COST Action FA1403

Progress Report of the STSM "FA1403-060317-082146"

"*In silico* prediction of interplays between flavanone metabolites and cell signalling-modulators in the vasculo-protective effect of polyphenols"

Previous studies from our group have demonstrated the contribution of flavanones, a flavonoid subclass of polyphenols, in the vascular protection in humans (REF) and preclinical models (REF). To decipher the underlying mechanisms of action of flavonones, we performed transcriptomics analyses. These analyses have shown that a dietary exposition to flavanones results in the modulation a gene expression towards an anti-inflammatory and anti-atherogenic profile. In addition, results from the in vitro experiment support the hypothesis of a beneficial effect of flavanones to preserve the endothelial function and thus to maintain vascular integrity. Our main objective now is to identify and validate cell-signalling pathways involved in the genomic effects of these compounds. To this way and before further experimental investigations, we would like to determine the potential molecular targets of these plant food bioactives. **The main goal of this STSM is to prioritize the potential targets of flavanone metabolites using computational chemistry methods. This screening will be focused to interplay of flavanone metabolites with proteins involved in NFκB modulation.**

Methods & Results:

Three datasets of gene expression in response to flavanones were used in this STSM (REF). These data were subjected to a new bioinformatics analysis to establish a target's mechanism of action of flavanones (hesperetin and its 3'-O-glucuronide, 7-O-glucuronide, and 3'-O-sulfate metabolites; naringenin and its 4'-O-beta-D-glucuronide and 7-O-beta-D-glucuronide metabolites). Using MetaCore™ software, we identified the most probable transcription factors that could explain modulation in gene expression in response to flavanone consumption/exposition. Figure 1 presents the lists of the Top 5 transcription factors (Left side). CREB1, C-myc, SP1, Oct3/4, p53, cJun, Hif1A and p65 transcription factors may at least explain modulation observed in two of the three datasets. Only SP1 and c-myc modulations might explain gene expression modulations observed in the three datasets.

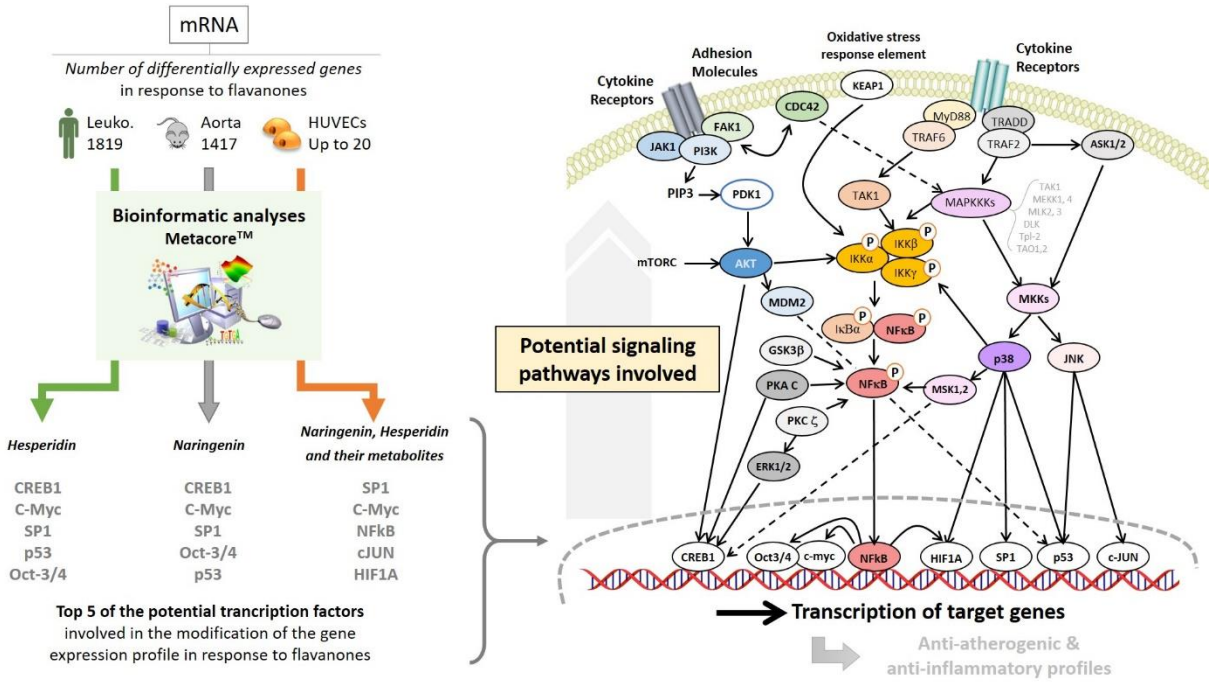

Figure 1: Identification of the potential targets that could explain the modulation of gene expression in response to flavanones.

Several signalling pathways may regulate the activity of these factors as shown in figure 1 (right side). We identified ~30 signalling proteins as potential targets involved in the cellular response to flavanones. These proteins belong to various families: protein adaptors (TRAF2, TRAF6, KEAP1, IκBα, MDM2, MyD88), Rho GTPase (CDC42), transcription factors (p65), Serine/Threonine kinases (*AGC kinases*: PKA, PDK1, AKT1/2/3, PKC ζ, MSK1/2; *CMGC kinases*: GSK3β, p38 MAPK, JNK1/2/3, ERK1/2, ASK1/2; *STE kinases*: TAK1, MEKK1/4/6, TAO1/2, MKK3/4/6, Tpl-2; *TLK Kinases*: DLK, MLK1/2), Tyrosine Kinases (FAK1, JAK1) and other kinases (PI3K, IKKα/β/γ).

To develop a hypothesis on how flavanones can interact with these targets, the strategy used was 1/ to cross reference from on-line databases regarding to structural data of proposed targets and structural data of ligands, their homologs, and their affinities, 2/ to predict the binding using virtual screening (docking).

First, co-crystal structures of selected proteins/ligands were search in PDB and ChEMBL databases. Only the interaction of naringenin in the kinase domain of p38 MAPK was reported in PDB. Hesperetin was identified as ligand in the databases but it was not co-crystal structure with selected tragets. It is worth to note that databases did not include any phase II metabolite of flavanones. Databases were queried for co-crystal structures of selected targets with others polyphenols. Details on the binding mode of hesperidin and naringin to the kinase GSK3β, hesperidin and quercetin to p38 mapk and quercetin to PI3K were identified.

The PDB database was queried for available co-crystal structures of selected targets. Only co-crystal structures with the resolution better than 3Å were retained. Further, since certain similarity is expected between co-crystallized ligands and our flavanons of interest, we further refined our selection of PDB files by excluding structures without ligands or with ligands of very different size (number of carbon atoms < 15 or > 25) or too different 2D/3D features from flavanons.
For examples, there are 24 PDB structures of KEAP1, one non-kinase of interest, but only 3 have sufficient similarity with flavanons. Two are the structures of KEAP1 BTB domain and contain covalently attached ligands. Since our flavanons do not contain reactive enone group which acts as a Michael receptor in co-crystallized ligands, there is little chance that flavanons would react in the similar way and thus bind to the same binding site in the BTB domain. The third one is the structure of KEAP1 KELCH domain and is considered suitable for further explorations.

We also evaluated the shape/pharmacophore similarity between flavanons and co-crystallized ligands. This was performed using ROCS (version 3.2.1.4, OpenEye Scientific Software). For each co-crystallized ligand, we constructed the ROCS query based on its binding conformation and using vROCS query generation wizard. We generated ensemble of 3D conformers for flavanons and their metabolites using Omega2 (version 2.5.1.4, OpenEye). ROCS optimized the alignment of each conformer with the query and calculated Shape and Color Tanimoto score for the shape and pharmacophore match respectively. Final ranking was based on the combination of these two scores (Tanimoto Combo). For each flavanone we kept up to 4 best alignments.

For each target, selected PDB files were downloaded. Protein structures were prepared for docking using Protein Preparation Wizard in Maestro (version 10.7.015, Schrödinger, LLC) which includes: assigning bond order to ligand, adding all hydrogen atoms, adjusting protonation state of ionazible groups, optimizing the hydrogen-bond network within the complex, removing crystallographic water molecules with fewer than 3 interactions with the protein or ligand, and constrained geometry minimization to relax the complex structure within the OPLS3 force field which will be used for subsequent docking. On the other side, the 2D structures of hesperetin, naringenin and their metabolites were converted into 3D ensuring proper assignment of stereocenters, hydrogen

atoms were added, the acidic groups such as sulfate and carboxylate were deprotonated and the final structures were geometry minimized in OPLS3 force field. This procedure provided a ligand file ready for docking.

Docking was performed using Glide (Schrödinger, LLLC). For all kinases, the receptor grid was calculated using a hydrogen-bond constraint to the hinge-region backbone hydrogen-bond donor, the interaction present in a vast majority of kinase inhibitors targeting the ATP-binding site. Glide docking was run using the SP ("standard precision") option and top 4 docked poses, following post-docking minimization, were retained for final analysis.

Conclusion & Perspectives:

Computational modeling can yield a large number of false positive results. For that reason it is important to corroborate them by the experimental data, which is why we searched for such data in public databases such as ChEMBL and PDB. The fact that we could identify experimental data for roughly only a half of proposed targets somewhat limits our predictions, but it nevertheless ensures improved accuracy for prioritization of the remaining targets.

Among the nineteen considered targets, KEAP1 belongs to the protein adaptator family, while the rest are kinases. The flavanons of interest and their metabolites show a good shape match to the 5FNR ligand, although the pharmacophore match is only mediocre. Nevertheless, flavanone metabolites contain acidic group similar to most of KEAP1 ligands interacting with the KELCH domain. We could successfully dock most of our compounds. It is not possible, at the moment, to define a definitive putative binding mode of these compound, but we could frequently observe a pi-pi stacking of the flavanons aromatic rings either with Tyr-525 or with the guanidinium groups of Arg-415 or Arg-483 as well as the salt bridge between the negative acidic groups of the metabolites and the aforementioned arginine sidechains. For those reasons we believe that a direct interaction between the flavanons, and especially their metabolites, and KEAP1 KELCH domain is likely.

Regarding the kinases, our compounds in general dock well to these targets and present a number of possible binding modes to the ATP binding site. Due to the relative symmetry of the molecules in terms of the pharmacophore locations the alternative binding modes cannot be excluded. A study of the binding modes of naringenin and hesperetin to other targets, which are not on our selected list, but for which co-crystallized structures are available, reveals that there is often only 1 and not more than 2 direct hydrogen-bonds between the ligand and the target aminoacids. Hydrophobic interactions with the ring systems also play a part (figure 2). We could also identify co-crystal structures of high interest directly showing the binding mode of flavanons to kinases. PDB structure 4EH3 is a complex of naringenin to p38MAPK, while 1E8W is a close flavon analog, quercetin, co-crystallized with PI3Kγ (figure 3). Both naringenin and quercetin orient themselves in the same fashion in the ATP binding pocket and their carbonyl group forms the important hydrogen-bond with the hinge hydrogen-bond donor. Thanks to the availability of strong data indicating how flavanons interact with kinases we concluded that the ligand similarity assessment would not bring much value for this target class and proceeded only with docking.

For a number of kinases our docked poses could reproduce this experimentally proven binding mode. What remains is to evaluate generated docked poses of our compounds to all kinase targets and to assign their relative likelihood of binding, which will enable us to rank them.

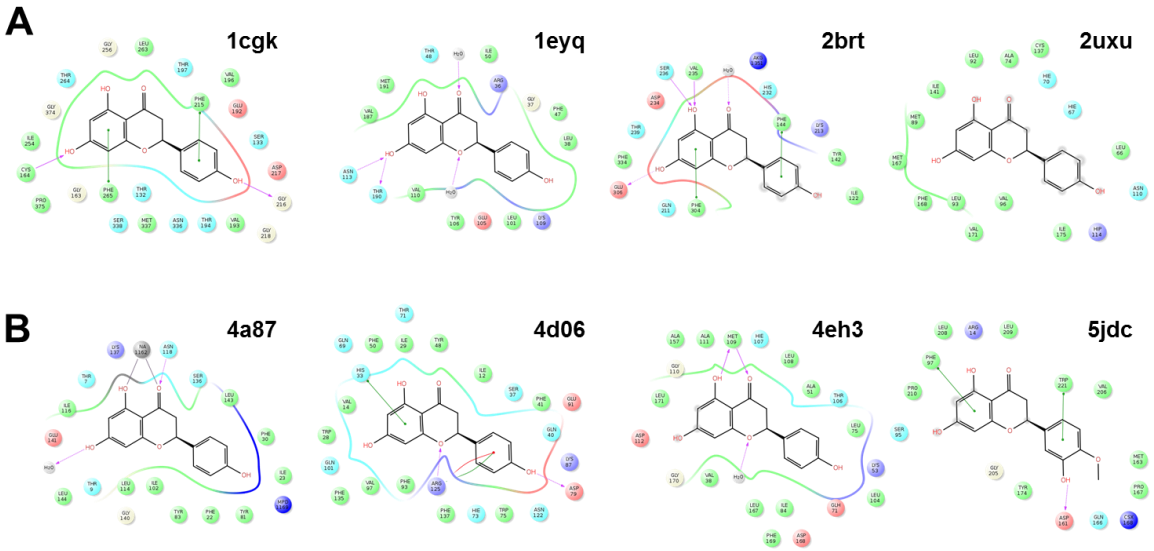

Figure 2: Examples of flavanon binding modes to other targets.
A: Naringenin; B: Hesperetin.

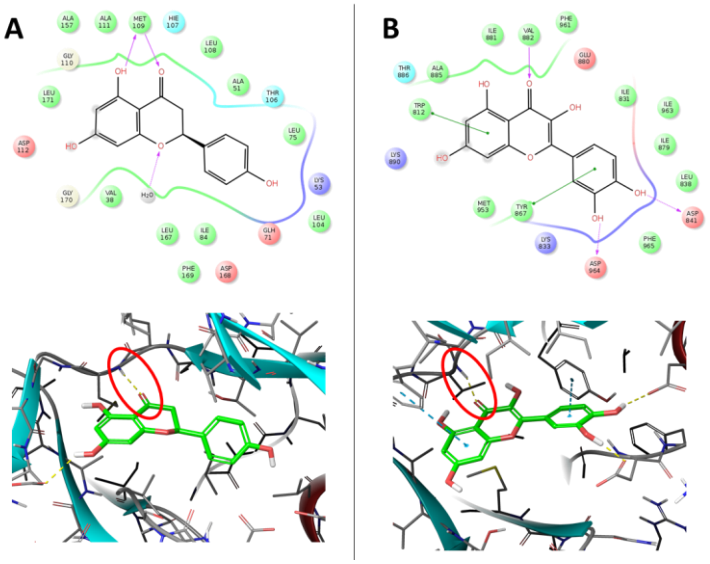

Figure 3: kinase binding modes of flavanon and flavon ligands.
A: Naringenin–p38MAPK complex (PDB Id: 4eh3), B: Quercetin–PI3Kγ complex (PDB Id:1e8w)

One expected outcome of this STSM is the preparation of one publication reporting the prioritization of flavanone targets and their docking mode obtained by computation approaches, and their experimental validation.

Identification and priorization of polyphenols targets is one objective of the working group 2 in the COST Action POSITIVe. This will allows to pinpoint cellular pathways, candidate genes and their variants involved in inter-individual variation. One perspective is to screen database to identify single nucleotide polymorphism that could affect polyphenol/target interplay and could be link to interindividual variation in the response to the consumption to flavanones.

COST Action FA1403

STSM Partners

Home Institution:

UMR 1019 – Human Nutrition Unit
INRA Centre de recherché Auvergne-Rhône-Alpes, 63122 Saint-Genès-Champanelle, France

Date: 14/04/2017
Dr Laurent-Emmanuel Monfoulet