A calcium ghost in the castle

The second *ECS workshop* took place in the castle of Smolenice, a small village in Slovaquia. The castle was built during the 14th century in order to protect the little Carpathians where the vampires tale was born. The castle was burnt by Napoleon when he was invading Russia and then rebuild during the 20th century. Since June 26, 1953 it is the property of the Slovak Academy of Sciences and devoted to scientific meetings gathering scientists from all over the world.

Many thanks to Albert Breier, Branislav Uhrik (and to the whole local committee) and to Claus Heizman that have been key persons in the organization of this exquisite ECS workshop.

The atmosphere of the castle is romantic with plenty of corridors in which you get lost and , if you travel between rooms at night, encounters ghost or vampire. We were more than sixty attending to this workshop with gender equilibrium.

At the evening of our arrival, the keynote lecture was given by **Joseph Metzger**. He presented a renewed vision of parvalbumin. This protein, the first member of the EF hand family to be described and purified, is a relaxing factor of the calcium signal. The elegant idea of Joseph was to use it to modify the calcium signal of a pathological cardiac myocyte in order to get back to a calcium signature of a healthy myocyte. He perfectly illustrates the emerging biology integrating the balance between modelisation and experimental validation. In some ways, he sets up a new challenge for our scientific community: How to design the right calcium binding protein in order for this mutated protein to make evolving a cellular calcium signal from a given shape to a completely different signature?

Then we got a beer.

The second day was devoted to annexins interacting with S100s and others targets.

Carl Creutz, the annexin grandpa (as introduced by Steve Moss) was presenting some of the relatives in a remake of the Chinese movies "*Spouses and Concubines*"[1]. In this story, the sorcins were spouses and the copin(e)s were playing concubines although it was not obvious if one sorcin was playing with two annexins or was tied to one annexin breaking the annexin dimer. The sorcin was interacting with the extended N-terminus of the annexin A7 and A11 through a putative new structure made of a beta helix (composed of proline and aromatic aminoacids). Now, we are stuck with the following questions: what is the function of this sorcin with five (EF) hands and his marriage with annexin? Some hints about a putative regulation of alternative splicing regulation by calcium were proposed by **Joachim Krebs** (because of some filiations between sorcin and alg2, a protein involved in such regulation).

On the other hand, using this new fancy tool (the atomic force microscope), Carl Creutz was presenting evidences that copins were helping annexins in the structuration of membranes and can play a role of anchoring proteins to nucleate lipoproteic supramolecular complexes.

Following this same general idea that annexin complexed with a calcium binding protein may be the nucleus of a macromolecular complex, **Volker Gerke** told us about the love affair between annexin II

[1] by the movie maker Zhang Yimou

and S100 A10 (I told you that the atmosphere of the castle was truly romantic). A dimer of annexin A2 was twinning with a dimer of S100A10 but S100A10 was exhibiting some free space for others partners. TRV5 and TRV6, special ion channels, were among them. The role of sorcin was devoted to S100A10 or A11 in the case of annexin II but no copins were exhibited.

Then, we got a beer.

Jean Gruenberg illustrates the role of annexin A2 in the biogenesis of endosome. Annexin A2 has also the ability to structure the membrane. **Richmond Muimo** shows that others ionic channels may also be a target of the hetero tetrameric complex annexin A2-S100A10.

Then, we got a beer and a lunch.

Beginning of the afternoon, Volker Gerke introduced **Katherine Hajjar**. She tolds us how annexin A2 and S100A10 participate to the fibrinolysis regulation. Again, annexin II by interacting with major players of the fibrinolysis, was structuring some of the mess of the clothing pathways.

Then, we got a beer.

And **Steve Moss** came to tell us that you have the choice between not seeing others becoming older (because you are loosing visual acuity) or not seeing yourself becoming older (because you are dying from kidney failure). The choice is between having or not having annexin A2,"that is the question".

Then, **Gary Shaw** wanted to explain all the previous talks from a structural point of view. It was a real challenge as most of the audience was real biologists.

Then we got (not a beer) but plenty of glasses of Tokay. If you go for only one, take the classic Tokay 6 putanas or putnovy. Putnovy is a barrel (about 25 kg when full) that slovakian women fill with grape seed contaminated with botrytis. When you mix 6 of those barrels with 136 l of dry white wine, you are getting a superb wine that you can drink with a "foie gras", stilton or Roquefort and with chocolate.

And the day went out with a grill party (again, a romantic one with barbecue and sausage –missing marshmallow and guitar).

Friday came and **Claus Heizmann** introduced the S100 day. In this family of 26 genes, S100B is now clearly accepted as a biomarker of brain damage. Whatever the brain aggression (stroke, neurodegenerative damage, …), S100B seems to be secreted by astrocytes and glial cells being a signal of danger. Then S100B concentration increased in the plasma and it seems possible to correlate the level of aggression of your brain and the concentration increase of S100B in blood.

Then, the link between calcium toolkit (the set of genes that codes for proteins participating to the generation and the decoding of calcium signal), inflammation and cancer was illustrated by **Jacques Ghysdael** from the Curie Institute that told us the tale of the leukemia cell, thymocytes that escape from their normal fate. By playing with the activation of calcineurin (protein revealed to the world by Claude Klee in the early 80s), you may rescue poor mice condemned to develop lymphomas. So, the obvious next step would be to use the calcineurin inhibitor of calcineurin (FK506) as a new anticancer therapy. But FK506 by inhibiting calcineurin is an immunosupressor that decreases the ability of the

organism to fight against tumor cells. It will then be necessary to exquisitely balance between acute and chronic treatment to optimize the putative action of such treatment in specific leukemia.

Then we switch to Alzheimer with **Octavio Arancio**. It exists in this domain several sects, each one believing in a specific cause for the development of the disease. We have the plaque believers, the tangle prayers, the epigenomic backgroundists and so on. However, a general working hypothesis in neurodegenerative disease is emerging. Aggression of the brain induces an inflammatory response ending in a chronic inflammation, neuron damage and therefore, the progressive apparition of specific symtoma depending of the temporal and spatial localization of the aggression and its type. What Octavio told us was that in Alzheimer disease, calpain (a calcium activated protease) is a key element in this chain of event. The calpain activity is correlated with neuronal damage. Therefore, by inhibiting calpain with small inhibitor molecules, it was possible to prevent neuronal damage and therefore, cognitive impairment at least in mouse model. What is going to be the efficacy of such drugs in human remains an open question as well as the toxicity of such lifelong treatment?

Peter Lackner gaves us a clear lecture in bioinformatics on how to align proteins and to predict structural domains or motifs. He then presented the concept of hypoallergen, a “degraded allergen” that can be used to desensitize an allergic patient. What you have to do is to conserve the “primary sequence information “and to degrade the “structural information” as much as possible. He illustrated this concept on calcium binding proteins that are classical allergens and their interaction with IgE. It may be possible to use such concept to weaken the interactions between calcium binding proteins and their targets therefore creating hypoactive mutants that may help to decipher the role specific calcium binding proteins in a given signaling pathway.

Ulrike Stein elegantly reported the link in colorectal cancer between S100A4 expression and metastasis. Beta-catenin is responsible of the upregulation of S100A4. Whatever the exact molecular mechanism of this cascade of event, it is clear that the inhibition of the S100A4 expression is a way to prevent metastasis. By screening collection of molecules, Ulrike has found either molecule inhibiting beta-catenin to trigger the expression of S100A4 or molecules that interferes with the S100A4 promoter. It is a gorgeous illustration that calcium binding proteins are biomarkers of important diseases but also constitutes a very attractive set of new therapeutic targets.

Finally, **Matthias Rothermundt** summarizes his work on the level of S100B and the prognostic in psychiatric disorders, namely schizophrenia and melancholic depression. In brief, high level of S100B is associated with less clinical improvement.

If we consider that S100B is a marker of an inflammatory response, what are the links between psychiatric disorder and inflammation?

Then we got a light lunch, a beer and took the bus to go to Spa town, Piestany. We had the choice between visiting the town and the Spa island (what I did) or to be tortured by heavyweight Slovakian wrestlers (they called that a massage) (what Roland Pochet got and aknowledged as a lifelong experience).

We started our gala dinner at five o’clock without beer and we enjoyed traditional Slovakian music given by a unique family , husband and wife with fantastic voice and their seven children playing all kind of old Slovakian instruments.

Coming back to the castle, the poster session started at 8 (with draft beer) and was the acme of such workshop and what we expect in this ECS meeting, a lively speed dating between young scientists from all over the world with (I hope) the generation of a bunch of collaborative and multidisciplinary works.

Saturday morning was our last session that was devoted to calcium and endoplasmic reticulum. **Albert Breier**, our host, pinpoint a link between MDR activity and calcium homeostasis in tumor cell. He was showing striking correlations such as the one between inhibition of MDR and binding to calmodulin for a set of small molecules. Is there a cause-effect relationship remains to elucidate ?

Helen Coe, a graduated student from Michalak's lab gave a brilliant presentation on the molecular role of calreticulin as a chaperon of the endoplasmic reticulum. The lab is using transgenic mutant mice to gain or loss of function of calreticulin. They are able to manipulate the temporal and spatial concentration of calreticulin and to link those modifications with several cardiac phenotypes. In absence of careticulin, it is lethal. After birth, the concentration of calreticulin decreases. If this concentration is kept high (respectively, if after a while, the calreticulin concentration is increased), the mouse gets a congenital heart block (respectively, a cardiac hypertrophy). It will be extremely interesting to have more presentation from graduated students of this caliber in the ECS workshops.

Finally, **Jan Lehostky** pinpoints the role of mitochondria as a calcium store during ischaemia and ischemic tolerance and **Adriana Gibadulinova** stressed the role of S100P and its transcriptional regulation of S100P in Hela cells, another member of the S100P family usable as biomarker in cancer.

What I kept from this meeting is a friendly and lively atmosphere in a fantastic location. Thanks to the Slovakian Academy of Sciences and to Albert Breier.

From a scientific point of view, I got the feeling that cancer, neurodegenerative disease, ischaemia, cardiac failure are symptoma that develop in response to a specific stress (DNA damage, impairment in the protein folding, oxidative stress, …). In **the heart of the inflammatory response** induced by such stresses, the **calcium signal is a "chef d'orchestre"** and among the calcium toolkit, the S100 familly , the annexin family and calcineurin (but also calmodulin and probably some others factors) are key actors. Therefore, I left Smolenice with yet a stronger conviction that the proteins of the calcium toolkit constitutes a potential set of biomarkers and therapeutic targets in at least cancer, neurodegenerative disease and cardiac pathology.

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