. The next obligatory step is to assay those food components on animal models that resemble certain pathologies using normal or transgenic animals. Finally, to test the beneficial effects of food components, human trials using randomized, placebo controlled and double-blind standards are recommended. In many cases, most of the compounds responsible for such effects are unknown.

In the last decade, the modern technologies developed in the scientific field have circumvented some of the steps in this established workflow and have redesigned the way of working.

First, identifying blood compounds derived from food (metabolites) rather than food components have become one of the main targets in the research on bioactive food components. Data based on studies that directly use botanical extracts or food stuff on cellular models, without considering metabolic aspects, have limited applicability. In contrast, studies exploring the absorption process, metabolites in the blood circulation and, more importantly, the intracellular final effectors that are responsible for bioactivity are becoming the right choice. Therefore, the combination of studying the cellular protein profile (proteome) together with the resulting metabolomic profiling (set of small organic molecules of a cell or organism, also called metabolome) correlates better with the beneficial effects of food components. This strategy is becoming the most suitable approach to understand the effects of food and diet in human health. Metabolome includes not only endogenous metabolites that are naturally produced by an organism (amino acids, organic acids, nucleic acids, fatty acids, sugars, vitamins, etc.) as well as exogenous chemicals (drugs, contaminants, food components such as plant polyphenols, toxins, etc.).

These –omics sciences represent the future for the discovery of reliable biomarkers associated to disease diagnosis, selection of treatments, reduction of therapeutic errors and monitoring in nutritional and pharmaceutical therapies.

Second, omics technologies allow us to identify hundreds or thousands of molecules that might be responsible for the beneficial effects of food bioactive compounds. These molecules are being incorporated in public accessible chemical libraries such as Super Natural II (http://bioinf-applied.charite.de/supernatural_new/index.php), Human Metabolome Database (http://www.hmdb.ca/) or Metlin database (https://metlin.scripps.edu/). Traditional cellular or animal models are clearly insufficient to approach such complexity and variety. As an alternative, computational molecular docking techniques are a unique tool for virtually screening a large number of compounds on selected protein targets in order to elucidate their potential mechanisms and effects. These in silico approaches allow virtual screening of millions of compounds against known molecular targets with a reasonable economic cost. Docking experiments usually start with the crystallographic structure of a protein with medical interest and can predict bound conformation and binding free energy of small molecules to the catalytic or allosteric binding sites of the protein (1). Fast docking methods with atomic resolution may take only a few minutes per ligand or compound (2), whereas molecular dynamics-based approaches may require hundreds or thousands of hours per ligand (3). From these studies, the Gibbs free energy variation (ΔG, Kcal/mol) for each ligand is obtained and those showing lowest energy variations can be selected as putative biomarker modulators, either inhibitors or agonists (4, 5). Usually, ΔG values ≤−10 kcal/mol imply dissociation constants (K_D) values in the nanomolar or subnanomolar range, therefore it can be used as a threshold to first filter the docking results.

Third, some human diseases have been related to deregulation of crucial metabolic enzymes such as the AMP-activated protein kinase (AMPK) (6). The cellular energy state is detected by various dynamic mechanisms that regulate the balance between catabolism and anabolism. AMPK is a cellular fuel sensitive kinase activated in deficient bioenergetic states that are caused by a lack of nutrients or hypoxia. It is well-known that AMPK is activated by energy demanding conditions such as starvation or physical exercise. Activation of several protein targets by AMPK promotes inactivation of the energy-consuming pathways and activates the catabolism of fatty acids and other fuels. Therefore, this mechanism increases the available energy for the cell and decreases its content of reserves. AMPK is involved in the regulation of carbohydrate and lipid metabolism, resulting in the inhibition of ATP-consuming anabolic pathways. In parallel, AMPK activation stimulates ATP production by increasing fatty acid oxidation, muscle glucose transport, mitochondrial biogenesis and caloric intake. It also plays a major role in hormonal signaling and is a central node of such signaling pathways, including the endocrine system (7). Therefore, AMPK is postulated as a molecular target in obesity-related pathologies.

As an example of these strategies, we have applied these *in silico* screening techniques to select compounds as potential modulators of two proteins of pharmacological interest in obesity: the transcription factor receptor PPAR-gamma (4) and the AMPK (8). In the first case, we have identified through a computational study a number of plant-derived phenolic compounds that can modulate the activity of PPAR-gamma and therefore may regulate lipid and energy metabolism. Currently, research is ongoing to verify the potential of the selected compounds to modulate lipid metabolism in cellular models for obesity. In the second example, we have used a different target related to obesity, the AMPK. An olive leaf extract showed the capability to reduce triglyceride content in adipocyte cellular model concomitantly to AMPK modulatory activity (8). Then, a bioassay-guided approach was utilized to isolate the fractions from the extract that exhibited AMPK modulatory activity on the adipocyte cell model and to further identify the potential compounds responsible for such activity. Molecular docking experiments revealed that several polyphenols may function as AMPK modulators: secoiridoids, cinnamic acids, phenylethanoids and phenylpropanoids, flavonoids and lignans. Ongoing research is focused on corroborating the direct effect of these isolated compounds on AMPK using cell models.

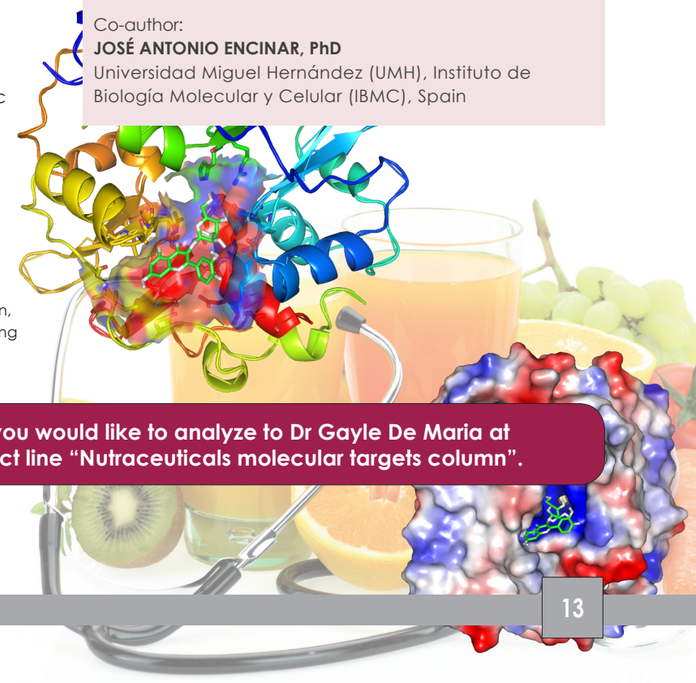
Most probably, in the development of the next generation of nutraceuticals, we won't be expecting the results of tedious cellular or animal models for those food components showing experimental evidences of human beneficial effects. In contrast, we will be virtually screening databases containing thousands of both, known and new molecules, on selected molecular targets related to human diseases. Cell or animal studies will be only utilized to prove the efficacy or the absence of toxicity of the selected ingredients. This novel work-flow will bring us into a new era where magic optimized combinations of nutrients can be designed for specific molecular targets or pathologies. Will the legislative framework, European food authorities or the market be prepared for this challenge?

REFERENCES

1. S. Forli, R. Huey, M.E. Pique, M.F. Sanner, D.S. Goodsell, A.J. Olson, Computational protein-ligand docking and virtual drug screening with the AutoDock suite, Nat Protoc, 11 (2016) 905-919.
2. O. Trott, A.J. Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, Journal of computational chemistry, 31 (2010) 455-461.
3. H. Perez-Sanchez, W. Wenzel, Optimization methods for virtual screening on novel computational architectures, Curr Comput Aided Drug Des, 7 (2011) 44-52.
4. J.A. Encinar, G. Fernandez-Ballester, V. Galiano-Ibarra, V. Micol, In silico approach for the discovery of new PPARgamma modulators among plant-derived polyphenols, Drug design, development and therapy, 9 (2015) 5877-5895.
5. V. Galiano, P. Garcia-Valtanen, V. Micol, J.A. Encinar, Looking for inhibitors of the dengue virus NS5 RNA-dependent RNA-polymerase using a molecular docking approach, Drug design, development and therapy, 10 (2016) 3163-3181.
6. J.A. Menendez, J. Joven, G. Aragones, E. Barrajon-Catalan, R. Beltran-Debon, I. Borras-Linares, J. Camps, B. Corominas-Faja, S. Cufi, S. Fernandez-Arroyo, A. Garcia-Heredia, A. Hernandez-Aguilera, M. Herranz-Lopez, C. Jimenez-Sanchez, E. Lopez-Bonet, J. Lozano-Sanchez, F. Luciano-Mateo, B. Martin-Castillo, V. Martin-Paredero, A. Perez-Sanchez, C. Oliveras-Ferraros, M. Riera-Borrull, E. Rodriguez-Gallego, R. Quirantes-Pine, A. Rull, L. Tomas-Menor, A. Vazquez-Martin, C. Alonso-Villaverde, V. Micol, A. Segura-Carretero, Xenohormetic and anti-aging activity of secoiridoid polyphenols present in extra virgin olive oil: A new family of gerosuppressant agents, Cell Cycle, 12 (2013) 555-578.
7. D.G. Hardie, AMP-activated protein kinase: an energy sensor that regulates all aspects of cell function, Genes Dev, 25 (2011) 1895-1908.
8. C. Jimenez-Sanchez, M. Olivares-Vicente, C. Rodriguez-Perez, M. Herranz-Lopez, J. Lozano-Sanchez, A. Segura Carretero, A. Fernandez-Gutierrez, V. Micol, AMPK modulatory activity of olive–tree leaves phenolic compounds: Bioassay-guided isolation on adipocyte model and in silico approach, PLoS ONE, 12 (2017) e0173074.

VICENTE MICOL, PhD
Professor of Biochemistry and Molecular Biology
Universidad Miguel Hernández (UMH), Instituto de Biología Molecular y Celular (IBMC), Spain
CIBER: CB12/03/30038, Fisiopatología de la Obesidad y la Nutrición, CIBERobn, Instituto de Salud Carlos III (ISCIII), Spain

Co-author:
JOSÉ ANTONIO ENCINAR, PhD
Universidad Miguel Hernández (UMH), Instituto de Biología Molecular y Celular (IBMC), Spain



Please send your reaction/comments/topics you would like to analyze to Dr Gayle De Maria at gayle@teknoscienze.com placing in the subject line "Nutraceuticals molecular targets column".